ADVENT OF CHIMERIC ANTIGEN RECEPTOR-T CELL (CAR-T CELL) THERAPY

(Soumya Bandi[1], Medical content writer and Dr. Pranjal Bordoloi[2], SVP Medical and Clinical Affairs Jodas Expoim Pvt. Ltd)

INTRODUCTION:

The immune system recognizes foreign substances by finding antigens on cells' surfaces. T cells, immune cells, have receptors that attach to these antigens, triggering the immune system to destroy them. India recorded 1.46 million new cancer cases in 2022, with lung and breast cancer being prevalent among males and females, with lymphoid leukemia being the primary childhood cancer. By 2025, cancer cases are expected to rise 12.8% compared to 2020.[1] Mutations in genes that impact, protein expression and alter cells are the underlying cause of cancer. These mutations suggest prospective targets for adoptive cell therapies and neoantigen screening.[2] In CAR T-cell therapies, T cells are taken from the patient's blood and modified in the lab to attach to the cancer cell antigen. The article summarizes a brief introduction and applications of CART cell therapy and evolution of the five generations of CAR-T cells.

The consistent production of cancer-specific T cells for cancer immunotherapy is made possible by genetic-engineering technologies like engineered T cell Receptors (TCR-T) and Chimeric Antigen Receptor T cells (CAR-T)[3]. Chimeric antigen receptors (CARs) are fusion proteins that can be genetically modified to express and transfused into patients. CAR-redirected T cells offer a promising cell-based immunotherapy method that can enhance and maintain antitumor GVL response without major histocompatibility complex restriction, potentially leading to remission of refractory/relapsed hematological malignancies.[4]

STRUCTURE OF CHIMERIC ANTIGEN RECEPTOR:

An intracellular signaling domain, a transmembrane domain, and an extracellular domain constitute the CAR structure. Tumour surface antigens, which are divided into tumor-specific antigens (TSAs) and tumor-associated antigens (TAAs), are recognized by the extracellular domain, a single-chain variable fragment (scFv). CAR-T cells get activated once scFv identifies TAAs, and they then transmit activation signals to the intracellular domain.[5]

The extracellular domain of CARs includes an antigen-recognition domain, a single peptide on the cell surface, and a transmembrane domain essential for receptor stability and surface expression.[6,7] The transmembrane domain is a hydrophobic alpha helix that extends in the cell membrane. The intracellular domain, the endo-domain, clusters and undergoes conformational changes upon stimulation, enabling the recruitment and phosphorylation of downstream signaling proteins.[7,8]

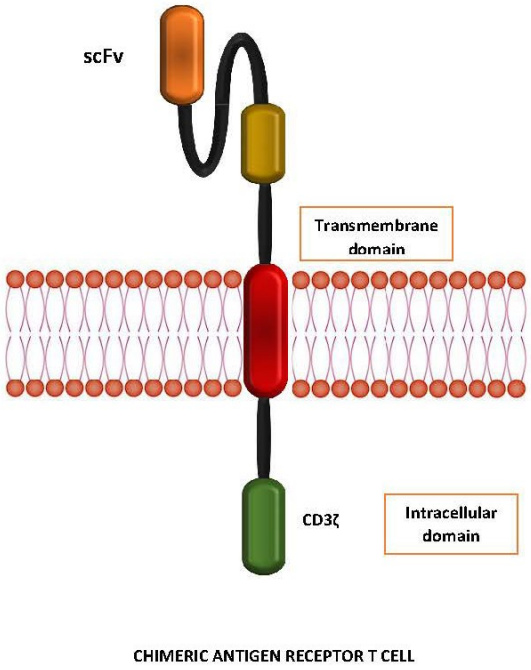

Fig 1: CAR has an ectodomain with an antigen recognition domain (scFv) and a hinge region connecting it to the TMD (spacer). It also has a lipophilic alpha-helical domain (TMD) and an endodomain with a CD3ζ containing three ITAMs for primary signal transmission and secondary signals for costimulation[9].

