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Living Medicines Engineered to Fight: A Comprehensive Review on CAR T-Cell Therapy

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ABSTRACT

Plethora of advancements in cancer treatment have resulted in designing unique signature and personalized therapies, tailored to patient-specific needs, using medications that target specific markers on cancer cells or even retrain the body's immune system. Chimeric Antigen Receptor T-cell, also called as CAR T-cell, is one such approach wherein a patient's own cells are modulated to kill the cancer cells. Conventional cancer treatments invite issues like low specificity, increased chances of relapse, or issues with radiotoxicity and tolerance in the case of chemotherapy. Currently, CAR T-cell therapy is under clinical investigation and different countries are still coming up with their guidelines for the pragmatic application of CAR T. In this review, we present all one needs to know about CAR T-cell therapy, its types, components, side effects, current authorization status in different countries along with the technology being used with respect to the therapy. We also brief upon the recent regulations or guidelines released by Europe and the USA, the countries who have actively initiated the CAR T idea. This review aims to provide insight into this targeted therapy having the potential to boost cancer research and to help researchers to develop more such patient-specific treatments to improve clinical outcomes in cancer.

Keywords: Immunotherapy; CAR T-cell therapy; Cancer; Immunology

INTRODUCTION

CAR T-cells also known as Chimeric Antigen Receptor T-cells (CARs, also known as Chimeric immunoreceptors or artificial T-cell receptors) are T-cells engineered with chimeric receptor proteins that increase the specificity to target a particular antigen[1]. The term chimeric is used because these are the fusion proteins that are constructed by linking two genes that code for different proteins i.e., antigen-binding gene and T-cell activating function of a gene into a single receptor. CAR T-cells are known to specifically identify cancer cells and destroy them via their interaction with the Tumor-Associated Antigens (TAAs) which are present on the tumor cell surface. CAR- TAA binding occurs, then T-cell activation takes place through the phosphorylation of immune receptor tyrosine-based activation motifs (ITAMs). This consequently induces T-cell proliferation, cytokine secretion, and cytotoxicity[2]. The components of a CAR T-cell include four domains as follows:

1. Ectodomain- which is an extracellular antigen recognition domain of the single-chain Fragment variant (scFv) obtained from an antibody
2. Hinge region
3. Transmembrane domain- which is an anchor to the T-cell membrane
4. Endodomain- which is an intracellular T-cell activation domain of CD3ζ[4].

CAR bridges the cell membrane systematically. It is constructed in a way that partly the receptor lies outside the cell and some part of it is within the cell.

The part of the CAR that sticks out from the cell's surface is composed of domains or fragments of lab-made antibodies. On the other hand, the internal part of each CAR has signaling as well as costimulatory domains. After receptor- antigen interaction, these convey signals into the cell[5].

CD4+ T-cells have the ability to enhance the efficiency of CD8+ T-cell-mediated cytotoxicity. CAR T-cells multiply in the patient's body and with the aid from their engineered receptor, recognize and later kill any cancer cells that harbor the target antigen on the surfaces[6]. By including various activation domains in the CAR design, the T-cell response intensity may be adjusted, making this device highly tunable[7]. To enhance patient outcomes, researchers have developed different generations of CAR T-cells altering their domains. Table 1 shows the characteristic features of every generation.

Table 1: Evolution of CAR T-cell therapy from First Generation to Fifth Generation

Generation	Features	Reference
First generation	Contains a single CD3 ζ- chain or FcεRIγ intracellular domain with the requirement of other costimulatory domains.	[8]
Second generation	CARs contain additional cytoplasmic domains like CD28, 4-1BB, or OX-40 which deliver a secondary signal when a tumor antigen is encountered. This overcomes insufficient proliferation, less cytokine production, and the short lifespan of conventional CAR T-cells by dual signaling.	[8]
Third generation	This generation was made by combining multiple costimulatory signaling domains within the endodomain which included CD3ζ-CD28-OX40 or CD3ζ-CD28-41BB. The efficacy achieved was not very different compared to second generation CAR T-cells.	[8]
Fourth generation	They have a constitutive or inducible expression cassette which contains a transgenic protein like a cytokine. These were designed in order to deliver the transgenic product to the targeted tumor site and are called T-cells Redirected for Universal Cytokine-mediated Killing (TRUCK) CAR T-cells.	[8]
Fifth generation	They integrate an additional membrane receptor. Principle is based on the addition of IL-2 receptors that allows JAK/STAT pathway activation which is antigen-dependent. This generation showed a broader therapeutic window and a better safety profile than the previous generations.	[9]

Fabrication of CAR T-cells

CAR T-cells can be either derived from T-cells in a patient's own blood- autologous treatment or derived from the T-cells of another healthy donor- allogeneic treatment[10]. The manufacturing of CAR T-cells has been illustrated in Figure 1 and Table 2.

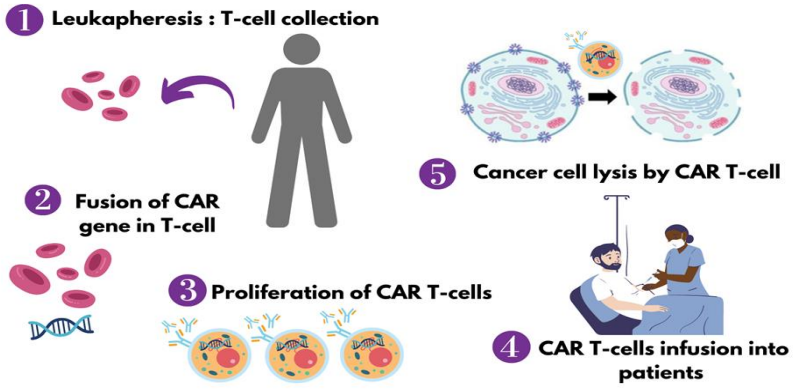

Figure 1. Manufacturing of CAR T-cells

Table 2: Making of CAR T-cells

Steps	Description	References
Isolation of T-cells	Leukapheresis (the separation and collection of white blood cells that include T-cells)	[11]
Incorporation of CAR gene	CAR gene is incorporated followed by growth and multiplication. Checkpoint inhibitors (mabs) are added, the binding then leads to the elimination of a mostly tumor-induced inhibition of T- lymphocytes and thereby, therapy response.	[12]
Infusion of CAR T-cells	CAR T-cells are infused back into the patient and a weak chemotherapy regimen is subjected to them as the cells are prone to work well under some cancer cells.	[13]
Post infusion process	CAR T-cells start binding with cancer cells and proliferate, destroying a greater number of cancer cells. To mitigate inflammatory toxicities caused during post-infusion of CAR T-cells, checkpoint inhibitors like anti-PD-1 or anti-PD-L1 are used.	[14,15]

