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Review Article

CAR-T CELL THERAPY FOR CHRONIC LYMPHOCYTIC LEUKEMIA: A PROMISING IMMUNOTHERAPY APPROACH

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Abstract: *Chronic Lymphocytic Leukemia (CLL) is a prevalent hematological malignancy with limited curative options, necessitating innovative and effective therapeutic approaches. Chimeric Antigen Receptor (CAR) T cell therapy has emerged as a transformative treatment modality, leveraging genetically modified T cells for targeted antitumor activity. This study explores the advancements in CAR T cell therapy for CLL, emphasizing its clinical potential, key manufacturing processes, structural design, therapeutic mechanisms, safety considerations, and strategies for optimizing efficacy. A comprehensive analysis of current literature and clinical data was conducted to examine CAR T cell manufacturing processes, including T cell source selection, activation, genetic modification, and expansion. Detailed evaluation of CAR T cell structural components and generational developments provided insights into their design and functionality. Additionally, therapeutic mechanisms, antigen selection strategies, and toxicity management approaches were critically reviewed. Key findings highlight the iterative advancements across four generations of CAR T cells, enhancing their antitumor efficacy, targeting specificity, and safety profile. Optimal target antigen selection and refinement of structural components, such as the ectodomain, transmembrane domain, and endodomain, significantly contributed to improved functionality. While CAR T cell therapy demonstrated robust antitumor activity in CLL, associated toxicities remain a challenge. Ongoing efforts focus on mitigating adverse events and optimizing clinical outcomes. CAR T cell therapy represents a promising paradigm shift in CLL treatment, combining precision targeting with potent antitumor effects. Continued advancements in design, manufacturing, and safety strategies underscore its transformative potential to improve patient outcomes and revolutionize CLL therapeutics. Further clinical trials and research are warranted to fully realize its therapeutic promise.*

Keywords: Hronic Lymphocytic Leukemia Chimeric Antigen Receptor T-Cells Hematologic Neoplasms Immunotherapy Antigens, Neoplasm

Introduction

Chronic Lymphocytic Leukemia (CLL) is a form of malignancy that originate in bone marrow, causing the overproduction of lymphocytes. It is characterized by the development of cancerous cells originating from mature B cells that have encountered antigen in the past (1). These abnormal cells accumulate in the blood, and different component of the lymphatic system (2). CLL is the predominant type of leukemia observed in adult population, particularly prevalent in western nations. The estimated prevalence of CLL ranges from 2 to 4 cases per 100,000 individuals globally. This type of leukemia is more commonly diagnosed in older individual, with typical age range is 75 at the time of diagnosis (3). Managing CLL in a clinical setting is complex and involves considering various factors. These factors include the age of the patient, any other existing health condition they may have (comorbidities), and specific characteristics of the CLL cells. These features include whether there are mutations in the heavy chain of antibodies, the presence of 17p deletion and the mutation in TP53 gene (4). The growth factors which involve are tumor necrosis factors α and interleukin 10. In case of CLL progression, certain other genetic changes have been observed such as overexpression of c-myc oncogene, which is a gene that has the potential to promote uncontrolled cell growth. Additionally, deletions of RB1 gene, which normally act as tumor suppressor by regulating cell division, have been found (5). These factors play an important role in determining the most appropriate treatment approach for each patient of CLL.The chosen treatment strategies may differ based on the specific stage of the disease. In the early phases of Chronic Lymphocytic Leukemia, when symptoms are minimal or absent, one of the primary treatment choices is the Watch and Wait method also known as Active Surveillance. Chemotherapy which involves the use of drugs for CLL include fludarabine, cyclophosphamide, and bendamustine, Targeted Therapy specially target certain molecules or pathways involved in CLL. Examples include inhibitor of Bruton Tyrosine kinase and inhibitor of posphatidylinositol 3 kinase, Immunotherapy which utilize the innate power of immune system of the body to combat cancerous cells,

