CAR-T Cell Therapy: Hope and Healing in the Battle Against Cancer

ABSTRACT

With numerous forms and a myriad of genetic and environmental factors cancer continues to be a leading cause of morbidity and mortality worldwide. Advances in cancer research have led to a deeper understanding of the molecular mechanisms driving cancer progression, facilitating the development of targeted therapies and immunotherapies. Chimeric Antigen Receptor T-cell therapy, commonly known as CAR-T cell therapy, represents a groundbreaking approach in the field of cancer immunotherapy. This innovative treatment involves genetically engineering a patient's T cells to express chimeric antigen receptors (CARs) that target specific cancer cells. CAR-T therapy has demonstrated remarkable success in the treatment of certain hematologic malignancies, particularly B-cell malignancies like acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), and non-Hodgkin lymphoma (NHL). CAR-T cell therapy also has challenges, such as managing side effects like cytokine release syndrome and neurotoxicity, high treatment costs, and the need for further research to broaden its applicability to solid tumors. Additionally, the long-term durability of responses requires continuous monitoring and research.

Keywords: CAR-T cell, Neurotoxicity, Autologous Therapy, Adoptive Cell Therapy, Immunotherapy, Leukaemia, Cytokine Release Syndrome (CRS).

1. INTRODUCTION

The term malignant growth envelops a wide assortment of illnesses, recognizable by organ or tissue of beginning and cell type and morphology (Carbone, 2020). Cancer is the world's subsequent driving reason for death after a coronary episode. Regardless of advances in standard treatments, including a medical procedure, radiation, and chemotherapy (Guedan*et al.,* 2019), cancer patients' general endurance has not improved (Muhammad *et al.,* 2017). Furthermore, it is predicted to overtake ischemic heart disorders as the primary cause of fatalities in the coming decades (Mattiuzzi and Lippi, 2019). The human guard framework can effectively distinguish self and non-selfparticles, including microbes, infections and strange disease cells. The recognition of cancer cells depends on their procured antigenicity and immunogenicity through the presence of unfamiliar antigens (Galluzzi and Martin, 2017). Be that as it may, disease cells can undermine the safe framework for their potential benefit, bringing about lacking antitumor invulnerability, and growth endurance and movement (Perales *et al.,* 2018). The capacity of T-cells to target and kill cancer cells is essential for the cancer immune cycle (Jogalekar *et al.,* 2022). Lymphocytes are a basic part of the adaptive immune system as they coordinate cytotoxic impacts, yet additionally give long cell 'memory' of explicit antigens (Pennock *et al.,* 2013). Immunotherapy has been perceived as another age of an antitumor weapons and will be the main power in future disease treatment. Immunotherapy is a sort of treatment that objectives the human defense system rather than focusing on cancers. By turning on a patient's defences, it can fend off and kill tumour cells (Emens *et al.,* 2017). One of the freshest and most

encouraging cancer therapies, chimeric antigen receptor (CAR) T-cell treatment, supports the body's resistant framework to battle disease. The progression of CAR T treatment has been made conceivable by the combination of quality sequencing, developing hereditary information, new strategies for genome manipulation, and the improvement of novel gene exchange advancements (Huang *et al.,* 2023). Antigen recognition and T-cell signalling domains combine to form the fusion protein known as CAR. (Brudno and Kochenderfer, 2019). CAR-T cells are autologous lymphocytes produced through the laboratory process of leukapheresis, which is the process of separating white blood cells from a sample of blood. and genetically altered to express a CAR (often via lentiviral or retroviral transduction; Roex *et al.,* 2020). With CD19 and CD20 as its primary targets, CAR T cell therapy has revolutionised the treatment of haematological malignancies. The patient's existing T lymphocytes are modified in CAR T cell treatment so that they can identify and eradicate cancer cells (Mazinani and Rahbarizadeh, 2022). For many years, biomarkers have been a crucial part of understanding cancer, and with the development of CAR T cell therapy, a new class of therapeutic biomarkers has emerged. CAR T cells can be directed to cancerous target cells using these markers (Townsend *et al.,* 2018). A powerful cytotoxic immune response is combined with tumour selectivity when the CAR is expressed on the T cell (Porter *et al.,* 2018). CAR-T cells get stimulated to multiply and release cytokines when certain antigens are recognised. According to Gonzalez et al. (2004) and Louis et al. (2011), CAR-T cells can aid in the destruction of cancer and have showed promise for the immunotherapy of several human malignancies. Patients who receive CAR T cell therapy can have tremendous efficacy, but there is a chance of serious side effects as well. Patients have experienced brain toxicity and the cytokine release syndrome, which serve as examples of the negative effects of powerful immune detection of antigenic sites and the ensuing vigorous immune response (Akce*et al.,* 2018).

2. CAR STRUCTURE

CARs are engineered receptors with rational structure on T-Cells that target exterior antigens of the target malignant cell. The restriction typically imposed by the major histocompatibility complex (MHC) can be avoided by T cells that have been genetically modified to express CAR (Zhao *et al.,* 2018). Therefore, it is essential to build CARs with the proper affinity to distinguish between cancerous and healthy cells without causing any toxicity (Caruso *et al.,* 2015). Chimeric antigen receptors (CARs) are synthetic receptors that typically include the transmembrane domain of the T cell receptor, the cytoplasmic signalling domain of the CD3 zeta chain, and the antigen-binding portion of a monoclonal antibody (mAb).

