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CAR-T Cell Therapy for Solid Tumors: A Review of Challenges and Emerging Solutions

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ABSTRACT

Chimeric antigen receptor (CAR)-T cell therapy is a promising immunotherapy for hematological malignancies. However, its application to solid tumors is limited by challenges such as the immunosuppressive tumor microenvironment, poor T-cell trafficking and infiltration, and on-target off-tumor toxicity. This review article discusses innovative strategies to address these limitations and enhance CAR-T cell efficacy against solid tumors. One approach involves engineering CAR-T cells to express checkpoint blockade inhibitors or dominant negative receptors to counteract immunosuppressive signals. Alternatively, CAR-T cells can be modified to secrete immunostimulatory cytokines or resist immunosuppressive factors like TGF-β. To improve T-cell trafficking and infiltration, regional delivery methods such as intrapleural or intracerebroventricular injection can be employed. Additionally, equipping CAR-T cells with chemokine receptors that match tumor-derived chemokines can enhance their homing ability. Overall, these emerging strategies hold the potential to overcome the current obstacles and expand the therapeutic applications of CAR-T cell therapy for solid tumors.

Introduction to CAR-T Cell Therapy

Cancer treatments have traditionally relied on chemotherapy, radiation therapy, and surgery. While these remain widely used, immunotherapy has emerged as a promising non-surgical alternative. Immunotherapy harnesses and enhances the body's immune system to fight cancer, either by stimulating an immune response against tumor cells or by engineering immune cells to target them. For example, immune checkpoint inhibitors, a class of drugs that block proteins that inhibit immune responses, have demonstrated effectiveness in treating various malignancies (National Cancer Institute, 2022). These drugs essentially release the brakes on the immune system, allowing it to more effectively recognize and attack cancer cells. James P. Allison and Tasaku Honjo were awarded the 2018 Nobel Prize in Physiology or Medicine for their groundbreaking work on cancer immunotherapy. Their research led to the development of many mechanisms for using immune checkpoint inhibitors for cancer therapy and preventing the immune system from attacking healthy cells (Zang, 2018).