The Call for CAR T-cells

The CAR identifies the antigen differently from natural, unaltered T-cells, which do so by utilizing the human leukocyte antigen (HLA) complex. CAR T-cells can develop a memory phenotype as regular T-cells, resulting in long-term persistence in the patient[16,17]. CAR T-cell treatments, in contrast to conventional therapy, are **living medicines** that use the immune system to lyse the tumor and prevent tumor relapse[17,18]. So far, CAR T-cell therapy is usually used as a second line of treatment[19]. Patients who are incompatible with prolonged and intensive chemotherapy may benefit from directed therapies like CAR T[20]. CAR T-cell therapy has demonstrated long-lasting remissions in patients, extending over several years[16].

The delivery takes place through a single infusion, necessitating a maximum of two weeks of inpatient care. This treatment option is advantageous due to its shorter duration, as there is no need for preparatory procedures or recovery periods. It is preferred over chemotherapy and stem cell transplants since it avoids the use of aggressive chemotherapy. Consequently, patients often experience a faster recovery compared to those undergoing stem cell transplants involving aggressive chemotherapy[21,22].

The battle between Conventional and CAR T

Table 3: Comparison of Conventional Therapy for Cancer vs CAR T-cell Therapy[23,24]

Conventional Therapy	CAR T Therapy
30-80% chances of relapse in cancer patients.	Long term efficacy following infusion and lower chances of relapse
Less specificity	Highly specific
Issues with radiotoxicity and drug tolerance	Lower chances of drug resistance or tolerance
Accompanied by fatal toxicities and side effects	Side effects can be clinically dealt with

The Downside of CAR T-cell Therapy

Although CAR T-cell therapy is valued for its patient-centric character, it adds to the complexity of the platform and increases the length of manufacturing time[25]. Owing to the intricacies of CAR T-cell therapy, the following are the major complications:

- **Cytokine Release Syndrome (CRS)**: Inflammatory cytokines like interleukin 6 wàA(IL-6) and tumor necrosis factor alpha (TNF-α) get overproduced overtime when immune cells like macrophages are indirectly activated by the proliferation of CAR T-cells[26].
- **CAR T-cell-related encephalopathy syndrome (CRES)**: Incidence of this therapy lies between 12-55% accounting for the second most common adverse event[27]. This condition is commonly seen with a few days of infusion. Symptoms of CRES vary rom moderate disorientation and confusion to deadly cerebral edema and regularly encompass speech difficulties[28].
- **Immune effector cell-associated neurotoxicity syndrome (ICANS)**: ICANS involves different neurological toxicities in conjunction with CRES rising from other therapies like bispecific antibodies and cellular immunotherapies that present same kind of side effects mediated by immune cells[29].
- '**On-target/off-tumor' toxicity**: Substantial on-target off-tumor risk is enhanced by wanting tumor antigen specificity[30]. When the target antigen expressed cells which are non-tumor in nature are attacked, then this toxicity occurs. Cancerous B-cells and normal cells, both possess CD19, which is the target for CAR T-cells because of which, healthy antibody-producing B-cells are also affected along with cancer cells increasing the risk of infection in the patient.
- **Tumor Lysis Syndrome (TLS):** Metabolic complications can manifest as a group when dying cells break down, often observed during the initiation of aggressive cancer treatments. TLS, which can lead to organ damage and pose a life-threatening risk, can arise as a complication in any therapy that triggers the cancer cell break down, along with CAR T-cells[31].
- **B-Cell Aplasia-** CAR T-cell therapy specifically targets antigens present on B cells' surfaces, leading to the elimination of both cancerous and healthy B cells. Consequently, a reduction in B cell count, known as B cell aplasia, is an anticipated outcome indicating the effectiveness of CD19-specific CAR T-cell treatment. This outcome can result in a diminished capacity to produce antibodies that play a crucial role in safeguarding the body against infections[32].

CAR T-cell Therapies approved by FDA

1. Abecma

Abecma is the brand name with Idecabtagene vicleucel being the generic name. This medication is prescribed for individuals with multiple myeloma who have previously undergone four or more treatment regimens that have been unsuccessful or are no longer effective[33]. It is a form of personalized immunotherapy in which for the expression of Chimeric Antigen Receptor, lentiviral vector is used for modification of patient's own T-cells genetically[34].

Mechanism of Action

The CAR construct includes

- a transmembrane domain,
- a 4-1BB costimulatory domain,
- an anti-BCMA scFv-targeting domain for antigen specificity,
- a CD3-zeta T-cell activation domain.

Activation of Abecma, which targets specific antigens, leads to the activation of CAR-positive T-cells. This activation leads to the production of cytokines and the cytolytic death of cells expressing BCMA (B-cell maturation antigen) [34]. BCMA, a highly promising therapeutic target, is found on the surface of both healthy and cancerous plasma cells involved in multiple myeloma[35]. Increased expression of this antigen promotes the growth, proliferation, and survival of myeloma cells. Activation of Abecma, a specific antigen-targeting therapy, leads to the development of CAR T-cells, production of chemical

messengers, and induction of cell death in BCMA-expressing cells[36].