2.1 Antigen Binding Region

The antigen recognition domain is derived from the variable region of monoclonal antibodies (Lam *et al.,* 2020). This area interacts with the target antigen and is constantly revealed to the outside of the cell (Zhang *et al.,* 2017; Rafiq *et al.,* 2020). The variable heavy (VH) and light (VL) chains of monoclonal antibodies are what give rise to single-chain variable fragments (scFv), a component of CARs. These scFvs often target membrane-bound cancer cell surface receptors and trigger T cell activation independent of the MHC. Although the sequences of linkers vary greatly, those frequently utilised in CARs contain repeats of the amino acids glycine and serine to give antigen-binding sites the flexibility they need to change orientation and remain stable (Yan and Sun, 1997). The presence of Gly and Ser residues also inhibits secondary structure development and lessens the possibility that the linker may obstruct the folding and functionality of the scFv (Van Rosmalen*et al.,* 2017). In the external domain, a hinge or spacer region is also present. This region serves to link the scFv to the transmembrane domain and to provide flexibility to overcome steric hindrance (Jayaraman *et al.,* 2020). It also adds to the length to enable the antigen-binding domain to access the targeted epitope (sterner and sterner, 2021).This enhances antigen binding and synapse formation between the CAR T cells and target cancer cells (Hudecek *et al.,* 2015) as

increasing epitope-paratope distance can also result in decreased delivery of granzymes and perforins to the target cell, limiting lytic efficacy (Woodsworth *et al.,* 2015).

2.2 Transmembrane Domain

The ectodomain and endodomain are joined by the transmembrane domain, which also acts as the cell membrane's anchor (Huang *et al.,* 2020). The transmembrane's primary role is to hook the CAR to the T cell membrane. The transmembrane domain of the CAR is perhaps the least well-studied part of the structure, consisting of a hydrophobic helix that traverses the cell membrane (Guedan*et al.,* 2019). In a study by Morin et al. (2015), CD28's extracellular and transmembrane domains can substantially activate T cells. It has been demonstrated that transmembrane domains control other crucial processes associated with CAR asembly, activation and aggregation. Furthermore, it is known that this domain influences the release of cytokines, which, if it is excessive, can result in significant non-specific toxicity. The majority of these domains come from CD3ζ, CD4, CD8α, or CD28each of which gives CAR-T cells radically distinct features (Harris and Kranz, 2016).

2.3 Intracellular Signalling Domain

The endodomain of the receptor is located within the cell and contains an internal T cell signalling domain (Chandran and Klebanoff, 2019). CD3ζendodomain present in initially designed car has a conserved amino acid sequence known as immunoreceptor tyrosine based activaing motifs which upon phosphorylation creates binding site for zap70, a signalling kinase (Courtney *et al.,* 2018). But relying solely on these patterns for signalling cannot result in efficient T cell responses (Brocker and Karjalainen, 1995). The generation of a co-stimulatory domain in series with the CD3 intracellular signalling domain increased IL-2 production and proliferation under repeated antigen exposure (Maher *et al.,* 2002).The release of proinflammatory cytokines such IL-17A, IL-17F, IL-22, and IFN- is increased when inducible co-stimulatory is used, which improves CAR T cell persistence (Guedan*et al.,* 2014). High response rates in patients have been linked to CAR T cells with the CD28 and 4-1BB domains. In preclinical trials, new costimulation domains like ICOS and CD27 have effectively eliminated tumour cells (Guedan*et al.,* 2014; Song *et al.,* 2012). Co-stimulatory receptors 4-1BB, ICOS, and OX40 influence metabolic cycles, apoptosis, and activation-induced cell death in addition to T-cell differentiation processes (Weinkove*et al.,* 2019).

3. MANUFACTURING CAR T-CELL

CAR T-cells are the immune cells that have been genetically modified to identify target antigens on the surface of targets and destroy them after adoptive transfer. The process includes the following steps (Fig 1).

3.1 Leukapheresis

Blood from the patient is drawn, and the lymphocytes are separated using apheresis (also known as leukapheresis), which is the first step in the creation of CAR T cell. Clinicians arrange collection based on the treatment regimen being used by the patient to guarantee that there are enough T cells present (Wang and Rivière, 2016). The operation is carried out at an approved clinic or infusion facility under the direction of the patient's medical professional (Batlevi*et al.,* 2016).