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Hematopoietic Stem Cells Transplantation (HSCT), the procedure in which healthy stem cells are injected into the body of patient who has defective bone marrow (6). Despite the excellent results observed from chemotherapy and targeted therapy, however, patients do relapses during treatment. Complications of HSCT involves the infection with encapsulated bacteria and Graft vs Host Disease. This condition occur when the T Cells originating from the donor, perceive the patient`s organ or tissues as foreign and attack them (7). In recent years, a treatment called cell-based immunotherapy has gain importance in the fight against cancer. This approach comprises of extracting the patient's cells, modify them in the lab and after that put them again in the cancerous patient. This procedure aids in boosting their ability to identify and kill the cancer cells. There are different types of cell-based immunotherapy. One approach comprises of using T lymphocytes present within the tumors of individual, diagnosed with cancer. We extract these cells from the cancerous patients and then grow in labs and when they are expended, they put back into the patients where they can fight against cancer cells more efficiently. Another popular approach called CAR-T cell therapy belongs to the category of immunotherapy that involves the use of modified cells for treatment. The procedure comprises of to isolate the T cells from the patients having tumor and genetically engineered to have special receptors called CARs. These receptors have the ability to recognize and attach to the special proteins found on the tumor cells. Following the genetic modification, the altered cells are reintroduced into the patient`s bloodstream. These cells circulate in the body and when they find the cancer cells, they attach on that specific protein with specific receptors CARs and kill the cancer cells (8). CAR T cells therapy has shown more superior outcome and provide long lasting results as compare to other cell-based immunotherapy (TIL, TCR modified T cells) (9). CARs are actually protein that are designed in the laboratory. These proteins consist of fragment of an antibody called single chain variable fragment and domain that activate T cells. The function of that antibody in the body is to identify and neutralize the foreign substance. And the domain is another crucial component of CAR which perform the function of triggering the T cell. When our engineered cells bind with the malignant cells in the body the single chain variable fragment identify the tumor cells and T cell activation domain send signals to the T cells to activate them and trigger their immune response against the cancer cells (10).

CAR-T Cell Therapy: An Exciting Breakthrough in Cellular Immunotherapy

In leukemia, particularly in CLL, tumor cell expresses various proteins on their surface, these proteins include kappa light chain, CD5, CD19, CD20 and CD23 (11). CD19 is considered as more prominent and successful target antigen because of limited expression on normal cells and more expression on the cancerous cells (12). The patient enrolled in phase 1 clinical trial in July 2010, where he received treatment using T cell modified with CARs. In the days leading up to the infusion of CAR-T cells, the patient underwent chemotherapy which deplete the number of other immune cells specially lymphocytes. This helps the CAR T-Cells better opportunity to fight with cancer cells. After four days of chemotherapy the patient was infused by 1.46×10^5 cells/kg of the weight of the body. As a result of the treatment, patient attained a state of complete remission (11). Recent reports have indicated that the patient maintain his remission status for over ten years following the therapy with engineered cells persisting in his body which are identified as CD4+ cells. These CD4+ CAR-T cells exhibited cytotoxic property (13).

More than 100 patients diagnosed with CLL have been treated utilizing CAR T-Cell Therapy. Studies have documented that individuals who didn't undergo lymphodepletion therapy before infusion of engeneered cells exhibited lowest overall response rate (ORR). It is believed that this procedure not only reduce the size of the tumor but it also decrease the presence of suppressor cells which could potentially weakens the effect of the administered altered cells in fighting against cancer (14).

Clinical Manufacturing of CAR T-Cells

Promising strategies for cancer immunotherapy involve the use of adoptive cell therapy, which can utilize two different approaches. The first approach involves using preexisting tumor-infiltrating lymphocytes. The second approach involves genetically modifying T cells which causes the expression of T lymphocytes (15) which we indicate as Chimeric Antigen Receptor (16). Both of these approaches have shown great potential in cancer treatment. The promising outcomes observed in initial stages of clinical study using CD19-targeted CAR-T cells to treat hematologic malignancies, have generated a significant enthusiasm and interest in the field of CAR-T cell-based therapies (11, 17-21). Despite possible variations in CAR cell designs, cell production follows a standardized approach. It involves several key steps, starting with the collection and processing of T cells from a suitable source. Afterward, the CAR-T cells are prepared through T-cell selection then activation, followed by genetic modification using a CAR cDNA. The modified cells are then subjected to large-scale expansion to increase their numbers. Finally, the manufacturing process concludes with the formulation of the CAR-T cells, ensuring their quality and readiness for use.