IMPORTANCE OF SCFV (SINGLE-CHAIN FRAGMENT VARIABLE) IN CAR T CELL THERAPY:

Antibodies are crucial in modern medicine, particularly in cancer treatment. A scFv antibody retains complete antigen-binding capability, making it a unique molecule for cancer treatment.[10] Its smaller fragments allow for faster and more even penetration to tumors and other tissues.[11] These fragments can be coupled with drugs and radionuclides, reducing exposure to healthy tissue. Additionally, they have no uptake by the kidney and can efficiently localize to tumors, making them crucial in cancer therapy.[12] The development of a new system in which an scFv library is able to be both rapidly and accurately screened based on

the antitumor functions of CAR-T cells would be expected to generate optimized scFv-expressing CAR-T cells with superior antitumor effects.[13] Optimised scFv-expressing CAR-T cells with enhanced antitumor effects should result from the creation of a novel technology that enables scFv to be quickly and precisely screened based on the antitumor activities of CAR-T cells.[13]

EVOLUTION OF CAR T CELLS:

CAR-T-cell construct designs have evolved over generations. Five generations of CAR-T cells have been created since 1989. The principal transmitter of signals from the endogenous T cell receptor (TCR), the CD3 ζ-chain or FcεRIγ, is represented by a single structure in the first generation.However, because to limited proliferation, a short in vivo life span, and insufficient released cytokines, the majority of studies using first-generation CAR-T cells have not produced the anticipated results.[8]

The costimulatory domains are required for the proliferation, differentiation and survival of the T-cell. Activation of the T-cells without costimulation may lead to the unresponsiveness of the T-cells, apoptosis or acquisition of the immune tolerance. Costimulatory molecules are promising therapeutic targets in transplantation, but understanding their cooperative function is crucial due to their complex relationships and shared ligands, as blocking a pathway may prevent the ligation of essential regulatory molecules.[14]

Second-generation CARs incorporate intracellular signaling domains from co-stimulatory protein receptors to enhance T cell proliferation, cytotoxicity, and response. These domains, including CD28, CD137, and CD134, improve cytotoxicity, sustain response, and prolong CAR-T cell life.[15] CD28-mediated co-stimulation regulates lymphocyte proliferation and survival, while CD134 sustains proliferation and strengthens IL-2 production. CD137 maintains T cell response signal, ensuring survival and memory.[16]

Third-generation CARs, combining multiple signaling domains, were used to treat lymphoma and colon cancer. However, outcomes were not improved compared to the second generation due to small case studies. Further studies are needed to explore safety and efficacy, as well as the selection of co-stimulatory molecules.

Fourth-generation CARs, T cell redirected for universal cytokine-mediated killing (TRUCKs), are created by adding IL-12 to second-generation constructs. These TRUCKs enhance T-cell activation and attract innate immune cells to eliminate antigen-negative cancer cells. They can also treat viral infections, metabolic disorders, and auto-immune diseases. These CAR-T cell therapies have gained significant interest in cancer treatment.[17]

Fifth-generation CAR-T cells have an extra intracellular domain, consisting of truncated cytokine receptors and a motif for binding transcription factors like STAT-3/5.[18] This receptor activates TCR, CD28 domain, and JAK-STAT3/5 signaling, triggering full T cell activation and proliferation. Additional variants, such as dual CARs, split CARs, and inducible-split CARs, enhance the specificity and control of transfused T cells.[19]