3.2 Engineering T cell with CAR gene

Eshhar and colleagues developed CARs (formerly known as T-bodies) in 1989 as fusions of antibody and TCR subunits which, when produced on T cells, facilitated MHC-independent T-cell activation. The scFv generated from phage display or the mAbs produced against cell-surface antigens are typically used to impart CAR specificity (Stastny *et al.,* 2007).CAR, a

protein made from the gene, binds to cancer cells and either permanently or temporarily expresses a therapeutic gene. The optimal target for CAR T cells is one that is expressed on the surface of all cancer cells but is not present on the surface of any normal cells. Based on its cell-surface expression and function in the majority of leukaemias and lymphomas, CD19 has been identified as a viable target (Sadelain*et al.,* 2017). The effective transport of the coding DNA is necessary for CAR T cell gene editing operations (Benmebarek*et al.,* 2019). There are currently two methods for incorporating genes into vectors: viral systems and non-viral systems (Zhang *et al.,* 2017). Adenovirus, adeno-associated virus, and retroviruses (including lentivirus) are some of the virus vectors. The most widely used of them for delivering genes are genetically modified retroviruses (Hu and Pathak, 2000). High gene transfer efficiency and consistent CAR expression are two benefits of these vectors (Ruella *et al.,* 2018). Naked DNA, liposomes, polymerizers, and molecular conjugates are examples of non-viral vectors. Minicircle DNA vectors, which are unique non-viral vectors developed in bacteria from a parental plasmid and can persistently express transgene at high levels in vivo (Kay *et al.,* 2010), are free of plasmid bacterial DNA sequences. The anti-CD20 antibody vector that contains a CD3-signaling cassette is used to clone single-chain variable segments to create the CD19 CAR construct (Kimman *et al.,* 2023). Finally, new strategies involving in situ T cell modification are being investigated as a way to streamline and lower the cost of CAR T cell manufacture. Pre-clinical models of B cell malignancies have demonstrated the safety and efficacy of in situ T cell programming utilizing DNA nanocarriers (Smith *et al.,* 2017) or lentiviral vectors selectively targeting human CD8+ T cells (Pfeiffer *et al.,* 2018). The immune cells have been altered to selectively combat cancer cells. The viral vector attaches to the patient's cells using viral machinery, and after entering the cells, the vector delivers genetic material in the form of RNA. As the patient cells proliferate and become more numerous in the bioreactor, the RNA is reverse-transcribed into DNA and permanently incorporated into their genomes. As a result, CAR expression is preserved. The patient's cells then translate and express the CAR, which is expressed on the cell surface (Levine *et al.,* 2016).

3.3 Grow and expand CAR T cells

The ideal culture mixing and gas exchange conditions required to produce a large amount of cells for clinical usage are provided by bioreactor culture systems. The CD19-targeted CAR T cell has been expanded using the WAVE Bioreactor, which includes a rocking platform (Somerville, 2012). The cell culture, which may have a volume of up to 5 L when the cell growth procedure is complete, needs to be condensed to a volume that can be infused into the patient. After product release, the cleaned and purified cells are cryopreserved in infusible media and transferred to and thawed at the facility where the patient is to be treated (Levine, 2015).

3.4 Infusion

CAR T cells will be returned to the patient once there are enough of them. The patient might get chemotherapy a few days before to the CAR T-cell infusion to assist reduce the amount of other immune cells. This increases the likelihood that the CAR T cells will become activated to fight the tumour. Because CAR T cells function best while there are still cancer cells to assault, this chemotherapy is typically not very potent. The CAR T cells begin to multiply and potentially aid in the destruction of additional cancer cells once they begin binding with malignant cells.

4. KILLING MECHANISM OF CAR T CELL

Through their T cell receptor, T cells identify and kill their target, which are mostly infections. The T cell receptor notifies the T cell to kill the invader when it detects a particular chemical on a bacterium

or virus. Engineered T cells' ability to move to cancer sites, multiply, and mediate effector functions that eradicate numerically greater tumour loads is the basis for their anti-tumor actions (Gattinoni*et al.,* 2012). When a target antigen is recognised and bound to by a CAR T cell's antigen binding domain, the CAR T cell is activated. Following this engagement, CAR molecules on the surface of T cells group together, which causes the CAR molecule to become immobile and creates an immunological synapse (Liu *et al.,* 2020). With enhanced tumour cell targeting and persistence for long-term tumour control, CAR T cells have been engineered to precisely identify and lyse target cells on their own while also stimulating T cell growth and differentiation—two critical processes for CAR T cell efficacy. According to Korell et al. (2022) immunological synapses, target antigen density, CAR affinity for the target, and signal transduction strength all affect how much CAR T cells are activated. For effective signal transduction and activation, CARs need noticeably greater antigen concentrations (Harris *et al.,* 2018). Different cytokines promote T cell differentiation into effector cells and boost T cell proliferation. The signal transduction is begun by CD3, with co-stimulatory molecules providing signal amplification (Fraietta*et al.,* 2018). Due to rapid degranulation and perforin release compared to natural T cells, CAR T cells destroy tumour cells more quickly (Davenport *et al.,* 2015). Additionally, given that CAR T cells can kill antigen-negative tumour cells through this mechanism and that Fas-negative tumour cells are resistant to the apoptosis produced by CAR T cells, they may be more dependent on Fas/FasL for the destruction of tumour cells. IFN- is a protein that CAR T cells also secrete to help kill tumour cells. Currently, leukaemia and lymphoma are the most common blood malignancies treated with car-t cell therapy in India. Many other types of car-t cell therapy have received FDA approval, and many more are awaiting approval (Ravindranath *et al.,* 2022). A few of these are listed in table 1.