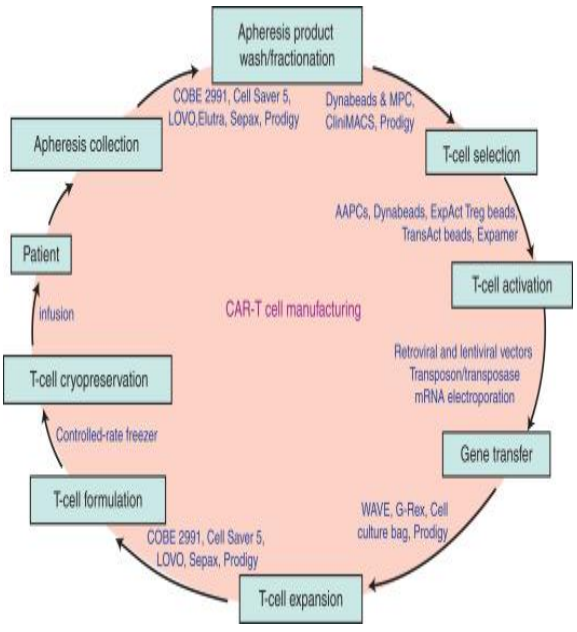

Figure 1

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Fig. 1: The CAR-T cell manufacturing process involves several significant stages, each requiring specific technologies and devices. These steps include T-cell collection from the patient, genetic modification of T cells to express the CAR receptor, cell expansion and proliferation, formulation and quality control, and final preparation for patient infusion.

T Cell Source

CAR Tcell therapy, primarily an autologous cell-based therapy, initiates with the retrieval of peripheral blood mononuclear cells (PBMCs) from the patient. This collection is typically performed through a leukapheresis process. Physicians carefully select the optimal timing for collection, considering the treatment plan, to ensure an adequate number of T lymphocytes. The collected apheresis products undergo diverse processing techniques based on subsequent procedures in the manufacturing process (22, 23)

T Lymphocyte Activation

For successful in-vivo production of T cells, it is essential to ensure continuous and appropriate activation. T-cell activation relies on receiving two key signals. The primary signal, known as Signal 1, is triggered by the T-cell receptor and is specific to the target antigen. Signal 2 for T-cell activation must also be provided by costimulatory signals such CD28, 4-1BB, or OX40 in addition to Signal 1. The delivery of the CAR cDNA into T cells via retroviral vectors requires this simultaneous activation (24, 25).

Genetic Modification

Assuring persistent CAR activation following the insertion of genetic material via viral or nonviral gene transfer techniques is crucial for the success of existing CAR-T cell treatments. For clinical applications, three primary types of gene expression vectors are commonly utilized: σ-retroviral vectors, lentiviral vectors, and the transposon/transposase system. Another approach to introduce CARs into cells, while circumventing long-term expression, involves messenger RNA transfer-mediated gene expression. This approach provides a means to achieve transient CAR expression without maintaining sustained genetic modifications (26, 27).

Expansion of CAR T cells

Expansion is carried out by wave bioreactor. The WAVE system incorporates perfusion capabilities, allowing for automatic intake and wastage evacuation. With this system, cells may readily proliferate, reaching concentrations exceeding 107 cells/mL. Remarkably, a single bioreactor of this platform has the capacity to support cell cultures of up to 25 liters (25, 28).

Structure of CAR-T Cells

CAR-T cells consist of three domains (Fig 2).

Fig. 2 The Chimeric Antigen Receptor (CAR) consist of three distinct sections: the outermost part known as ectodomain, followed by the transmembrane domain situated in the middle, and finally the innermost section called the endodomain

Ectodomain

It is located on outside of the cells, facing the extracellular space. It is composed of the three parts: the signal peptide, the antigen recognition region, and the spacer.

The signal peptide plays a crucial role in guiding the protein during its synthesis to the endoplasmic reticulum, a cellular compartment involved in protein processing. In case of a CAR, the signal peptide is made up of a structure called a single-chain variable fragments. Antigen recognition region is typically another scFv, which is designed to specifically recognize and bind to a particular target, such as a tumor antigen. This region is engineered to have a high affinity for its target, allowing the CAR to effectively recognize and interact with the desired molecules.

To connect the antigen recognition domain to the transmembrane domain, a spacer is used. (29).