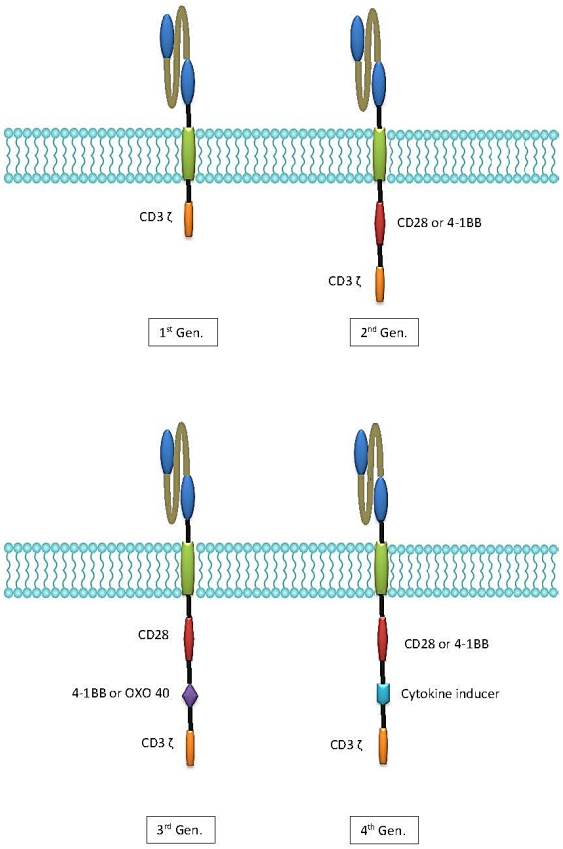

Fig 2: Four generation of the CART cells [20]

First-generation CARs had limited expansion and persistence due to lack of a costimulatory signal. Second-generation CARs included CD3ζ and co-stimulation signals, enhancing cytotoxicity and persistence. Third-generation CARs added additional costimulatory domains, enhancing T-cell expansion and persistence. Fourth-generation CARs activated NFAT, modulating immune effects.

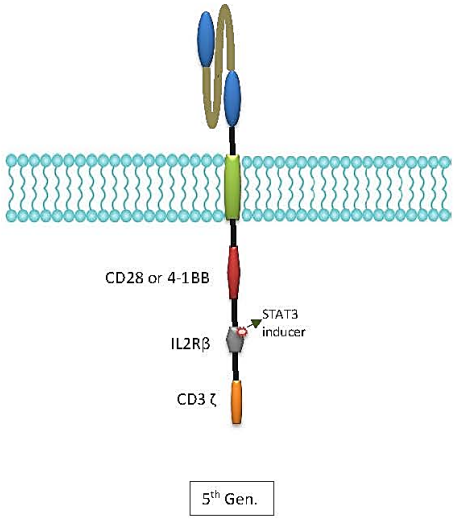

Fig 3: Fifth generation of CART cell [20]

Fifth-generation CARs require gene editing to inactivate the T-cell receptor alpha constant gene, triggering T-cell activation and proliferation.

PROCESS OF CAR-T CELL THERAPY:

CAR-T cells are produced through several steps, including leukocyte extraction, enrichment and washing, and separation of CD4/CD8 subsets using specific antibody markers. To activate the T cells, purified autologous antigen-presenting cells (APCs) are used, or beads coated with anti-CD3/anti-CD28 monoclonal antibodies, anti-CD3 antibodies alone or in combination with feeder cells and growth factors like IL-2. The culture conditions are further refined to polarize the T cells to a specific phenotype.[8]

The manufacturing process for CAR T cells involves stimulating, transducing, expanding, and cryopreserving T cells under Good Manufacturing Practices (GMP) conditions. This process leads to low throughput, product variability, and high costs. Efforts from the pharmaceutical industry and academic institutions have reduced manufacturing time from 14 days to a few days or even a day.[21]

Leukapheresis is an efficient method for obtaining T lymphocytes for CAR T cell culture. It separates whole blood into components, focusing on the Mono nuclear cells (MNC) layer. T cells are collected, activated, transduced, and expanded before reinfused into the patient. Robust T cell activation for CAR T cell manufacture can be achieved using soluble anti-CD3 monoclonal anitbodies, anti-CD3/anti-CD28 mAb coated paramagnetic beads, or cell-based

engineered artificial APCs. CAR T cell dosing regimens require post-modification expansion to achieve clinically significant numbers for treatment. GMP-compliant methods are crucial for expanding cells. Factors include providing adequate nutrient-rich media, optimizing gas exchange, and operating within a closed system. Efficient CAR T cell expansion can be achieved in static and dynamic culture systems, with next-generation systems offering real-time monitoring for optimal expansion.[22]