5. PROS & CONS ASSOCIATED WITH THERAPY

The advantages of CAR T-cell therapy, which is also a "living drug," can last for many years. Since the cells can survive in the body for a long time, if and when a recurrence occurs, they could be able to identify and combat cancer cells. The cost of and the amount of modification needed for the CAR T cells are significantly higher. Although CAR T cells have been effective in treating liquid (blood) cancers, they have not been as effective in treating solid tumours, and they are linked to toxicities that pose a serious risk to human life (Kandra *et al.,* 2022). Tumour resistance to single antigen targeting CAR constructions is one of the most difficult limitations of CAR-T cell treatment. Antigen escape is the term used to describe this process (Majzner and Mackall, 2018). It may be beneficial to use human or humanised antibody fragments instead of murine-derived CARs to minimise CAR immunogenicity because the host immune system's detection of CAR constructions may be a factor in cytokine-related toxicities (Sommermeyer*et al.,* 2017). The effectiveness of CAR-T cell therapy in solid tumours, such as lung cancer, is constrained in part by CAR-T cell depletion (Kasakovski*et al.,* 2018). Aphasia, tremor, ataxia, myoclonus, and CRS are among the neurological toxicities associated with the use of CAR-T cell treatment. Organ damage could conceivably result in CAR-T cells when they react with an antigen expressed in normal tissue that is identical to the target antigen expressed by cancer (Brudno and Kochenderfer, 2016) as without the assistance of HLA expression, CAR-T cells are capable of recognising cell surface chemicals (Zhao *et al.,* 2018).

6. CONCLUSION

In conclusion, CAR-T cell therapy stands as a transformative advancement in the realm of cancer treatment, holding the potential to reshape the prognosis for patients with certain blood cancers. One may predict similar results with additional leukemia targets and haematological cancers given the success of CD19 CARs. The way is being prepared for CAR T cell treatment to be used more widely and with greater success. CAR T cells' functionality, selectivity, and effectiveness are constantly being enhanced. CAR T cells hold potential for cancer treatment that has not yet been fully realized when combined with developments in cell engineering and gene editing. The recent approval of two CAR T cell therapies heralds the beginning of a new age in cell therapy, where it will be necessary to show that such methods are broadly applicable. On-target off-tumor effects can happen even with proper

antigen targeting and result in related toxicity. Getting CAR-T cells to make their way to and infiltrate solid tumors is difficult. Future CAR T-cell therapy solutions will specifically target two, three, or even more different molecules on a given tumor. As a result, CAR T-cells would be able to identify malignancy even if one of their targets was missed. It is anticipated that the toxicity and adverse effects of this new CAR T cell therapy would be reduced. The ultimate goal is to replace chemotherapy and stem cell transplantation with CAR T-cell therapy. However, it is not a panacea, and further research is needed to extend its benefits to a wider range of cancer types and to address safety and accessibility concerns. Ongoing research and clinical trials continue to refine and expand the application of CAR-T therapy, offering hope for more widespread use and improvements in safety and affordability.

Consent for publication

Not applicable.

REFERENCES

[1] Akce M, Zaidi MY, Waller EK, El-Rayes BF & Lesinski GB. (2018). The potential of CAR T cell therapy in pancreatic cancer. Frontiers in immunology, 9: 2166.

[2] Antonia SJ, Villegas A, Daniel D, Vicente D, Murakami S, Hui R &Özgüroğlu M. (2018). Overall survival with durvalumab after chemoradiotherapy in stage III NSCLC. New England Journal of Medicine, 379(24): 2342-2350.

[3] Awasthi R, Maier HJ, Zhang J & Lim S. (2023). Kymriah®(tisagenlecleucel)–An overview of the clinical development journey of the first approved CAR-T therapy. Human Vaccines and Immunotherapeutics: 2210046.

[4] Batlevi CL, Matsuki E, Brentjens RJ & Younes A. (2016). Novel immunotherapies in lymphoid malignancies. Nature reviews Clinical oncology, 13(1): 25-40.

[5] Benmebarek MR, Karches CH, Cadilha BL, Lesch, S, Endres S & Kobold S. (2019). Killing mechanisms of chimeric antigen receptor (CAR) T cells. International journal of molecular sciences, 20(6): 1283.

[6] Brocker T & Karjalainen K. (1995). Signals through T cell receptor-zeta chain alone is insufficient to prime resting T lymphocytes. The Journal of experimental medicine, 181(5): 1653-1659.

[7] Brudno JN & Kochenderfer JN. (2016). Toxicities of chimeric antigen receptor T cells: recognition and management. Blood, The Journal of the American Society of Hematology, 127(26): 3321-3330.

[8] Brudno JN & Kochenderfer JN. (2019). Recent advances in CAR T-cell toxicity: mechanisms, manifestations and management. Blood reviews, 34: 45-55.

[9] Carbone A. (2020). Cancer Classification at the Crossroads. Cancers, 12 (4): 980.

[10] Caruso HG, Hurton LV, Najjar A, Rushworth D, Ang S, Olivares S & Cooper LJ. (2015). Tuning sensitivity of CAR to EGFR density limits recognition of normal tissue while maintaining potent antitumor activity. Cancer research, 75(17): 3505-3518.

[11] Chandran SS & Klebanoff CA. (2019). T cell receptor-based cancer immunotherapy: emerging efficacy and pathways of resistance. Immunological reviews, 290(1): 127-147.

[12] Cheever MA &Higano CS. (2011). PROVENGE (Sipuleucel-T) in prostate cancer: the first FDA-approved therapeutic cancer vaccine. Clinical Cancer Research, 17(11): 3520-3526.