Multiple Myeloma

Multiple Myeloma is distinguished by the buildup of atypical plasma cells within the bone marrow, resulting in the development of bone tumors[37]. The bone marrow fails to produce an adequate number of normal blood cells, leading to a compromised immune system[38,39]. Poor clinical outcomes, such as response rates that are only 20% to 30%, responses that last only two to three months, and short survival times are usually seem in individuals with relapsed or refractory multiple myeloma receiving treatment from all three major drug classes, such as an immunomodulatory agent, a proteasome inhibitor, and an anti-CD38[40, 41]. Development of Abecma REMS (Risk Evaluation and Mitigation Strategy) has also been initiated because of CRS and neurologic toxicities risks[42].

2. Kymriah

Kymriah® is the brand name while the generic name of this therapy is Tisagenlecleucel.

This two domains included:

- costimulatory domain- 4-1BB
- intracellular signaling domain (Endodomain)- CD3ζ[43]

Mechanism of Action:

The CAR contains a murine single-chain antibody variable fragment (scFv) that detects CD19, which is attached to 4-1BB (CD137) and CD3 intracellular signaling domains[44]. The intercellular signaling domain CD3 induces T-cell activation and anticancer action. 4-1BB promotes the growth and survival of Tisagenlecleucel cells[45]. Due to the nature of individual components present in therapy, the CAR binds to CD19-positive cells precisely and transmits a signal to drive activation and development of T-cell, enhances the long-term viability of Tisagenlecleucel cells[46-48].

Acute Lymphoblastic Leukaemia (ALL)

ALL is the result of a clonal buildup of immature blood cells in the bone marrow[49]. Clinical trials for Central Nervous System (CNS) Leukaemia have been conducted using CAR-T, and individuals with asymptomatic CNS illness have shown remission of cerebrospinal fluid blasts with intravenously delivered CD19 CAR T-cells[50]. Several patients reacted without experiencing considerable neurotoxicity, although others developed severe neurotoxicity, indicating that factors other than CNS disease load should be considered[51, 52].

3. Tecartus

Tecartus is the brandname of brexucabtagene autoleucel. It is prescribed to adults who have refractory mantle cell lymphoma[53]. Coupling of CD28 and CD3-zeta costimulatory domains to a murine anti-CD19 single-chain variable in the anti-CD19 CAR[54].

Tecartus comprises a hinge, which connects an exterior domain containing a single-chain variable segment to a transmembrane domain and two intracellular CD28/CD3 domains.

- CD28 is a costimulatory domain
- CD3ζ is a signaling domain
-

Mechanism of Action

To transduce it into T-cells, a gamma-retrovirus vector is used. CAR T-cells stimulate T-cells by promoting pathways of signalling via the CD3 domain after adhering to the CD19 receptor on lymphoma cells[55]. By releasing the granzyme B and perforin, CAR T-cells start a chain reaction of cytokines leading to neoplasm breakdown and lymphoma demise. The costimulatory domain of CD28 boosts CAR T-cell development and anti-CD19 function[56, 57].

Mantle cell lymphoma

A non-Hodgkin lymphoma variant. Mantle cell lymphoma frequently starts gradually (indolently) and then expands quickly (aggressively)[58]. The FDA granted approval based on the consequences of a clinical study entitled ZUMA-2, which investigated brexucabtagene in 60 patients with mantle cell lymphoma who had previously received up to five therapies. In the experiment, 87% of patients reacted to a single administration of brexucabtagene, and 62% had a complete response, which means they no longer exhibited indications of the illness, at least momentarily[59]. All of the patients had previously received treatment with a medication

that inhibits the action of Bruton tyrosine kinase, a protein that contributes to the proliferation and recurrence of some malignancies[60].

4. Breyanzi

Breyanzi is used to treat Diffuse Large B-Cell Lymphoma, Primary Mediastinal Large B-Cell Lymphoma, and Follicular Lymphoma Grade 3B.

Mechanism of Action

It is an amalgam of two distinct kinds of white blood cells from the patient cells. It has a 4-1BB costimulatory domain, which activates promotion of Breyanzi development and endurance[61]. When the patient is administered Breyanzi, the transformed T-cells interact with CD19 proteins on tumor cells and destroy them, assisting in the removal of malignancies from the patient's body[62,63].

B-Cell Lymphoma

It occurs when healthy B-cells transform into rapidly multiplying cancer cells that do not perish. Cancer cells multiply and eventually overpower cells that are healthy[64-66].

5. Yescarta

Yescarta, generic name being Axicabtagene Ciloleucel, is a prescription medicine used to treat two kinds of non-Hodgkin lymphoma:

- When the initial treatment did not work leading to resurfacing of cancer within a year, or when a minimum of two forms of treatment are unable to manage the malignancy[67].
- follicular lymphoma when a minimum of two forms of treatment are unable to suppress the disease[68, 69].

Mechanism of Action

Yescarta is a CD19-directed and genetically altered allogeneic T-cell immunotherapy, adheres to CD19-expressing cancer cells and normal B cells. Following anti-CD19 CAR T-cell involvement with CD19-expressing target T-cells, the CD28 and CD3-zeta co-stimulatory domains are triggered, resulting in T-cell activation, development, takeover of effector functions, and discharge of inflammatory cytokines and chemical messengers. As a consequence of this, CD19-expressing cells are eliminated[70].

Follicular lymphoma

It is a subtype of B-cell lymphoma, a form of non-Hodgkin's lymphoma. It progresses via transformation and relapse[71]. CAR T-cell therapy provides clinicians with another treatment option for follicular lymphoma[72]. It may be beneficial for people whose cancer did not respond to the initial therapies they attempted, a condition known as refractory follicular lymphoma. It may also benefit people who went into remission on another treatment but had their illness return, a condition known as relapsed follicular lymphoma[73, 74].

Green Signal for CAR T-cell Therapy Worldwide

The various approved CAR T-cell therapies in respective countries are listed in Table 4.