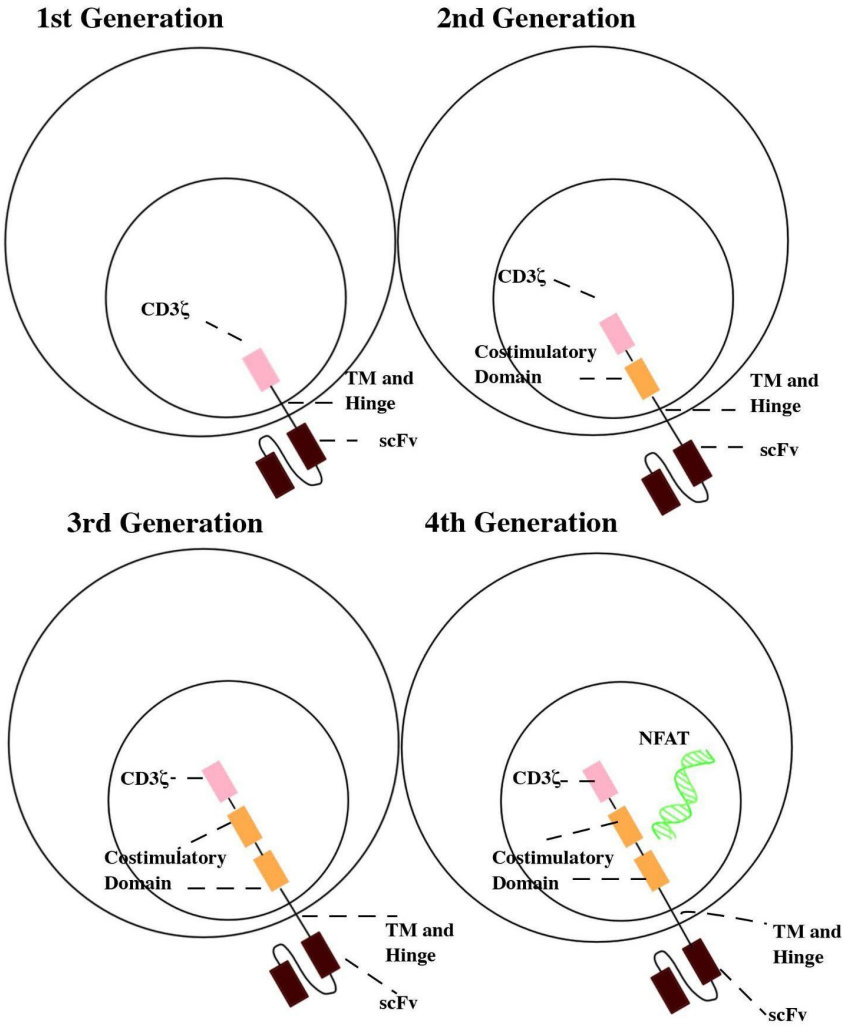

Figure 1. This figure illustrates the development of CAR-T cells for greater efficacy against cancer. The first generation consists of a single CD3ζ signaling domain, which improves activation and signaling after recognizing the target antigen (e.g., CD19). The second and third generations incorporate additional costimulatory domains to further enhance immune responses. The fourth generation introduces a unique feature: inducible expression of cytokines such as IL-12, which stimulates CD8+ T cell function, through a promoter, NFAT, that is responsive to the nuclear factor of activated T cells (Poorebrahim et al., 2021)

CAR-T cell therapy entails multiple steps: leukapheresis to isolate T cells, T-cell stimulation and genetic modification to express the CAR, expansion of the modified cells, and rigorous quality control before reinfusion (Dana et al., 2021). Primarily used for hematological cancers like leukemia and lymphoma, CAR-T therapy offers advantages over conventional treatments due to the ability of T cells to form immunological memory, leading to sustained responses against targeted antigens. The duration of CAR-T cell treatment is generally shorter than traditional methods like chemotherapy and radiation, as the engineered T-cells can rapidly recognize and eliminate cancer cells upon reintroduction. Moreover, CAR-T cells exhibit a high degree of precision in targeting tumor cells with the specific antigen they are designed to recognize. However, CAR-T therapy is not without limitations, including antigen escape (where cancer cells lose the targeted antigen), on-target off-tumor effects (where healthy cells expressing the antigen

are also attacked), challenges in T-cell trafficking and infiltration into solid tumors, the presence of immunosuppressive tumor microenvironments, and the potential for CAR-T cell-related toxicities like cytokine release syndrome (Sterner et al., 2021)

How CAR-T Cell Therapy Can Be Used in an Immunosuppressive Microenvironment

CAR-T cell therapy can be used in conjunction with checkpoint blockade immunotherapy to improve T-cell functions. Solid tumors release inhibitory molecules, such as PD-L1, a protein that codes for programmed cell death (Xiang et al., 2017). These inhibitory molecules are expressed on both host and tumor cells and can disrupt CAR-T cell therapy, as PD-1 receptors expressed on active T-cells interact with PD-L1 and inhibit T-cells from eliminating target cells (Jiang et al., 2019). Therefore, using cell-extrinsic and intrinsic strategies to block the interaction between PD-1 and PD-L1 can help overcome this issue.

Cell-extrinsic strategies involve the use of HER2-targeted CAR-T cells (Grosser et al., 2019). In a 2013 study, upregulated PD-1 resulted in increased proliferation of in vitro T-cells, IFN-γ (interferon-gamma, which induces macrophages and enables rapid phagocytosis and tumoricidal activities), granzyme B expression (a protease enzyme produced by cytotoxic lymphocytes with virucidal and tumoricidal properties), and increased activity of CAR-T cells in general (John et al., 2013). Additionally, PD-L1 checkpoint blockade (CPB) was also found to be effective for CAR-T cell therapy by blocking PD-L1 expression on MDSCs (Huang et al., 2017). While the administration of CPB can lead to potentially toxic PD-L1 levels, this can be mitigated by combining anti-HER2 CAR-T cell therapy (T-cells specifically programmed to target HER2-expressing proteins) with an oncolytic adenovirus, which allows for the production of PD-L1 antibodies within the tumor microenvironment (Tanoue et al., 2017). Clinical trials by Borghaei and his colleagues show encouraging results: Nivolumab (a fully human anti-PD-L1 antibody) resulted in a one-year survival rate of 51% and a response rate of 19% in patients with NSCLC (Non-Small Cell Lung Cancer) (Borghaei et al., 2015).