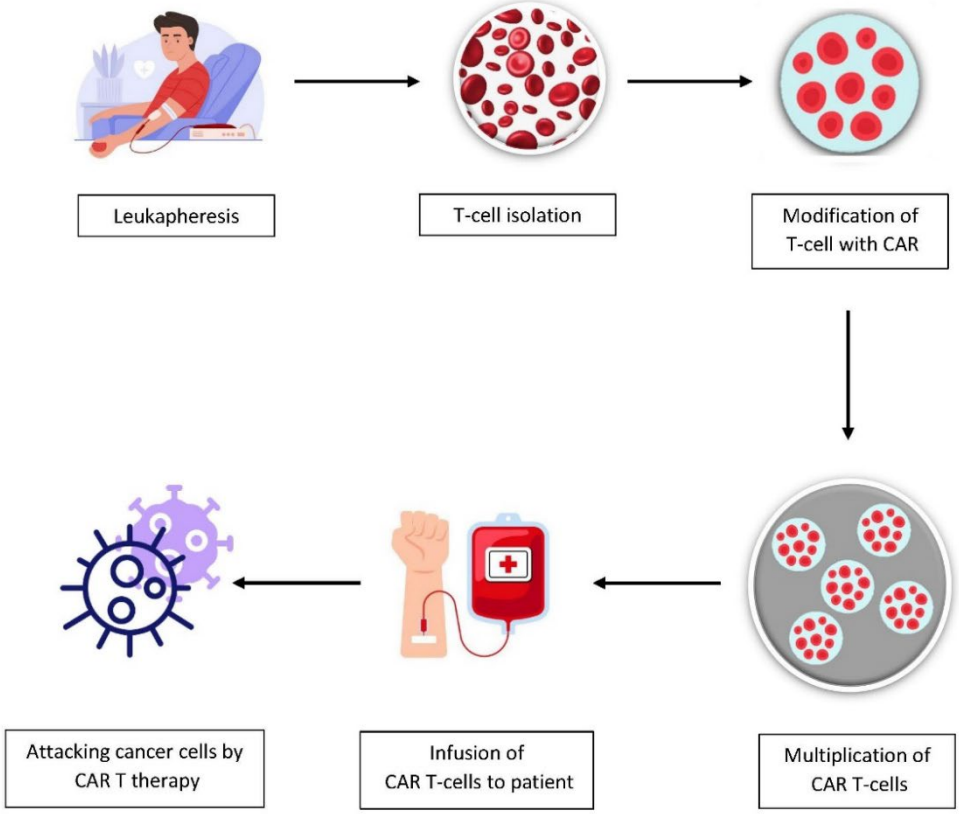

Fig: 2 Production of CAR T cells

The CAR-T cell manufacturing process involves a series of steps, including leukapheresis, activation, transduction, expansion, and infusion. Isolated autologous T cells are isolated, activated using artificial antigen-presenting cells, antibody coated magnetic beads, and growth factors, and then expanded in bioreactors. The harvested cells are then infused back to the patient.[23]

CLINICAL APPLICATIONS OF CART CELL THERAPY:

CAR-T Cell products are approved by the FDA for various indications, including B-cell precursor acute lymphoblastic leukemia (ALL), adult patients with relapsed or refractory large B-cell lymphoma, relapsed or refractory follicular lymphoma, relapsed or refractory mantle cell

lymphoma, relapsed or refractory B-cell precursor acute lymphoblastic leukemia, relapsed or refractory multiple myeloma, and relapsed or refractory multiple myeloma. These products include Kymriah® (tisagenlecleucel), Yescarta® (axicabtagene ciloleucel), Tecartus® (brexucabtagene autoleucal), Breyanzi® (lisocabtagene maraleucel), Abecma® (idecabtagene vicleucel), and Carvykti® (ciltacabtagene autoleucel). The FDA has approved these products for patients up to 25 years of age with refractory or relapsed disease, relapsed or refractory disease after two or more lines of systemic therapy, and relapsed or refractory disease after two or more lines of therapy.[24]

India's Central Drugs Standard Control Organization approved NexCAR19 as India's first approved CAR-T cell therapy in October 2023. The therapy, based on two clinical trials, showed 67% objective response in patients with advanced lymphoma or leukemia, with half experiencing complete response.[25]

CONCLUSION:

Cancer mutations in genes cause protein expression and cell alteration, making them potential targets for adoptive cell therapies and neoantigen screening. Genetic-engineering technologies like TCR-T and CAR-T enable consistent cancer-specific T cell production for immunotherapy. CAR-T cells enhance antitumor GVL response without restriction, potentially leading to remission of refractory/relapsed hematological malignancies.