Table 4: Approved CAR T-cell Therapies Worldwide

Countries	Approved Therapies	References
Singapore	Kymriah for B cell Acute Lymphoblastic Leukaemia	[80]
UK	Kymriah, Yescarta, and Tecartus for B-cell Acute Lymphoblastic Leukaemia and specific types of lymphomas	[81]
China	Yescarta for Non-Hodgkin's lymphoma	[81]
Australia	Kymriah, Yescarta, Tecartus for Acute Lymphoblastic Leukaemia, large B cell lymphoma, and mantle cell lymphoma respectively	[81]

Techy CAR T

Currently, the production of CAR T-cells requires two weeks to make a clinical dose. They are collected for cryopreservation through a series of procedures before being infused[82]. The CliniMACS Prodigy is a closed automated system that abides by Good Manufacturing Practises (GMPs) and can make CAR T-cells outside of a cleanroom environment in an unclassified location[83]. O'Connor, et al. demonstrated sufficient ex vivo expansion of CAR T-cells. This eight-day experiment showed better anti-leukemic activity of CAR T-cells with shorter culture durations[84]. This technology not only offers sample

monitoring, but also enables the delivery of non-frozen cells, and rapidly yields a functional product within two weeks[85]. Miltenyi Prodigy® and the Lonza Cocoon®, are closed, semi-automated systems which follow a "one-device-per-patient approach" to minimize the risk of cross-contamination[86].

Artificial Intelligence (AI) and CAR T

With more control and customized process rules, AI can get vital process insights about the properties and behavior of the cells[87]. Furthermore, AI can optimize manufacturing schedules and resource management. As a result, several AI applications will be developed as part of AIDPATH (AI driven Decentralised Production for Advanced Therapies in the Hospital) across the CAR T-cell production and therapy process[88]. A digital twin will follow the product through the full production process and run simulations on the cell behavior to gain deeper process insights into the CAR T-cell process and comprehend how patient-specific characteristics influence it. The control software may adaptively control the bioreactor during the time-consuming cell expansion process based on these observations. The status of the cells can be ascertained from the bioreactor's recorded process data, such as lactose or oxygen, and potential expansion methods can be predicted[89]. Metabolomics data are added to the process data due to their promising qualities for quality control in personalized medication[90]. Notably, compared to standard non-automated CAR T-cell manufacturing campaigns, the operator hands-on time is significantly reduced[91].

A possible approach to increase the accessibility of CAR immunotherapy is the flexible connection of various devices that perform distinct stages of the CAR T manufacturing process in a machine-driven street. This would enable each device to operate at high capacity and allow the processing of material from multiple patients concurrently[92, 93]. In manual handling, production failure and product contamination are frequent occurrences. On an extended CliniMACS Prodigy platform with an in-line electroporation unit, an automated T-cell engineering procedure can generate off-the-shelf CAR T-cells[94]. In this configuration, a Transcription Activator-Like Effector Nuclease (TALEN) that targets the *TRAC* locus was transferred along with mRNA that encoded a CD19-targeting CAR by lentiviral delivery. In summary, it is possible to develop an automated, Good Manufacturing Practices (GMP)-compliant procedure that combines lentiviral transduction with electroporation of TALEN mRNA to create functional, TCR/-free CAR19 T-cells on a clinical scale[95, 96].

Navigating Regulatory Hurdles

The regulatory approach taken for these cell therapies is dictated not only by the manufacturing of the products but also by their intended clinical use and method of clinical delivery[79, 97, 98].

US FDA

According to the FDA's current framework for biological products, CAR T-cells are governed as Gene Therapy (GT). The general considerations for GT product manufacturing and testing are outlined in the FDA guidance document "Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs): Guidance for Industry".

For the manufacturing process to be controlled and kept under control, appropriate in-process testing is essential. Multiple factors, including vitality, cell quantity, cell phenotype, and CAR expression, are frequently evaluated as part of CAR T-cell in-process testing regimens. The specificity and affinity for the target antigen should be evaluated as part of the preclinical assessment of the antigen recognition domain in order to determine the possibility of on-target/off-tumor and off-target toxicity[75].

The variables that determine the safety and activity of CAR T-cells are multifaceted. The vector construct's design, vector distribution technique, cell source, biological activities (such cytokine expression patterns, cytotoxicity, and proliferation), and the incorporation of novel components like immunomodulatory factors are all factors to take into account[76]. Sponsors are encouraged to use National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) for the purpose of checking the severity of adverse events. The underlying condition, the durability of the CAR T-cells, and the CAR vector all influence how

long patients who have received CAR T-cells must be followed up. After receiving CAR T-cells with an incorporated transgene, subjects should be monitored for 15 years[77].

European Union

CAR T-cell Therapy is considered Gene Therapy Medicinal Products (GTMPs) under Advanced Therapy Medicinal Products (ATMP)[78]. The two authorized CAR T Therapies are Yescarta and Kymriah as of yet. The starting materials are procured by hospitals or blood banks and operated apheresis activities products are passed through EU Tissues and Cells Directives after which, it is considered for ATMP Regulation.

All phases of the transplant procedure are covered by the Tissues and Cells Directives, including donation, sourcing, evaluation, processing, preservation, storage, and distribution. When it comes to patient or donor care, starting material procurement, processing, intermediate storage, packing, release, and testing, document completion, and ATMP receipt, storage, thawing, and administration, manufacturers typically train individuals involved. In order to ensure that patients have access to the innovative therapies covered by ATMP, the Hospital Exemption (HE) is established under Article 28 (Individual Prescription). Minimizing patient risks related to CAR T-cell treatment is a shared objective of the responsible authorities and manufacturers[3].