[13] Courtney AH, Lo WL & Weiss A. (2018). TCR signaling: mechanisms of initiation and propagation. Trends in biochemical sciences, 43(2): 108-123.

[14] Davenport AJ, Jenkins MR, Cross RS, Yong CS, Prince HM, Ritchie DS & Neeson, PJ. (2015). CAR-T cells inflict sequential killing of multiple tumor target cells. Cancer immunology research, 3(5): 483-494.

[15] Dhillon S & Syed YY. (2019). Atezolizumab first-line combination therapy: a review in metastatic nonsquamous NSCLC. Targeted oncology, 14(6): 759-768.
[16] Emens LA, Ascierto PA, Darcy PK, Demaria S, Eggermont AM, Redmond WL &Marincola FM. (2017). Cancer immunotherapy: opportunities and challenges in the rapidly evolving clinical landscape. European journal of cancer, 81: 116-129.
[17] Fraietta JA, Lacey SF, Orlando EJ, Pruteanu-Malinici I, Gohil M, Lundh S &Melenhorst JJ. (2018). Determinants of response and resistance to CD19 chimeric antigen receptor (CAR) T cell therapy of chronic lymphocytic leukemia. Nature medicine, 24(5): 563-571.
[18] Gaiser MR, Bongiorno M & Brownell I. (2018). PD-L1 inhibition with avelumab for metastatic Merkel cell carcinoma. Expert review of clinical pharmacology, 11(4): 345-359.
[19] Galluzzi L & Martin P. (2017). CARs on a highway with roadblocks. Oncoimmunology, 6(12): e1388486.
[20] Gattinoni L, Klebanoff CA & Restifo NP. (2012). Paths to stemness: building the ultimate antitumour T cell. Nature Reviews Cancer, 12(10): 671-684.
[21] Gonzalez S, Naranjo A, Serrano LM, Chang WC, Wright CL & Jensen MC. (2004). Genetic engineering of cytolytic T lymphocytes for adoptive T-cell therapy of neuroblastoma. The Journal of Gene Medicine: A cross-disciplinary journal for research on the science of gene transfer and its clinical applications, 6(6): 704-711.
[22] Graziani G, Tentori L & Navarra P. (2012). Ipilimumab: a novel immunostimulatory monoclonal antibody for the treatment of cancer. Pharmacological research, 65(1): 9-22.
[23] Guedan S, Calderon H, Posey AD & Maus MV. (2019). Engineering and design of chimeric antigen receptors. Molecular Therapy-Methods and Clinical Development, 12: 145-156.
[24] Guedan S, Chen X, Madar A, Carpenito C, McGettigan SE, Frigault MJ & June CH. (2014). ICOS-based chimeric antigen receptors program bipolar TH17/TH1 cells. Blood, The Journal of the American Society of Hematology, 124(7): 1070-1080.
[25] Guedan S, Ruella M & June CH. (2019). Emerging cellular therapies for cancer. Annual review of immunology, 37: 145-171.
[26] Han D, Xu Z, Zhuang Y, Ye Z & Qian Q. (2021). Current progress in CAR-T cell therapy for hematological malignancies. Journal of Cancer, 12(2): 326.
[27] Hansen DK, Sidana S, Peres LC, Colin Leitzinger C, Shune L, Shrewsbury A & Patel KK. (2023). Idecabtagenevicleucel for relapsed/refractory multiple myeloma: real-world experience from the myeloma CAR T consortium. Journal of Clinical Oncology, 41(11): 2087-2097.
[28] Harris DT & Kranz DM. (2016). Adoptive T cell therapies: a comparison of T cell receptors and chimeric antigen receptors. Trends in pharmacological sciences, 37(3): 220-230.
[29] Harris DT, Hager MV, Smith SN, Cai Q, Stone JD, Kruger P & Kranz DM. (2018). Comparison of T cell activities mediated by human TCRs and CARs that use the same recognition domains. The Journal of Immunology, 200(3): 1088-1100.
[30] Heo YA & Duggan ST. (2018). Niraparib: a review in ovarian cancer. Targeted oncology, 13: 533-539.
[31] Hu WS & Pathak VK. (2000). Design of retroviral vectors and helper cells for gene therapy. Pharmacological Reviews, 52(4): 493-512.
[32] Huang R, Li X, He Y, Zhu W, Gao L, Liu Y & Zhang X. (2020). Recent advances in CAR-T cell engineering. Journal of Hematology and Oncology, 13(1): 1-19.
[33] Huang Z, Dewanjee S, Chakraborty P, Jha NK, Dey A, Gangopadhyay M & Jha SK. (2023). CAR T cells: Engineered immune cells to treat brain cancers and beyond. Molecular Cancer, 22(1): 1-27.
[34] Hudecek M, Sommermeyer D, Kosasih PL, Silva-Benedict A, Liu L, Rader C & Riddell SR. (2015). The nonsignaling extracellular spacer domain of chimeric antigen receptors is decisive for in vivo antitumor activity. Cancer immunology research, 3(2): 125-135.
[35] Jaklevic MC. (2021). CAR-T therapy is approved for non-Hodgkin lymphoma. JAMA, 325(11): 1032-1032.

