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CAR T CELL THERAPY

Dr. Renuka[1*], Dr. Juhi Aggarwal[2], Dr. Eram H. Pasha[3']

[1]*Post graduate student Department of Biochemistry, Santosh Medical College & Hospital
[2]Prof & Head of Department of Biochemistry
[3]Assistant Professor Department of Biochemistry

***Corresponding Author:** Dr. Renuka
[1]*Post graduate student Department of Biochemistry, Santosh Medical College & Hospital

CAR T cells refer to T lymphocytes that undergo genetic engineering to generate artificial T cell receptors, serving a crucial role in immunotherapy[1]. Chimeric antigen receptors (CARs) are specialized cell-surface receptors designed to identify specific proteins (antigens)[2]. These receptors, considered chimeric because they incorporate components from various receptors, are created by extracting T cells from the patient and modifying them in a laboratory setting[3]. The modified T cells then display chimeric antigen receptors, or CARs, on their surface.

CARs primarily target certain proteins or antigens present on the surface of cancer cells, forming a recognition and binding mechanism[4]. The structure of CARs encompasses three essential components: an intracellular CD3ζ T cell activation domain, a transmembrane domain, and the extracellular antigen recognition domain derived from the single-chain fragment variant (scFv) produced from an antibody.

The detailed composition of CARs includes an ectodomain, transmembrane domain, and endodomain. The ectodomain, situated outside the cytoplasm and exposed to the extracellular space, is a crucial component of membrane proteins[5]. The transmembrane domain, originating from the most membrane proximal part of the endodomain, features a hydrophobic alpha helix spanning the membrane. Finally, the endodomain completes the CAR structure.[6]

EVOLUTION OF CAR T CELL

The initial generation of CAR-T cells typically featured a single structure from the CD3 ζ-chain or FcεRIγ in the intracellular domain[7]. While effective in transmitting signals from the endogenous T cell receptor, these cells had limitations, such as insufficient interleukin-2 (IL-2) production, necessitating exogenous IL-2 administration for effective tumor cell eradication[8]. Recent studies indicated that modifying CD3ζ signaling could enhance transgene expression by reducing apoptosis signals[9]. However, clinical focus remained on CD3ζ-chain CAR-T cells due to their superior T cell activation and tumor cell-killing capabilities, despite lower in vitro expression levels. The transmembrane domain of CAR-T cells, composed of homologous or heterologous combinations of CD3, CD8, and CD28, facilitates cellular activation and interaction with the endogenous TCR.[10] Various first-generation CAR-T cells targeting specific antigens showed promise in treating tumors, although challenges like inadequate proliferation, short in vivo lifespan, and insufficient cytokine secretion persisted in many studies.

Second-generation CARs integrate intracellular signaling domains from co-stimulatory receptors such as CD28 or CD137, enhancing proliferation, cytotoxicity, and sustained response, thereby extending CAR-T cell lifespan in vivo.[11] While CD28ζ-CAR-T cells offer continuous stimulation, proliferation, and growth, 4-1BBζ-CAR-T cells may lead to early exhaustion, potentially limiting their antitumor efficacy, though direct comparisons are yet to be established.

The third-generation CARs, incorporating multiple signaling domains like CD3ζ-CD28-OX40 or CD3ζ-CD28-41BB, aimed to enhance potency with increased cytokine production and killing ability. However, scFv CD20-CD28-CD137-CD3ζ-CAR-T cells and HER2-CAR-T cells, employed in lymphoma and colon cancer treatment, did not show improved outcomes compared to the second generation, possibly due to limited case studies. Further research is essential to investigate the safety and efficacy of these treatments, emphasizing the significance of co-stimulatory molecule selection.

Fourth-generation CARs, termed T cell redirected for universal cytokine-mediated killing (TRUCKs), involve adding IL-12 to second-generation constructs. TRUCKs enhance T-cell activation, activate innate immune cells, and eliminate antigen-negative cancer cells in targeted lesions. Exploring TRUCKs role in shaping the tumor environment and treating viral infections, metabolic disorders, and autoimmune diseases holds promise.

HOW DOES CAR T CELL THERAPY WORK?