Tran's membrane Domain

Transmembrane domain is a part of the membrane protein positioned in immediate proximity to the membrane of the cell. Comprises of helical structure with hydrophobic properties, this element traverses the membrane, providing stability to the protein. In case of CARs, the choice of transmembrane domain influences the stability and function of the receptor.

The native CD3-zeta transmembrane domain has been found to interact with the cell`s natural T-cell receptor (TCR), leading to the integration of the synthetic TCR of the CAR into the existing TCR complex. This can potentially interfere with the signaling and function of the CAR.

To overcome this issue, alternative transmembrane domains have been explored. Currently, the CD28 transmembrane domain is considered one of the most promising options for CAR procedure. It provides better stability and reduces the chances of unwanted interaction with the native TCR (29, 30).

Endodomain

This domain serves as the operational component of the receptor and typically contain CD3 zeta, which consist of three immunoreceptor-tyrosine-based activation motifs (ITAMs). Upon antigen recognition, it clusters together and activates signaling, that subsequently propagate to the T cell. Co-stimulatory signaling is required for this process to occur (29, 30).

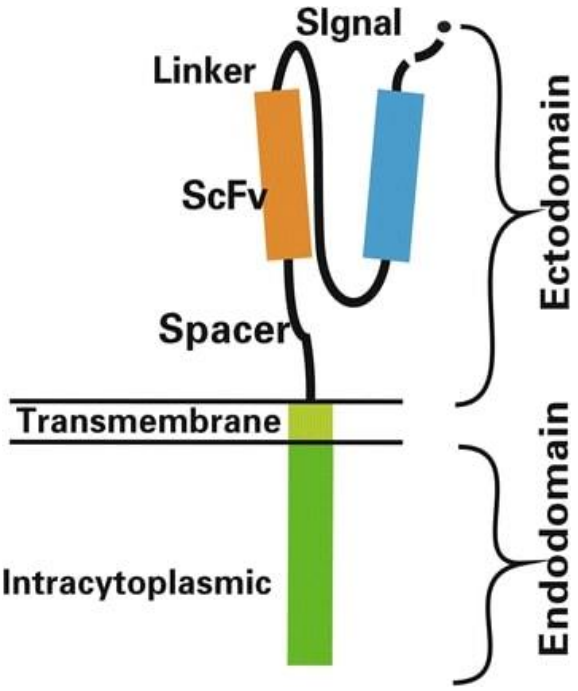

Figure 2

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Evolution of CAR T cells

The classification of the CAR cells is based on the construction of their inner domain or endodomain which allows them to organize into the four distinct generations (Fig 3).