[36] Jayaraman J, Mellody MP, Hou AJ, Desai RP, Fung AW, Pham AHT & Zhao W. (2020). CAR-T design: Elements and their synergistic function. EBioMedicine, 58.

[37] Jogalekar MP, Rajendran RL, Khan F, Dmello C, Gangadaran P & Ahn BC. (2022). CAR T-cell-based gene therapy for cancers: new perspectives, challenges, and clinical developments. Frontiers in immunology, 13: 925985.

[38] Kamath SD, Kalyan A & Benson III AB. (2018). Pembrolizumab for the treatment of gastric cancer. Expert review of anticancer therapy, 18(12): 1177-1187.

[39] Kandra P, Nandigama R, Eul B, Huber M, Kobold S, Seeger W & Savai R. (2022). Utility and drawbacks of chimeric antigen receptor T Cell (CAR-T) therapy in lung cancer. Frontiers in Immunology, 13: 903562.

[40] Kasakovski D, Xu L & Li Y. (2018). T cell senescence and CAR-T cell exhaustion in hematological malignancies. Journal of hematology and oncology, 11: 1-9.

[41] Kay MA, He CY & Chen ZY. (2010). A robust system for production of minicircle DNA vectors. Nature biotechnology, 28(12): 1287-1289.

[42] Kimman T, Slomp A, Martens A, Grabherr S, Li S, van Diest E &Peperzak V. (2023). Serpin B9 controls tumor cell killing by CAR T cells. Journal for Immunotherapy of Cancer, 11(3): e006364.

[43] Korell F, Berger TR & Maus MV. (2022). Understanding CAR T cell-tumor interactions: paving the way for successful clinical outcomes. Med, 3(8): 538-564.

[44] Lam N, Trinklein ND, Buelow B, Patterson GH, Ojha N & Kochenderfer JN. (2020). Anti-BCMA chimeric antigen receptors with fully human heavy-chain-only antigen recognition domains. Nature communications, 11(1): 283.

[45] Levine BL. (2015). Performance-enhancing drugs: design and production of redirected chimeric antigen receptor (CAR) T cells. Cancer gene therapy, 22(2): 79-84.

[46] Levine BL, Miskin J, Wonnacott K & Keir C. (2017). Global manufacturing of CAR T cell therapy. Molecular Therapy-Methods and Clinical Development, 4: 92-101.

[47] Liu D, Badeti S, Dotti G, Jiang JG, Wang H, Dermody J & Liu C. (2020). The role of immunological synapse in predicting the efficacy of chimeric antigen receptor (CAR) immunotherapy. Cell communication and signaling, 18(1): 1-20.

[48] Louis CU, Savoldo B, Dotti G, Pule M, Yvon E, Myers GD & Brenner MK. (2011). Antitumor activity and long-term fate of chimeric antigen receptor–positive T cells in patients with neuroblastoma. Blood, The Journal of the American Society ofHematology, 118(23): 6050-6056.

[49] Maher J, Brentjens RJ, Gunset G, Rivière I &Sadelain M. (2002). Human T- lymphocyte cytotoxicity and proliferation directed by a single chimeric TCRζ/CD28 receptor. Nature biotechnology, 20(1): 70-75.

[50] Majzner RG & Mackall CL. (2018). Tumor antigen escape from CAR T-cell therapy. Cancer discovery, 8(10): 1219-1226.

[51] Mattiuzzi C & Lippi G. (2019). Current cancer epidemiology. Journal of epidemiology and global health, 9(4): 217.

[52] Mazinani M &Rahbarizadeh F. (2022). CAR-T cell potency: from structural elements to vector backbone components. Biomarker Research, 10(1): 1-24.

[53] Mian A & Hill BT. (2021). Brexucabtageneautoleucel for the treatment of relapsed/refractory mantle cell lymphoma. Expert Opinion on Biological Therapy, 21(4): 435-441.

[54] Morin SO, Giroux V, Favre C, Bechah Y, Auphan-Anezin N, Roncagalli R & Nunes JA. (2015). In the absence of its cytosolic domain, the CD28 molecule still contributes to T cell activation. Cellular and molecular life sciences, 72: 2739-2748.

[55] Muhammad N, Mao Q & Xia H. (2017). CAR T-cells for cancer therapy. Biotechnology and Genetic Engineering Reviews, 33(2): 190-226.

[56] Papadouli I, Mueller-Berghaus J, Beuneu C, Ali S, Hofner B, Petavy F &Pignatti F. (2020). EMA review of AxicabtageneCiloleucel (Yescarta) for the treatment of diffuse large B-cell lymphoma. The Oncologist, 25(10): 894-902.