CONCLUSION

CAR T-cells are rightly attributed as living medicines because of their ability to utilize the patients' immune system to target tumor antigens. Currently, CAR T-cell therapy is the second- line treatment for advanced cancers following chemotherapy. Although highly specific, this therapy suffers the disadvantage of on- target/off- target toxicity. The advancements in technology in manufacturing CAR T-cells will help in overcoming the challenges of this therapy. Research in India on this therapy is at a primitive stage but is likely to gain momentum in the near future. On the contrary, the US is the leading country where abundant research and development have been carried out to leverage this therapy for maximizing patient efficacy and safety. Amongst the existing CAR T-cell therapies, Australia, China, Singapore, and the UK have approved one or more therapies. To summarize, CAR T-cell therapy withstands a lot of potential in cancer treatment and hence should be further explored in terms of extensive research to develop it into a robust and cost-effective regimen.

List of Abbreviations

CAR	Chimeric Antigen Receptor
CAR T-cells	Chimeric Antigen Receptor T-cells
CAR- TAA	Chimeric Antigen Receptor - Tumor Associated Antigens
CRES	CART-cell-related encephalopathy syndrome
ICANS	Immune effector cell-associated neurotoxicity syndrome
TLS	Tumor Lysis Syndrome
BCMA	B-cell maturation antigen
CRS	Cytokine Release Syndrome
CNS	Central Nervous System
TALEN	Transcription Activator-Like Effector Nuclease
GT	Gene Therapy
ATMP	Advanced Therapy Medicinal Products

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

REFERENCES

1. Gils Roex, Tom Feys, Yves Beguin, et al. Chimeric Antigen Receptor-T-Cell Therapy for B-Cell Hematological Malignancies: An Update of the Pivotal Clinical Trial Data. Pharmaceutics. 2020; 12(2) :194.

2. Yu s, Li A, Liu Q, et al. Chimeric antigen receptor T-cells: a novel therapy for solid tumors. J Hematol Oncol. 2017;10(1):78.

3. E. McGrath and P. Machalik. The Regulatory Framework for CAR T-cells in Europe: Current Status and Foreseeable Changes AND Centre Qualification by Competent Authorities and Manufacturers," in The EBMT/EHA CAR T-cell Handbook, N. Kröger, J. Gribben, C. Chabannon, I. Yakoub-Agha, and H. Einsele, Eds., Cham (CH): Springer, 2022. Accessed: Apr. 26, 2023. [Online]. Available: http://www.ncbi.nlm.nih.gov/books/NBK584158/.

4. Sterner RC , Sterner RM. CAR T-cell therapy: current limitations and potential strategies. Blood Cancer J. 2021 Apr 6;11(4):69.
5. Kevin J Curran [1] , Hollie J Pegram, Renier J Brentjens. Chimeric antigen receptors for T-cell immunotherapy: current understanding and future directions. J Gene Med. 2012;14(6):405-15.
6. C Zhang, Liu J, Zhong JF, et al. Engineering CAR T-cells. Biomark Res. 2017;5:22.
7. Mancikova V, Smida M. Current State of CAR T-Cell Therapy in Chronic Lymphocytic Leukemia. Int J Mol Sci. 2021;22(11):5536.
8. Chmielewski M, Abken H. TRUCKs: the fourth generation of CARs. Expert Opin Biol Ther. 2015;15(8):1145-54.
9. A. R. PhD. From the first to the fifth generation of CAR T-cells. ProteoGenix. https://www.proteogenix.science/scientific-corner/CAR T/CAR T-generations/ (accessed Apr. 26, 2023).
10. Autologous vs. allogeneic CAR T: Decisions must be made 'carefully and deliberately.' https://www.healio.com/news/hematology-oncology/20201028/autologous-vs-allogeneic-cart-decisions-must-be-made-carefully-and-deliberately (accessed Apr. 26, 2023).
11. National Cancer Institute (NCI), Leukapheresis for CAR or Adoptive Cell Therapy Manufacturing. clinicaltrials.gov, Clinical trial registration NCT03226704, Mar. 2023. Accessed: Apr. 24, 2023. [Online]. Available: https://clinicaltrials.gov/ct2/show/NCT03226704
12. N. Hosseinkhani, Derakhshani A, Kooshkaki O, et al. Immune Checkpoints and CAR T-cells: The Pioneers in Future Cancer Therapies? Int J Mol Sci. 2020;21(21):8305.
13. CAR T-cell Therapy and Its Side Effects. https://www.cancer.org/treatment/treatments-and-side-effects/treatment-types/immunotherapy/CAR T-cell1.html (accessed Apr. 26, 2023).
14. Frontiers | CAR T-cell Therapy: Mechanism, Management, and Mitigation of Inflammatory Toxicities. https://www.frontiersin.org/articles/10.3389/fimmu.2021.693016/full#B16 (accessed Apr. 26, 2023).
15. Jafarzadeh L, Masoumi E, Fallah-Mehrjardi K, et al. Prolonged Persistence of Chimeric Antigen Receptor (CAR) T-cell in Adoptive Cancer Immunotherapy: Challenges and Ways Forward. Front Immunol. 2020; 11: 702.
16. Advantages of CAR T-cell Therapy | Rutgers Cancer Institute of New Jersey. https://www.cinj.org/patient-care/advantages-CAR T-cell-therapy (accessed Apr. 26, 2023).
17. Wolf P, Alzubi J, Gratzke C, et al. The potential of CAR T-cell therapy for prostate cancer. Nat Rev Urol. 2021;18(9): 556-571.
18. Miliotou AN, Papadopoulou LC. CAR T-cell Therapy: A New Era in Cancer Immunotherapy. Curr Pharm Biotechnol. 2018; 19(1): 5–18.
19. CAR T-cells: Second-Line Treatment Option for NHL? – NCI https://www.cancer.gov/news-events/cancer-currents-blog/2022/nhl-CAR T-cells-belinda-transform-zuma7 (accessed Apr. 26, 2023).
20. Wei G, Hu Y, Pu c, et al. CD19 targeted CAR T therapy versus chemotherapy in re-induction treatment of refractory/relapsed acute lymphoblastic leukemia: results of a case-controlled study. Ann Hematol. 2018; 97(5): 781–789.
21. Expanding CAR T-cell Therapy Beyond Cancer | Inside Precision Medicine. https://www.insideprecisionmedicine.com/news-and-features/expanding-CAR T-cell-therapy-beyond-cancer/?gclid=CjwKCAjwzNOaBhAcEiwAD7Tb6Fztzv2jnDk0qVBvbNUhWwEtYq_r4R9Lb2hTrJq-yWobUGDQSl5TRRoCA4AQAvD_BwE# (accessed Apr. 26, 2023).
22. What is CAR T-Cell Therapy? | Advantages, Design, & Toxicity. https://www.allucent.com/resources/blog/what-are-CAR T-cell-therapies (accessed Apr. 26, 2023).
23. W. Yan, Hu H, Tang B. Advances Of Chimeric Antigen Receptor T-cell Therapy In Ovarian Cancer. Onco Targets Ther. 2019; 12: 8015–8022.
24. Zhao L , Cao YJ. Engineered T-cell Therapy for Cancer in the Clinic Front. Immunol 2019 10 Accessed: Apr. 26, 2023. [Online]. Available: https://www.frontiersin.org/articles/10.3389/fimmu.2019.02250.
25. Next-Generation Chimeric Antigen Receptor T-Cell Therapy: Going off the Shelf | SpringerLink. https://link.springer.com/article/10.1007/s40259-017-0247-0 (accessed Apr. 26, 2023).
26. Remodeled CAR T-Cell Therapy Causes Fewer Side Effects - NCI https://www.cancer.gov/news-events/cancer-currents-blog/2020/CAR T-cell-therapy-lymphoma-reduced-side-effects (accessed Apr. 26, 2023).
27. C Jérôme. Prise en charge pratique d'une encéphalopathie liée au traitement par cellules CAR T chez l'adulte et l'enfant : recommandations de la Société francophone de greffe de moelle et de thérapie cellulaire (SFGM-TC)" Bulletin du Cancer 2020 107, no. 1, p. 12
28. Ali S, Kjeken R, Niederlaender ch, et al. The European Medicines Agency Review of Kymriah (Tisagenlecleucel) for the Treatment of Acute Lymphoblastic Leukemia and Diffuse Large B-Cell Lymphoma. Oncologist. 2020; 25(2):e321–e327.
29. Abbasi S, Asghari Totmaj M,Abbasi M, et al. Chimeric antigen receptor T-cells: Novel cell therapy for