Step 1 : Apheresis : patient's white blood cells are isolated, collected & sent to the laboratory.

Step 2: Reprogramming : once at laboratory, T cells are genetically modified with chimeric antigen receptors(CAR) to kill cancer cells.

Step 3 : Expansion : the cells are then expanded until there are millions of disease attacking cells.

Step 4 : Infusion: Modified CAR T Cells are infused through an intravenous route, into the patient's blood.

Step 5: Cancer Cell Death : CAR T Cells track down and kill the patients tumor cells.

INDICATION OF CAR T CELL THERAPY

The primary advantage of CAR T cell therapy, distinguishing it from other cancer treatments, lies in its prompt intervention and a single infusion of CAR T cells[12]. A mere 2–3 weeks of careful monitoring suffices for the patient. Regarded as a contemporary therapeutic approach, CAR T cell efficacy can persist for decades, with sustained ability to locate and eliminate cancer cells during relapse. Currently authorized for patients whose transplants haven't been curative and who relapse post-transplant, CAR T cell therapy is envisioned as a substitute for various transplant types. Clinical trials on blood cancer demonstrate success, even in refractory cases where cancer resurged post-multiple transplants. CAR T cell therapy offers a relapse-free life and the potential for additional remedial treatments like stem cell transplantation, earning it the moniker 'living drug.'

1.For treating Acute Lymphoblastic Leukemia (ALL), particularly in fatal relapsed/refractory B-ALL, CAR-T therapy is most appropriate. The optimal CAR is anti-CD19, a crucial biomarker in B cell lineage with higher expression in B-ALL[13]. Additionally, anti-CD20 and immunoglobulin light chains are potential targets. CD22 is another viable target, and recent clinical trials have tested two different anti-CD22 agents for B-ALL, addressing the limitations of anti-CD19 therapy.

2. CD19 CAR-T cells were employed for relapsed and high-risk CLL patients, yielding comparable complete and partial response rates. Besides CD19, investigations into targets like tyrosine-protein kinase trans-membrane receptors have been conducted. Studies have explored CAR-T cell efficacy in CLL patients and identified its potential in treating B-cell malignancy relapses post allogeneic hematopoietic stem cell transplantation (Allo-HSCT), offering an alternative to traditional donor lymphocyte infusions.[14]

3. B cell malignancies: CD19 is an ideal target due to its high expression in B cell malignancies and limited presence outside the B cell lineage[15]. CD19 CAR T cells have shown success in treating relapsed leukemia, although resistance mechanisms, such as CD19 loss, pose challenges. Trials targeting other antigens, like BCMA, exhibit off-tumor reactions, highlighting translational challenges in advancing CAR T cell therapy.