CAR-T-cell construct designs have evolved over five generations since 1989. The first generation has a single structure, but limited proliferation and short in vivo life span. Second-generation CARs enhance T cell proliferation and response. Third-generation CARs combined multiple signaling domains but did not improve outcomes. Fifth-generation CARs have an extra intracellular domain and a motif for binding transcription factors. Additional variants enhance specificity and control of transfused T cells. While immune checkpoint inhibitors are more often utilised, CAR T-cell treatment has shown to be just as effective in curing advanced leukaemias and lymphomas, therefore preventing cancer from coming back for years.

The FDA has approved CAR-T Cell products for various cancers that include, B-cell precursor acute lymphoblastic leukemia, adult patients with relapsed or refractory large B-cell lymphoma, relapsed or refractory follicular lymphoma, relapsed or refractory mantle cell

lymphoma, relapsed or refractory B-cell precursor acute lymphoblastic leukemia, multiple myeloma, and multiple myeloma. India's Central Drugs Standard Control Organization approved NexCAR19 in October 2023, showing 67% objective response in patients with advanced lymphoma or leukemia.

REFERENCES:

1. Sathishkumar K, Chaturvedi M, Das P, Stephen S, Mathur P. Cancer incidence estimates for 2022 & projection for 2025: Result from National Cancer Registry Programme, India. *Indian J Med Res*. 2022;156(4):598. doi:10.4103/ijmr.ijmr_1821_22

2. Wang Z, Cao YJ. Adoptive Cell Therapy Targeting Neoantigens: A Frontier for Cancer Research. *Front Immunol*. 2020;11:176. doi:10.3389/fimmu.2020.00176

3. Jiang X, Xu J, Liu M, et al. Adoptive CD8+ T cell therapy against cancer:Challenges and opportunities. *Cancer Lett*. 2019;462:23-32. doi:10.1016/j.canlet.2019.07.017

4. Liu J, Zhong JF, Zhang X, Zhang C. Allogeneic CD19-CAR-T cell infusion after allogeneic hematopoietic stem cell transplantation in B cell malignancies. *J Hematol OncolJ Hematol Oncol*. 2017;10(1):35. doi:10.1186/s13045-017-0405-3

5. Zhang X, Zhu L, Zhang H, Chen S, Xiao Y. CAR-T Cell Therapy in Hematological Malignancies: Current Opportunities and Challenges. *Front Immunol*. 2022;13:927153. doi:10.3389/fimmu.2022.927153

6. Goulart LR, Santos PS, Carneiro AP, Santana BB, Vallinoto AC, Araujo TG. Unraveling Antibody Display: Systems Biology and Personalized Medicine. *Curr Pharm Des*. 2016;22(43):6560-6576. doi:10.2174/1381612822666160923112816

7. Alnefaie A, Albogami S, Asiri Y, et al. Chimeric Antigen Receptor T-Cells: An Overview of Concepts, Applications, Limitations, and Proposed Solutions. *Front Bioeng Biotechnol*. 2022;10:797440. doi:10.3389/fbioe.2022.797440

8. Zhang C, Liu J, Zhong JF, Zhang X. Engineering CAR-T cells. *Biomark Res*. 2017;5:22. doi:10.1186/s40364-017-0102-y

9. Verma A, Rafiq S. Chimeric Antigen Receptor (CAR) T Cell Therapy for Glioblastoma. *Cancer Treat Res*. 2022;183:161-184. doi:10.1007/978-3-030-96376-7_5

10. Hudson PJ. Recombinant antibody constructs in cancer therapy. *Curr Opin Immunol*. 1999;11(5):548-557. doi:10.1016/s0952-7915(99)00013-8

11. Colcher D, Pavlinkova G, Beresford G, Booth BJ, Choudhury A, Batra SK. Pharmacokinetics and biodistribution of genetically-engineered antibodies. *Q J Nucl Med Off Publ Ital Assoc Nucl Med AIMN Int Assoc Radiopharmacol IAR*. 1998;42(4):225-241.