hematological malignancies. Cancer Med. 2023; 12(7): 7844-7858.
30. Martinez M, Kyung Moon E, et al. CAR T-cells for Solid Tumors: New Strategies for Finding, Infiltrating, and Surviving in the Tumor Microenvironment. Front Immunol. 2019; 10: 128.
31. Bonifant CL [1], Jackson HJ [2], Brentjens RJ, et al. Toxicity and management in CAR T-cell therapy. Mol Ther Oncolytics. 2016:3:16011.
32. "multil." https://www.lls.org/treatment/types-treatment/immunotherapy/chimeric-antigen-receptor-CAR T-cell-therapy (accessed Apr. 26, 2023).
33. CAR T-cell Therapy for R/R Multiple Myeloma | ABECMA® (idecabtagene vicleucel). https://www.abecma.com/ (accessed Apr. 26, 2023).
34. EMA, Abecma, European Medicines Agency, Jun. 23, 2021. https://www.ema.europa.eu/en/medicines/human/EPAR/abecma (accessed Apr. 26, 2023).
35. Shah N, Chari A, Scott E, et al. B-cell maturation antigen (BCMA) in multiple myeloma: rationale for targeting and current therapeutic approaches. Leukemia. 2020;34(4): 985–1005.
36. Fala L, Abecma (Idecabtagene Vicleucel) First-in-Class BCMA-Directed CAR T-Cell Therapy Approved for Relapsed or Refractory Multiple Myeloma. Accessed: Apr. 26, 2023. [Online]
37. Fairfield H, Falank C, Avery L, et al. Multiple myeloma in the marrow: pathogenesis and treatments. Ann N Y Acad Sci. 2016; 1364(1): 32-51.
38. CAR T-cell therapy for Multiple Myeloma | Rho Chi Post. https://rhochistj.org/RhoChiPost/CAR T-cell-therapy-for-multiple-myeloma/ (accessed Apr. 26, 2023).
39. Rajkumar SV, Kumar S. Multiple Myeloma: Diagnosis and Treatment. Mayo Clin Proc. 2016; 91(1): 101–119.
40. Barlogie B; Shaughnessy J, Tricot G,et al. Treatment of multiple myeloma. Blood. 2004; 103(1): 20–32
41. U.S. Food and Drug Administration Approves Bristol Myers Squibb's and bluebird bio's Abecma (idecabtagene vicleucel), the First Anti-BCMA CAR T-cell Therapy for Relapsed or Refractory Multiple Myeloma - bluebird bio (NASDAQ:BLUE), Bristol-Myers Squibb (NYSE:BMY) - Benzinga
42. ABECMA® Is a Personalized Immune Cell Therapy That Targets BCMA With a One-Time Infusion1" https://www.abecmahcp.com/mechanism-of-action#:~:text=ABECMA%C2%AE%20Is%20a%20Personalized,With%20a%20One%2DTime%20Infusion&text=Antigen%2Dspecific%20activation%20of%20ABECMA,killing%20of%20BCMA%2Dexpressing%20cells. (accessed May, 2023)
43. Tisagenlecleucel (Kymriah) CAR T-Cell Therapy - Drug Information - Chemocare. https://chemocare.com/chemotherapy/drug-info/tisagenlecleucel.aspx (accessed Apr. 26, 2023).
44. E Roselli, R Faramand, ML Davila. Insight into next-generation CAR therapeutics: designing CAR T-cells to improve clinical outcomes. J Clin Invest. 2021;131(2):e142030.
45. Regression of Glioblastoma after Chimeric Antigen Receptor T-Cell Therapy | NEJM. https://www.nejm.org/doi/full/10.1056/nejmoa1610497 (accessed Apr. 26, 2023).
46. Clinical trials of dual-target CAR T-cells, donor-derived CAR T-cells, and universal CAR T-cells for acute lymphoid leukemia | SpringerLink https://link.springer.com/article/10.1186/s13045-019-0705-x (accessed Apr. 26, 2023).
47. Maude SL, Laetsch TW , Buechner J, et al. Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia. N Engl J Med. 2018;378(5):439-448.
48. Rosenbaum L. Tragedy, Perseverance, and Chance - The Story of CAR T Therapy. N Engl J Med. 2017; 377(14):1313-1315.
49. R. A. Larson and J. Anastasi. Acute Lymphoblastic Leukemia: Clinical Presentation, Diagnosis, and Classification in Acute Leukemias E. H. Estey, S. H. Faderl, and H. M. Kantarjian, Eds in Hematologic Malignancies. Berlin, Heidelberg: Springer, 2008, pp. 109–118.
50. Samra B, Jabbour E, Ravandi F, et al. Evolving therapy of adult acute lymphoblastic leukemia: state-of-the-art treatment and future directions. J Hematol Oncol. 2020 Jun 5;13(1):70.
51. Lee DW , Kochenderfer JN [2], Stetler-Stevenson M, et al. T-cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. Lancet. 2015; 385(9967): 517–528.
52. T-cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial - The Lancet. https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(14)61403-3/fulltext (accessed Apr. 26, 2023).
53. C. for B. E. and Research, TECARTUS (brexucabtagene autoleucel), FDA Accessed: Apr. 26, 2023. [Online]. Available: https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/tecartus-brexucabtagene-autoleucel
54. Brexucabtagene Autoleucel (Tecartus™) : Oncology Times. https://journals.lww.com/oncology-times/Fulltext/2022/08200/Brexucabtagene_Autoleucel__Tecartus__.7.aspx (accessed Apr. 26, 2023).