CHALLENGES TOWARDS SUCCESSFUL CAR T CELL THERAPY

1. Antigen escape : The primary challenge faced by CAR-T cell therapy is the emergence of tumor resistance to CAR constructs that target a single antigen[16]. While CAR-T cells designed for single antigen targeting initially yield high response rates, a substantial number of treated patients experience either partial or complete loss of the target antigen expression in malignant cells. This phenomenon, referred to as antigen escape, poses a significant limitation. For instance, despite the initial success of CD19-targeted CAR-T cell therapy in achieving durable responses in 70–90% of relapsed and/or refractory ALL patients, recent follow-up data indicate the development of a common resistance mechanism. This includes downregulation or loss of CD19 antigen in 30–70% of patients who experience recurrent disease after treatment.
2. On-target off-tumor effects: Successfully targeting solid tumor antigens poses a challenge due to their expression on normal tissues. Careful antigen selection in CAR design is essential to ensure therapeutic effectiveness and minimize "on-target off-tumor" toxicity.[17] A promising approach involves targeting tumor-restricted post-translational modifications, such as truncated O-glycans like Tn and sialyl-Tn. Several CAR-T cell targets, including TAG7228, B7-H3, MUC1, and MUC16, have been explored. Ongoing research investigates improved versions to enhance anti-tumor responses while mitigating toxicity concerns, aiming to broaden CAR-T cell therapy applications in hematological malignancy and solid tumors.
3. CAR-T cell trafficking and tumor infiltration: Solid tumor CAR-T cell therapy faces challenges in comparison to hematological malignancies, mainly due to the limited capacity of CAR-T cells to reach and infiltrate solid tumors[18]. The immunosuppressive tumor microenvironment and physical barriers, like the tumor stroma, hinder CAR-T cell penetration and mobility within solid tumors. Modifying integrin αvβ6-CAR-T cells to express CXCR2 or elevating CXCR1/CXCR2 expression in CAR-T cells enhances their trafficking, significantly improving antitumor efficacy. The effectiveness of CAR-T cell therapy is hindered by physical barriers like tumor stroma, mainly composed of heparin sulfate proteoglycan (HSPG). CAR-T cells engineered to express heparanase, an enzyme breaking down HSPG, exhibit enhanced tumor infiltration and increased antitumor activity.
4. Immunosuppressive microenvironment: In the tumor microenvironment, various immunosuppressive cell types, such as myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs), and regulatory T cells (Tregs), infiltrate solid tumors[19]. These cells, along with tumor cells, stimulate the production of cytokines, chemokines, and growth factors that support tumor growth. Immune checkpoint pathways like PD-1 or CTLA-4 further contribute to reduced antitumor immunity. The limited effectiveness or lack of response to CAR-T cell therapy is often attributed to insufficient T cell expansion and short-term T cell persistence, possibly linked to the activation of co-inhibitory pathways leading to T cell exhaustion.
5. CAR-T cell-associated toxicities: The occurrence and intensity of CRS, HLH/MAS, and/or ICANS in CAR-T cell therapy depend on factors like CAR design, target specificity, and tumor type[20]. The predominant toxicities have been extensively studied in patients who received the first FDA-approved CD19-directed CAR-T cell therapy. Even in trials with remarkable response rates, severe, life-threatening events occurred in some patients. For instance, in CAR-T cell therapy for acute lymphoblastic leukemia/lymphoma (ALL/LBL), almost all patients experienced some degree of toxicity, with 23–46% exhibiting severe cytokine production and extensive T cell expansion[21]. These toxicities manifest as cytokine-release syndrome (CRS), hemo-phagocytic lymphohistiocytosis/macrophage activation syndrome (HLH/MAS), and immune effector cell-associated neurotoxicity syndrome (ICANS), each presenting distinct symptoms and complications.

Modulating toxicity by CAR-T cell engineering

A CAR-T cell antigen-binding domain needs to engage its target epitope and reach a minimal threshold level to induce effective therapeutic responses[22]. Cytokine release and CAR-T cell activation. Yet, there is also a threshold level of activation that, when crossed, results in hazardously

high cytokine and immune system activation levels. Stated differently, the therapeutic window of the CAR-T cell must not be exceeded for the cell to be clinically effective, as this will result in toxicity[23]. From an engineering standpoint, the amount of tumor antigen expressed on malignant cells, tumor burden, the affinity of the antigen-binding domain to its target epitope, and the CAR's stability all have an impact on the degree of CAR-T cell activation and activation kinetics.

FUTURE PROSPECTIVES

Traditional CARs face limitations when dealing with cancer cells that undergo alterations or downregulation of targeted antigens, leading to antigenic loss or escape variations. To address this, scientists are developing Tandem CARs (Tan-CARs), which incorporate two antigen recognition sites on a single intracellular domain, enhancing T cell activation and therapeutic properties[24]. Recent advancements include trivalent CAR T cells, targeting multiple antigens like HER2, IL-13 receptor α2, and ephrin A2, aiming to address interpatient variability and target nearly 100% of tumor cells.

A bispecific receptor, featuring dual antigen recognition and intracellular signaling domains on a single cell surface, exemplified by the novel CD19/CD20 CAR, holds promise in recognizing multiple tumor antigens and countering evasion[25]. Successful applications, such as blinatumomab against B-ALL, highlight curative potential.

Utilizing CRISPR technology, the CAR gene can be delivered to the TRAC locus of T cells. Placing the anti-CD19 CAR at the TCR α constant locus enhances T cell potency and ensures consistent CAR expression in peripheral blood T cells[26]. CRISPR-modified cells demonstrate effective performance in a mouse model of ALL compared to conventionally engineered CAR T cells.