Studies have also shown that cell-intrinsic methods can assist in the performance of CAR-T cell therapy for solid tumors. According to Grosser and his colleagues, cell-intrinsic methods continuously provide CPB to block PD-1/PD-L1 immune checkpoint pathways (Grosser et al., 2019). These pathways prevent the immune system from attacking healthy cells but can also be exploited by cancer cells to evade T-cell attack (National Cancer Institute, 2022). This continuous blockade allows the dominant negative of PD-1 to compete with PD-L1 and PD-L2 (programmed death ligand 2; regulating CD4+ T-cell responses), leading to less suppression of T-cells, and therefore increased efficacy of CAR-T cell therapy, especially in lung cancer. Additionally, genome editing through CRISPR/Cas9 (a genome editing technology that allows alteration of sections of DNA) can be used to induce T-cell resistance to cancer cells exploiting PD-1 signaling. CRISPR technology can engineer TCR, b-2 microglobulin, and PD-1, allowing CAR-T cells to build resistance to PD-1 signaling, which occurs when PD-L1 reacts with PD-1 (Rupp et al., 2017). Overall, because solid tumors expressing PD-L1 create immunosuppressive environments for CAR-T cells, using CPBs has shown positive results in mitigating this hindrance.

Another way to apply CAR-T cell therapy to solid tumors is to modify CAR-T cells to provide immunostimulatory signals from cytokines or possess resistance to immunosuppressive factors (Krenciute et al., 2017). IL-12 is a cytokine that stimulates CD8+ T cell function (independently of T-cell ligands; displaying tumoricidal behaviors), resulting in increased proliferation and antitumor activity of T-cells (Curtsinger et al., 2003). In a study by Koneru and her colleagues, flexi linkers were utilized to modify 4H11-28z/IL-12 CAR. They genetically modified the human IL-12 p35 and p40 subunits, both known for producing IFN-γ and promoting Type 1 T helper (Th1) differentiation – Th1 cells are known for producing IL-2 and IFN-γ, aiding T-cell function. The results of this study have been promising, showing the effectiveness of CAR-T in treating SKOV3 tumor, a human ovarian cancer cell line. With these modifications, 4H11-28z/IL-12 T cells could lyse tumor cells with higher levels and precision. The study showed

promising results in vivo as well, with mice injected with 4H11-28z/IL-12 CAR-T cells having a significantly higher survival rate than mice without genetically modified T-cells (4H11-28z) after injection of SKOV3 (1 X 10^7) ovarian tumor cells (Koneru et al., 2015).

For pancreatic tumors, the cytokine IL-4 (a protein that regulates antibody production and development of T-cell responses) negatively affects T-cells by impairing protective responses (Mohammed et al., 2017). Researchers overcame this issue by utilizing inverted cytokine receptors to synthesize IL-4 with IL-7 – IL-7 being beneficial to T-cells by regulating genes with apoptotic functions. The CAR-PSCA T cells with synthesized IL-4 and IL-7 receptors bind with IL-4, allowing T-cells to proliferate due to the synthesized IL-7. An alternative modification involves building resistance to immunosuppressive factors such as TGF-β. In early cancer stages, TGF-β acts as a tumor suppressor, while in later stages, it acts as a tumor promoter. TGF-β also inhibits T-cell activity by inhibiting Th1 and Th2 (immune responses against parasites, bacteria, etc.) differentiation and cytotoxic T lymphocytes (Sanjabi et al., 2017). This TGF-β signaling can be prevented with coexpression of dnTGF-βRII using the T2A element, which has been found to lead to CAR-T proliferation, resistance to exhaustion, and cytokine secretion. With dnTGF-βRII synthesized with Pbbz (dnTGF-bRII-T2A-Pbbz) CAR-T, the T-cell was able to induce lysis in prostate cancer cells expressing prostate-specific membrane antigens (PSMA).