55. Davila ML , Brentjens RJ. CD19-Targeted CAR T-cells as novel cancer immunotherapy for relapsed or refractory B-cell acute lymphoblastic leukemia. Clin Adv Hematol Oncol. 2016; 14(10):802-808.
56. Mian A , Hill BT. Brexucabtagene autoleucel for the treatment of relapsed/refractory mantle cell lymphoma. Expert Opin Biol Ther. 2021; 21(4): 435–441.
57. Romejko-Jarosińska J. CAR T therapy in mantle cell lymphoma: a review of the current literature. Acta Haematol Pol. 2022; 53(3): 166–175.
58. Mantle Cell Lymphoma; Prognosis, Symptoms & Treatment. Cleveland Clinic. https://my.clevelandclinic.org/health/diseases/24030-mantle-cell-lymphoma (accessed Apr. 26, 2023).
59. CAR T-Cell Therapy Approved by FDA for Mantle Cell Lymphoma - NCI. https://www.cancer.gov/news-events/cancer-currents-blog/2020/fda-brexucabtagene-mantle-cell-lymphoma (accessed Apr. 26, 2023).
60. CAR T-Cell Therapy Approved by FDA for Mantle Cell Lymphomhttps://www.cancer.gov/news-events/cancer-currents-blog/2020/fda-brexucabtagene-mantle-cell-lymphoma.(accessed June 1, 2023)
61. EMA, Breyanzi. European Medicines Agency. https://www.ema.europa.eu/en/medicines/human/EPAR/breyanzi (accessed Apr. 26, 2023).
62. Understanding Cancer: Metastasis, Stages of Cancer, and More. https://www.rxlist.com/cancer_101_slideshow_pictures/article.htm (accessed Apr. 26, 2023).
63. What Is Breyanzi® (lisocabtagene maraleucel)?. Breyanzi® (lisocabtagene maraleucel) Patient Site. https://www.breyanzi.com/about (accessed Apr. 26, 2023).
64. S. Bailly et al. Targeting CD19 in diffuse large B-cell lymphoma: An expert opinion pper Hematol. Oncol 2022 40, no. 4, pp. 505–517
65. B-cell Lymphoma: Types, Symptoms & Prognosis. https://my.clevelandclinic.org/health/diseases/22030-b-cell-lymphoma (accessed Apr. 26, 2023).
66. CAR T-cell Therapy Breyanzi® Approved as Relapsed or Refractory Large B-cell Lymphoma Second-Line Therapy in Japan. https://news.bms.com/news/details/2022/CAR T-Cell-Therapy-Breyanzi-Approved-as-Relapsed-or-Refractory-Large-B-cell-Lymphoma-Second-Line-Therapy-in-Japan/default.aspx (accessed Apr. 26, 2023).
67. B Coiffier, C Sarkozy. Diffuse large B-cell lymphoma: R-CHOP failure-what to do? Hematology Am Soc Hematol Educ Program 2016 16(1), pp- 366-378
68. S. S. Neelapu et al. Axicabtagene Ciloleucel CAR T-Cell Therapy in Refractory Large B-Cell Lymphoma N. Engl. J. Med 2017 377, no. 26, pp. 2531–2544
69. Yescarta CAR T-cell therapy for non-Hodgkin lymphoma patients. https://www.yescarta.com/ (accessed Apr. 26, 2023).
70. Yescarta: Cost, Uses, Side Effects, Dosage, and More.https://www.healthline.com/health/drugs/yescarta#what-to-consider (accessed Apr. 26, 2023).
71. Luminari S, Bellei M, Biasoli I, et al. Follicular lymphoma - treatment and prognostic factors. Rev Bras Hematol Hemoter. 2012 34(1):54-9
72. Kase AM, Kharfan-Dabaja MA, Donaldson A, et al. Immunotherapy beyond cellular therapy in follicular lymphoma: A case of complete remission after failure of two CAR T. Clin Case Rep. 2022; 10(4):e05572.
73. CAR T has 'fundamentally changed' outcomes in follicular lymphoma. https://www.healio.com/news/hematology-oncology/20221004/cart-has-fundamentally-changed-outcomes-in-follicular-lymphoma (accessed Apr. 26, 2023).
74. Follicular Lymphoma: Stages, Symptoms, Treatment & Prognosis Cleveland Clinic. https://my.clevelandclinic.org/health/diseases/22606-follicular-lymphoma (accessed Apr. 26, 2023).
75. Patel K, Chotai Np. Documentation and Records: Harmonized GMP Requirements. J Young Pharm. 2011;3(2):138-50
76. Shah NN, Fry TJ. Mechanisms of resistance to CAR T-cell therapy. Nat Rev Clin Oncol. 2019; 16(6): 372-385.
77. C. for B. E. and Research, "Considerations for the Development of Chimeric Antigen Receptor (CAR) T-cell Products," U.S. Food and Drug Administration. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/considerations-development-chimeric-antigen-receptor-CAR T-cell-products (accessed Apr. 26, 2023).
78. Lu J, Xu L, Wei W, et al. Advanced therapy medicinal products in China: Regulation and development. MedComm(2020). 2013; 4(3):e251.
79. Locke FL , Davila ML. Regulatory challenges and considerations for the clinical application of CAR T-cell anti-cancer therapy. Expert Opin Biol Ther. 2017; 17(6): 659–661.
80. A unique hub-and-spoke model to optimize patient management in lymphoma using novel CAR-T-cell therapy in Southeast and South Asia - Wei Inng Lim - 2022 - Hematological Oncology - Wiley Online Library. https://onlinelibrary.wiley.com/doi/10.1002/hon.3065 (accessed Apr. 26, 2023).
81. Availability of CAR T-cell therapy: list of all countries. https://www.susupport.com/knowledge/cell-gene-therapy/which-countries-cell-therapy-available (accessed Apr. 26, 2023).