The exploration of adoptive regulatory T-cell (Treg) therapy, shows promise as a therapeutic strategy under current clinical investigation for treating various autoimmune diseases and preventing transplant rejection. the current status of Treg therapy delves into potential approaches for generating antigen-specific Tregs, including the prospective utilization of chimeric antigen receptors (CARs) in forthcoming clinical trials.

CONCLUSION

CAR T cell therapy, a groundbreaking immunotherapy, utilizes genetically engineered T lymphocytes with chimeric antigen receptors (CARs) that target specific cancer cell proteins. CARs, comprised of diverse receptor components, include an intracellular CD3ζ T cell activation domain, a transmembrane domain, and an extracellular antigen recognition domain from antibody fragments.

The evolutionary progression of CAR designs, advancing from initial limitations to second, third, and fourth generations (TRUCKs), reflects ongoing efforts to enhance efficacy. Second-generation CARs, incorporating co-stimulatory receptors, improved proliferation and response. Third-generation CARs, with multiple signaling domains, aimed for increased potency, and fourth-generation CARs added IL-12 for enhanced T-cell activation and immune modulation.

CAR T cell therapy involves apheresis, laboratory reprogramming of T cells with CARs, cell expansion, and subsequent infusion into patients for cancer cell destruction. Noteworthy advantages include a single infusion, rapid intervention, and durable efficacy, making it a potential "living drug" with enduring benefits. Current applications primarily target hematological malignancies, focusing on B-cell disorders like ALL and CLL, with ongoing research exploring targets beyond CD19.

Challenges persist, including antigen escape, on-target off-tumor effects, limited CAR-T cell trafficking in solid tumors, immunosuppressive microenvironments, and associated toxicities like CRS. Engineering approaches aim to balance CAR-T cell activation and toxicity thresholds for enhanced efficacy.

Future prospects involve innovative CAR designs such as Tan-CARs, incorporating multiple antigen recognition sites. Ongoing advancements include the exploration of bispecific receptors, CRISPR technology targeting the TRAC locus, and adoptive regulatory T-cell therapy utilizing CARs for autoimmune diseases and transplant rejection prevention.

In conclusion, CAR T cell therapy signifies a transformative era in cancer treatment, with ongoing research and technological advancements addressing challenges and expanding its applications. The convergence of novel CAR designs, precision genome editing, and immunotherapeutic strategies holds the potential for continued breakthroughs in this rapidly evolving field.