12. Kim M kon, Jeong HJ, Kao CHK, et al. Improved renal clearance and tumor targeting of 99mTc-labeled anti-Tac monoclonal antibody Fab by chemical modifications. *Nucl Med Biol*. 2002;29(2):139-146. doi:10.1016/s0969-8051(01)00296-7

13. Ochi T, Maruta M, Tanimoto K, et al. A single-chain antibody generation system yielding CAR-T cells with superior antitumor function. *Commun Biol*. 2021;4(1):273. doi:10.1038/s42003-021-01791-1

14. Magee CN, Boenisch O, Najafian N. The role of costimulatory molecules in directing the functional differentiation of alloreactive T helper cells. *Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg*. 2012;12(10):2588-2600. doi:10.1111/j.1600-6143.2012.04180.x

15. Croft M. The role of TNF superfamily members in T-cell function and diseases. *Nat Rev Immunol*. 2009;9(4):271-285. doi:10.1038/nri2526

16. Finney HM, Lawson AD, Bebbington CR, Weir AN. Chimeric receptors providing both primary and costimulatory signaling in T cells from a single gene product. *J Immunol Baltim Md 1950*. 1998;161(6):2791-2797.

17. Chmielewski M, Abken H. TRUCKs: the fourth generation of CARs. *Expert Opin Biol Ther*. 2015;15(8):1145-1154. doi:10.1517/14712598.2015.1046430

18. Mehrabadi AZ, Ranjbar R, Farzanehpour M, et al. Therapeutic potential of CAR T cell in malignancies: A scoping review. *Biomed Pharmacother Biomedecine Pharmacother*. 2022;146:112512. doi:10.1016/j.biopha.2021.112512

19. Tokarew N, Ogonek J, Endres S, von Bergwelt-Baildon M, Kobold S. Teaching an old dog new tricks: next-generation CAR T cells. *Br J Cancer*. 2019;120(1):26-37. doi:10.1038/s41416-018-0325-1

20. Rallis KS, Hillyar CRT, Sideris M, Davies JK. T-cell-based Immunotherapies for Haematological Cancers, Part B: A SWOT Analysis of Adoptive Cell Therapies. *Anticancer Res*. 2021;41(3):1143-1156. doi:10.21873/anticanres.14871

21. Blache U, Popp G, Dünkel A, Koehl U, Fricke S. Potential solutions for manufacture of CAR T cells in cancer immunotherapy. *Nat Commun*. 2022;13(1):5225. doi:10.1038/s41467-022-32866-0

22. Roddie C, Dias J, O'Reilly MA, et al. Durable Responses and Low Toxicity After Fast Off-Rate CD19 Chimeric Antigen Receptor-T Therapy in Adults With Relapsed or Refractory B-Cell Acute Lymphoblastic Leukemia. *J Clin Oncol Off J Am Soc Clin Oncol*. 2021;39(30):3352-3363. doi:10.1200/JCO.21.00917

23. Faeq MH, Al-Haideri M, Mohammad TAM, et al. CAR-modified immune cells as a rapidly evolving approach in the context of cancer immunotherapies. *Med Oncol Northwood Lond Engl*. 2023;40(5):155. doi:10.1007/s12032-023-02019-4

24. Chen YJ, Abila B, Mostafa Kamel Y. CAR-T: What Is Next? *Cancers*. 2023;15(3):663. doi:10.3390/cancers15030663

25. Linda Wang. India's First Homegrown CAR T-Cell Therapy Has Roots in NCI Collaboration. *National Cancer Institute*. February 7, 2024.

Image credit- Freepik