82. Wang X , L Popplewell L , Wagner JR, et al. Phase 1 studies of central memory–derived CD19 CAR T–cell therapy following autologous HSCT in patients with B-cell NHL Blood. 2016; 127(24): 2980–2990.
83. Mock U, Nickolay L, Philip B, et al. Automated manufacturing of chimeric antigen receptor T-cells for adoptive immunotherapy using CliniMACS Prodigy. Cytotherapy. 2016; 18(8):1002–1011.
84. Ghassemi S, Nunez-Cruz S, O'Connor RS, et al. Reducing Ex Vivo Culture Improves the Antileukemic Activity of Chimeric Antigen Receptor (CAR) T-cells. Cancer Immunol Res. 2018; 6(9): 1100–1109.
85. Zhang W, Jordan KR, Schulte B, et al. Characterization of clinical grade CD19 chimeric antigen receptor T-cells produced using automated CliniMACS prodigy system. Drug Des Devel Ther. 2018; 12: 3343–3356.
86. Hort S, Herbst L, Bäckel N, et al. Toward Rapid, Widely Available Autologous CAR T-cell Therapy - Artificial Intelligence and Automation Enabling the Smart Manufacturing Hospital. Front Med (Lausanne). 2022:9:913287.
87. Schork NJ. Artificial Intelligence and Personalized Medicine. Cancer Treat Res. 2019; 178: 265-283.
88. Frontiers | Toward Rapid, Widely Available Autologous CAR T-cell Therapy – Artificial Intelligence and Automation Enabling the Smart Manufacturing Hospital https://www.frontiersin.org/articles/10.3389/fmed.2022.913287/full#B6 (accessed Apr. 26, 2023).
89. Mehrian M, Lambrechts T, Marechal M, et al. Predicting in vitro human mesenchymal stromal cell expansion based on individual donor characteristics using machine learning. Cytotherapy. 2020; 22(2): 82–90.
90. Li S, Todor A, Luo R. Blood transcriptomics and metabolomics for personalized medicine. Comput Struct Biotechnol J. 2015; 14: 1–7.
91. Lock D , Monjezi R , Brandes C. Automated, scaled, transposon-based production of CAR T-cells. J Immunother Cancer. 2022; 10(9): e005189.
92. K. Aleksandrova et al. Functionality and Cell Senescence of CD4/ CD8-Selected CD20 CAR T-cells Manufactured Using the Automated CliniMACS Prodigy® Platform Transfus. Med. Hemotherapy Off. Organ Dtsch. Ges. Transfusionsmedizin Immunhamatologie 2019 46, no. 1, pp. 47–54.
93. Potential solutions for manufacture of CAR T-cells in cancer immunotherapy | Nature Communications. https://www.nature.com/articles/s41467-022-32866-0#ref-CR15 (accessed Apr. 26, 2023).
94. Levine BL, Miskin J, Wonnacott K, et al. Global Manufacturing of CAR T-cell Therapy. Mol Ther Methods Clin Dev. 2016; 4: 92-101.
95. Automated generation of gene-edited CAR T-cells at clinical scale: Molecular Therapy - Methods & Clinical Development. https://www.cell.com/molecular-therapy-family/methods/fulltext/S2329-0501(20)30256-4 (accessed Apr. 26, 2023).
96. Oluwole OO, Davila ML. At The Bedside: Clinical review of chimeric antigen receptor (CAR) T-cell therapy for B cell malignancies. *J Leukoc Biol.* 2016; 100(6):1265–1272.
97. BioInsights - The regulation of CAR T-cells. https://insights.bio/immuno-oncology-insights/journal/article/421/the-regulation-of-CAR T-cells (accessed Apr. 26, 2023).
98. Regulatory Challenges in Global CAR T-cell Therapy Development > Premier Research. https://premier-research.com/white-paper-regulatory-challenges-in-global-CAR T-cell-therapy-development/ (accessed Apr. 26, 2023).