REFERENCES

1. Chen L, Qiao D, Wang J, Tian G, Wang M. Cancer immunotherapy with lymphocytes genetically engineered with T cell receptors for solid cancers. Immunology Letters. 2019 Dec 1;216:51-62.
2. Sadelain M, Brentjens R, Rivière I. The basic principles of chimeric antigen receptor design. Cancer discovery. 2013 Apr 1;3(4):388-98.
3. Maher J. Immunotherapy of malignant disease using chimeric antigen receptor engrafted T cells. International Scholarly Research Notices. 2012;2012.
4. Chang ZL, Chen YY. CARs: synthetic immunoreceptors for cancer therapy and beyond. Trends in molecular medicine. 2017 May 1;23(5):430-50.
5. Asmamaw Dejenie T, Tiruneh G/Medhin M, Dessie Terefe G, Tadele Admasu F, Wale Tesega W, Chekol Abebe E. Current updates on generations, approvals, and clinical trials of CAR T-cell therapy. Human Vaccines & Immunotherapeutics. 2022 Nov 30;18(6):2114254.
6. Steyn A. Transmembrane glycoprotein gp41 of the Human Immunodeficiency Virus Type I: gene synthesis, recombinant expression and immunological characterization. University of Pretoria (South Africa); 2011.
7. Zhang C, Liu J, Zhong JF, Zhang X. Engineering car-t cells. Biomarker research. 2017 Dec;5(1):1-6.
8. Jin J, Cheng J, Huang M, Luo H, Zhou J. Fueling chimeric antigen receptor T cells with cytokines. American journal of cancer research. 2020;10(12):4038.
9. Sievers NM, Dörrie J, Schaft N. CARs: beyond T cells and T cell-derived signaling domains. International Journal of Molecular Sciences. 2020 May 15;21(10):3525.
10. Lanitis E, Poussin M, Klattenhoff AW, Song D, Sandaltzopoulos R, June CH, Powell Jr DJ. Chimeric antigen receptor T Cells with dissociated signaling domains exhibit focused antitumor activity with reduced potential for toxicity in vivo. Cancer immunology research. 2013 Jul 1;1(1):43-53.
11. Honikel MM, Olejniczak SH. Co-stimulatory receptor signaling in CAR-T cells. Biomolecules. 2022 Sep 15;12(9):1303.
12. Subklewe M, von Bergwelt-Baildon M, Humpe A. Chimeric antigen receptor T cells: a race to revolutionize cancer therapy. Transfusion Medicine and Hemotherapy. 2019 Feb 5;46(1):15-24.
13. Chatterjee G, Dhende P, Raj S, Shetty V, Ghogale S, Deshpande N, Girase K, Patil J, Kalra A, Narula G, Dalvi K. 15-color highly sensitive flow cytometry assay for post anti-CD19 targeted therapy (anti-CD19-CAR-T and blinatumomab) measurable residual disease assessment in B-lymphoblastic leukemia/lymphoma: Real-world applicability and challenges. European Journal of Haematology. 2024 Jan;112(1):122-36.
14. Liu J, Zhong JF, Zhang X, Zhang C. Allogeneic CD19-CAR-T cell infusion after allogeneic hematopoietic stem cell transplantation in B cell malignancies. Journal of hematology & oncology. 2017 Dec;10(1):1-8.
15. Spiegel JY, Patel S, Muffly L, Hossain NM, Oak J, Baird JH, Frank MJ, Shiraz P, Sahaf B, Craig J, Iglesias M. CAR T cells with dual targeting of CD19 and CD22 in adult patients with recurrent or refractory B cell malignancies: a phase 1 trial. Nature medicine. 2021 Aug;27(8):1419-31.
16. Majzner RG, Mackall CL. Tumor antigen escape from CAR T-cell therapy. Cancer discovery. 2018 Oct 1;8(10):1219-26.

17. Careful antigen selection in CAR design is essential to ensure therapeutic effectiveness and minimize "on-target off-tumor" toxicity
18. Hong M, Talluri S, Chen YY. Advances in promoting chimeric antigen receptor T cell trafficking and infiltration of solid tumors. Current Opinion in Biotechnology. 2023 Dec 1;84:103020.
19. Haist M, Stege H, Grabbe S, Bros M. The functional crosstalk between myeloid-derived suppressor cells and regulatory T cells within the immunosuppressive tumor microenvironment. Cancers. 2021 Jan 8;13(2):210.
20. Yang C, Nguyen J, Yen Y. Complete spectrum of adverse events associated with chimeric antigen receptor (CAR)-T cell therapies. Journal of Biomedical Science. 2023 Oct 21;30(1):89.
21. Sterner RC, Sterner RM. CAR-T cell therapy: current limitations and potential strategies. Blood cancer journal. 2021 Apr 6;11(4):69.
22. De Bousser E, Callewaert N, Festjens N. T cell engaging immunotherapies, highlighting chimeric antigen receptor (CAR) T cell therapy. Cancers. 2021 Dec 1;13(23):6067.
23. Watanabe K, Kuramitsu S, Posey Jr AD, June CH. Expanding the therapeutic window for CAR T cell therapy in solid tumors: the knowns and unknowns of CAR T cell biology. Frontiers in immunology. 2018 Oct 26;9:2486.
24. Mohanty R, Chowdhury CR, Arega S, Sen P, Ganguly P, Ganguly N. CAR T cell therapy: A new era for cancer treatment. Oncology reports. 2019 Dec 1;42(6):2183-95.
25. Nguyen A, Johanning G, Shi Y. Emerging novel combined CAR-T cell therapies. Cancers. 2022 Mar 9;14(6):1403.
26. Hiltensperger M, Krackhardt AM. Current and future concepts for the generation and application of genetically engineered CAR-T and TCR-T cells. Frontiers in Immunology. 2023 Mar 6;14:1121030.