Overcoming Immunosuppressive Factors: Regional Delivery and Local Administration

Even after mitigating the hostile effects of immunosuppressive tumor microenvironments, T-cells may still exhibit limited efficacy against solid tumors due to insufficient functional persistence or inadequate tumor infiltration. A method called regional delivery, however, can offer a potential solution by enhancing T-cell trafficking to the tumor site while minimizing systemic toxicity associated with intravenous delivery (I). According to a 2014 study, intrapleurally injected T-cells have shown superior performance compared to systemically injected T-cells (Adusimilli et al., 2014). Intrapleural injection of M28z CAR-T cells resulted in a significant, greater than 10-fold increase in T-cell accumulation at the tumor site compared to intravenous injection. Serial immunohistochemical analyses revealed that this enhanced accumulation was sustained over time. While both administration routes showed similar T-cell accumulation initially (3-5 days), the intrapleural group exhibited continued T-cell expansion, whereas the intravenous group (conventional injection) experienced a decline. Another study from 2021 revealed that intrapleural injection of anti-PD1 monoclonal antibodies (mAbs), along with CAR-T cells, increased the numbers of granzyme B+ CD8+ T-cells, DC69+ dendritic cells, and IFN-γ+ cells within the tumor (Li et al., 2021). This combination approach led to prolonged survival and reduced cancer nodules in mice, highlighting the potential synergy of local immunotherapy with CAR-T cell therapy. In the context of metastatic brain tumors, the risk of "on-target, off-tumor" toxicities can be mitigated by intracerebroventricular injection of HER2-CAR T-cells (Priceman et al., 2018). This approach allows for the direct targeting of brain metastases, bypassing the blood-brain barrier, which often restricts T-cell entry following intravenous injection. By concentrating T-cells at the tumor site, local administration minimizes the risk of unintended off-target effects in other organs. Therefore, these studies collectively underscore the potential of regional delivery to improve CAR-T cell therapy for solid tumors. By enhancing T-cell trafficking and tumor infiltration, local administration strategies may not only boost therapeutic efficacy but also reduce systemic toxicities, paving the way for safer and more effective cancer immunotherapy.

Expressing Chemokine Receptors for Enhanced Tumor Infiltration

Expressing specific chemokine receptors on CAR-T cells allows for enhanced trafficking and infiltration into solid tumors. The CXCR2 chemokine receptor, which typically controls cell migration, recruitment, and hematopoietic cell

homeostasis, and is not naturally attached to T-cells in humans, can be engineered onto T-cell surfaces through modification. This modification has been shown to increase T-cell infiltration and trafficking ability, allowing them to reach solid tumors more effectively. In a study by Liu et al., modified T-cells expressing CXCR2 demonstrated a rapid increase in tumor accumulation, responding as early as 3 days after infusion, compared to 5 days for unmodified T-cells. While the overall anti-tumor efficacy of the two groups was not significantly different, the faster response of CXCR2-modified T-cells suggests a potential advantage (Liu et al., 2020). Another approach involves synthesizing IL-8 with T-cells to guide them towards the tumor. While IL-8 does not directly attract T-cells, it does recruit neutrophils to inflammation sites, potentially enhancing the immune response.

Limitations

Despite promising potential solutions, further research is needed to address the serious side effects associated with CAR-T cell therapy, such as cytokine release syndrome (CRS). CRS occurs when a massive influx of proinflammatory cytokines is released during T-cell-mediated tumor destruction, or when T-cell therapy is used in conjunction with other immunotherapies. While most patients experience mild symptoms like fever, low blood pressure, and breathing difficulties, CRS can be life-threatening in some cases, leading to hemophagocytic lymphohistiocytosis/macrophage activation syndrome (HLH/MAS), a condition where the body's immune cells, lymphocytes and histiocytes, attack healthy tissues in the organs, bone marrow, and other blood cells (Martín-Rojas et al., 2022). Another detrimental side effect is immune effector cell-associated neurotoxicity syndrome (ICANS), which can progress from mild tremors to cerebral edema and may be fatal (DanaFarber Cancer Institute, n.d.; Johns Hopkins Medicine, 2024).

Although CAR-T cell therapy shows promise in its tumoricidal behaviors, its potential side effects cannot be ignored. Some studies suggest that inhibiting GM-CSF, a growth factor, can enhance T-cell function while reducing CRS and neuroinflammation (Sterner et al.). However, more research is needed to fully understand T-cell behavior and the immune system's response to CAR-T therapy in order to develop strategies for mitigating these serious side effects. Therefore, it is crucial for future research to focus on minimizing these risks to make CAR-T cell therapy a safer and more viable option for cancer treatment.

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