[57] Perales MA, Kebriaei P, Kean LS &Sadelain M. (2018). Building a safer and faster CAR: seatbelts, airbags, and CRISPR. Biology of Blood and Marrow Transplantation, 24(1): 27-31.
[58] Pfeiffer A, Thalheimer FB, Hartmann S, Frank AM, Bender RR, Danisch S & Buchholz CJ. (2018). In vivo generation of human CD 19-CAR T cells results in B-cell depletion and signs of cytokine release syndrome. EMBO molecular medicine, 10(11): e9158.
[59] Porter D, Frey N, Wood PA, Weng Y & Grupp SA. (2018). Grading of cytokine release syndrome associated with the CAR T cell therapy tisagenlecleucel. Journal of hematology and oncology, 11: 1-12.
[60] Rafiq S, Hackett CS &Brentjens RJ. (2020). Engineering strategies to overcome the current roadblocks in CAR T cell therapy. Nature reviews Clinical oncology, 17(3): 147-167.
[61] Ravindranath A, Dubey A, Suresh S, Chaudhuri G &Chirmule N. (2022). CAR-T cell therapy in India requires a paradigm shift in training, education and health care processes. Cytotherapy, 24(2): 101-109.
[62] Roex G, Timmers M, Wouters K, Campillo-Davo D, Flumens D, Schroyens W &Anguille S. (2020). Safety and clinical efficacy of BCMA CAR-T-cell therapy in multiple myeloma. Journal of hematology and oncology, 13(1): 1-14.
[63] Ruella M, Xu J, Barrett DM, Fraietta JA, Reich TJ, Ambrose DE &Melenhorst JJ. (2018). Induction of resistance to chimeric antigen receptor T cell therapy by transduction of a single leukemic B cell. Nature medicine, 24(10): 1499-1503.
[64] Sadelain M, Rivière I & Riddell S. (2017). Therapeutic T cell engineering. Nature, 545(7655): 423-431.
[65] Sanford M. (2015). Blinatumomab: first global approval. Drugs, 75: 321-327.
[66] Smith TT, Stephan SB, Moffett HF, McKnight LE, Ji W, Reiman D & Stephan MT. (2017). In situ programming of leukaemia-specific T cells using synthetic DNA nanocarriers. Nature nanotechnolog y, 12(8): 813-820.
[67] Somerville R, Devillier L, Parkhurst MR, Rosenberg SA & Dudley ME. (2012). Clinical scale rapid expansion of lymphocytes for adoptive cell transfer therapy in the WAVE® bioreactor. Journal of translational medicine, 10(1): 1-11.
[68] Sommermeyer D, Hill T, Shamah SM, Salter AI, Chen Y, Mohler KM & Riddell SR. (2017). Fully human CD19-specific chimeric antigen receptors for T-cell therapy. Leukemia, 31(10): 2191-2199.
[69] Song DG, Ye Q, Poussin M, Harms GM, Figini M & Powell Jr DJ. (2012). CD27 costimulation augments the survival and antitumor activity of redirected human T cells in vivo. Blood, The Journal of the American Society of Hematology, 119(3): 696-706.
[70] Stastny MJ, Brown CE, Ruel C & Jensen MC. (2007). Medulloblastomas expressing IL13Rα2 aretargets for IL13-zetakine+ cytolytic T cells. Journal of pediatrichematology/oncology, 29(10): 669-677.
[71] Sterner RC & Sterner RM. (2021). CAR-T cell therapy: current limitations and potential strategies. Blood cancer journal, 11(4): 69.
[72] Townsend MH, Shrestha G, Robison RA & O'Neill KL. (2018). The expansion of targetable biomarkers for CAR T cell therapy. Journal of Experimental and Clinical Cancer Research, 37: 1-23.
[73] ul Hussain H, Burney MH, Rehan ST & Hasan MM. (2022). Dostarlimab: A breakthrough in the field of oncology. Annals of Medicine and Surgery, 80: 104046.
[74] Van Rosmalen M, Krom M &Merkx M. (2017). Tuning the flexibility of glycine- serine linkers to allow rational design of multidomain proteins. Biochemistry, 56(50): 6565-6574.
[75] Wang X & Rivière I. (2016). Clinical manufacturing of CAR T cells: foundation of a promising therapy. Molecular Therapy-Oncolytics, 3.
[76] Weinkove R, George P, Dasyam N & McLellan AD. (2019). Selecting costimulatory domains for chimeric antigen receptors: functional and clinical considerations. Clinical and translational immunology, 8(5): e1049.

[77] Woodsworth DJ, Dunsing V & Coombs D. (2015). Design parameters for granzyme- mediated cytotoxic lymphocyte target-cell killing and specificity. Biophysical journal, 109(3): 477-488.
[78] Yan BX & Sun YQ. (1997). Glycine residues provide flexibility for enzyme active sites. Journal of Biological Chemistry, 272(6): 3190-3194.
[79] Zhang C, Liu J, Zhong JF & Zhang X. (2017). Engineering car-t cells. Biomarker research, 5(1): 1-6.
[80] Zhao J, Lin Q, Song Y & Liu D. (2018). Universal CARs, universal T cells, and universal CAR T cells. Journal of hematology and oncology, 11: 1-9.
[81] Zhao Z, Chen Y, Francisco NM, Zhang Y & Wu M. (2018). The application of CAR-T cell therapy in hematological malignancies: advantages and challenges. Acta Pharmaceutica Sinica B, 8(4): 539-51.

Table 1. CAR-T Cell Therapies in Cancer.

S.No.	Trade Name	Drug	Target Disease	References
1	ABECMA	IdecabtageneVicleucel	Multiple Myeloma	**Hansen *et al.,* 2023**
2	BREYANZI	LisocabtageneMaraleucel	B-Cell Non-Hodgkin Lymphoma (NHL)	**Jaklevic, 2021**
3	CARVYKTI	CiltacabtageneAutoleucel	Multiple Myeloma	**Martin *et al.,* 2023**
4	KYMRIAH	Tisagenlecleucel	Acute Lymphoblastic Leukemia, B-Cell Lymphoma, and Follicular Lymphoma.	**Awasthi *et al.,* 2023**
5	TECARTUS	BrexucabtageneAutoleucel	Mantle Cell Lymphoma (MCL)	**Mian and Hill, 2021**
6	YESCARTA	AxicabtageneCiloleucel	B-Cell Non-Hodgkin Lymphoma (NHL)	**Papadouli*et al.,*2020**
7	BAVENCIO	Avelumab	Metastatic Merkel Cell Carcinoma (MCC)	**Gaiser *et al.,* 2018**
8	TECENTRIQ	Atezolizumab	Non–Small-Cell Lung Cancer (NSCLC)	**Dhillon and Syed, 2019**
9	KEYTRUDA	Pembrolizumab	Metastatic Gastric Cancer	**Kamath *et al.,*2018**
10	IMFINZI	Durvalumab	Non–Small-Cell Lung Cancer (NSCLC)	**Antonia *et al.,*2018**
11	OPDIVO	Nivolumab	Melanoma, Non-Small Cell Lung Cancer (NSCLC), Urothelial Cancer, and Renal Cell Cancer.	**Prasad and Kaestner, 2017**
12	PROVENGE	Sipuleucel- T	Prostate Cancer	**Cheever and Higano, 2011**
13	YERVOY	Iplimumab	Metastatic Melanoma	**Graziani *et al.,* 2012**

14	BLINCYTO	Blinatumomab	Acute Lymphoblastic Leukaemia	**Sanford, 2015**
15	JEMPERLI	Dostarlimab	Colorectal Cancer	**ul Hussain *et al.*,2022**
16	LYNPARZA	Olaparib	Ovarian Cancer	**Gunderson and Moore, 2015**
17	ZEJULA	Niraparib	Ovarian Cancer	**Heo *et al.*,2018**

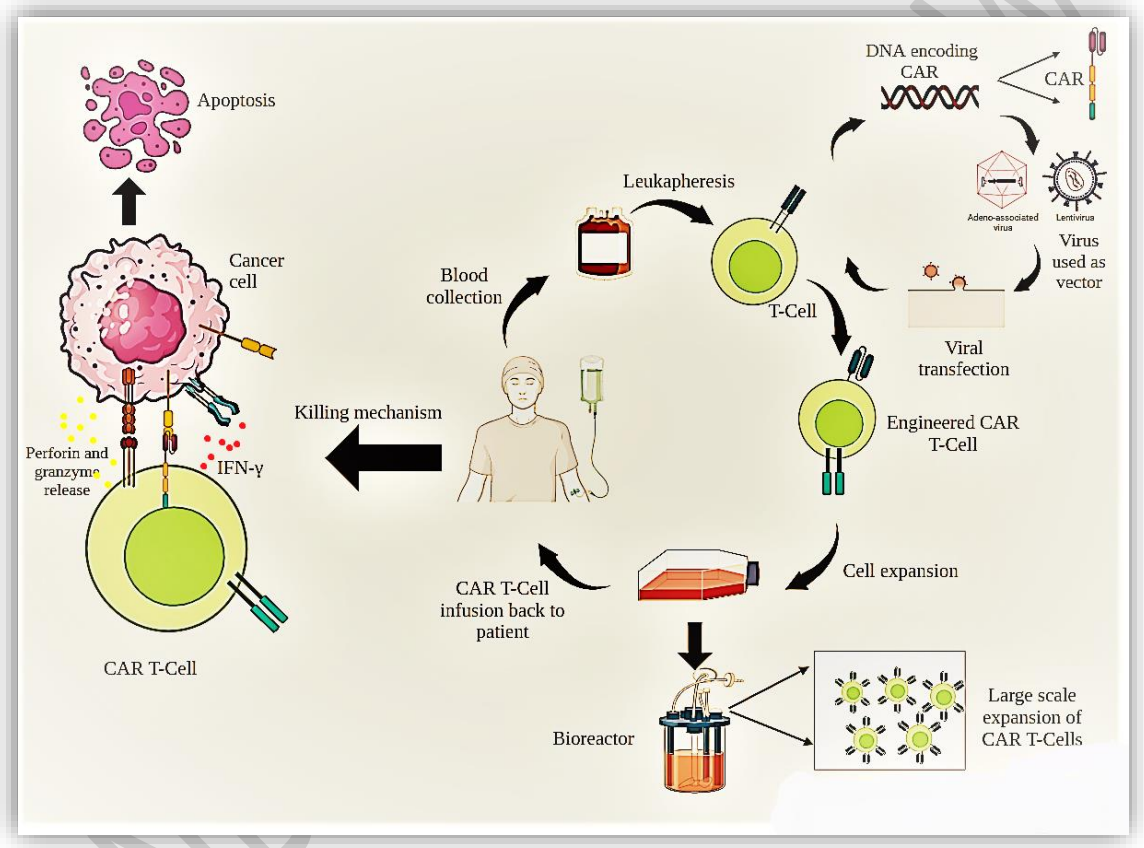

Fig 1. CAR T-Cell Therapy and its Mechanism of Action.