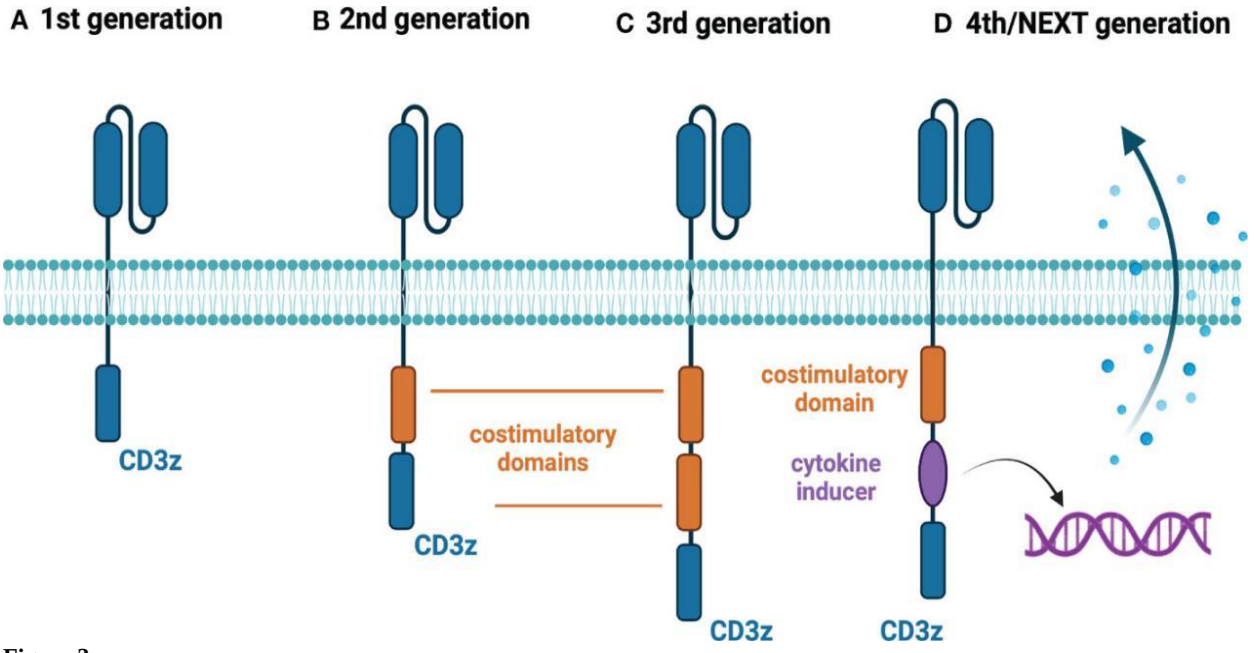

Figure 3

Fig. 3: The CAR has undergone significant advancements across different generations. In the first generations, a single chain antibody was used to connect the ITAMs located in the transmembrane region, enabling signal transduction. Moving to the second generation, an additional costimulatory molecule was introduced into the signal transduction region. This enhanced the activation signals and improve the performance of CAR therapy. The third generation further refined the CAR design by incorporating another costimulatory molecule, such as CD137 or CD134, along with existing CM1. This combination of costimulatory molecule provided enhance and sustained activation signals, leading to the improved CAR functionality.

First Generation of CARs

The first generation of CAR cells is characterized by their incorporation of a singular structure originating from either the CD3 zeta chain in the inner domain. This structure serves as the primary source of signal transmission from intrinsic TCR. Nonetheless, these CAR cells exhibit limited production of IL2, a critical factor for efficient tumor eradication. Consequently, the supplementation of exogenous IL-2 becomes imperative to bolster the capacity of these CAR cells in targeting and eradicating tumor cells (29, 31).

Second Generation of CARs

So, the CAR cells that contain only CD3 zeta chain in their inner domain is not enough for the activation of other cells without co stimulatory domain. Consequently, this issue is addressed by introducing the second generation. In this advancement the intracellular signaling domain (CD137 or CD28) is introduced into the cytoplasmic tail of CARs which derived from various co-stimulatory protein receptors. This results in increase of sustained response, cytotoxicity, multiplication, and prolongation of CAR cell survival in body (32, 33).

Third Generation of CARs To enhance the potency of CAR cells, the third generation of CAR cells were

Introduced by merging multiple signaling domains, such as CD3zeta-OX40-CD28. This strategic combination aims to bolster the effectiveness of CAR cells by amplifying their cytokines production and killing capacity (34).

Fourth Generation of CARs

The fourth generation CAR cells, known as T cell redirected for universal cytokine mediated killing, were created by integrating IL-12 into the core structure of second-generation constructs. These innovative TRUCKs are designed to boost T cell activation and trigger the mobilization of innate immune cells. This collaborative immune response is aimed at eliminating cancer cells that do not express the targeted antigen within the intended region. By harnessing the power of TRUCKs, a wider array of antigen-negative cancer cells can be effectively targeted and eradicated (35).

How CAR T-Cell Therapy Works

The process of CAR-T cell therapy begins by extracting T lymphocytes of patients from their bloodstream. The laboratory receives these cells next, where a special receptor known as a chimeric antigen receptor (CAR) is introduced into them through genetic engineering techniques. This engineered CAR protein is designed to be expressed on the surface of T lymphocytes, which results in the production of CAR T cells. In the laboratory, numbers of these cells are cultured and allowed to multiply, ensuring a substantial population of these modified cells. Once a sufficient number of CAR T cells have been produced, they are administered back to the patient through an intravenous infusion. After entering the patient's bloodstream, the CAR cells navigate

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through the whole blood and selectively attach to antigens, on the outer surface of cancer cells. After binding, these engeneered cells activate and initiate a targeted attack on the cancer cells, ultimately leading to their destruction. This process of CAR-T cell therapy combines the power of genetic modification and the body's natural immune response to combat cancer in a personalized and targeted manner.

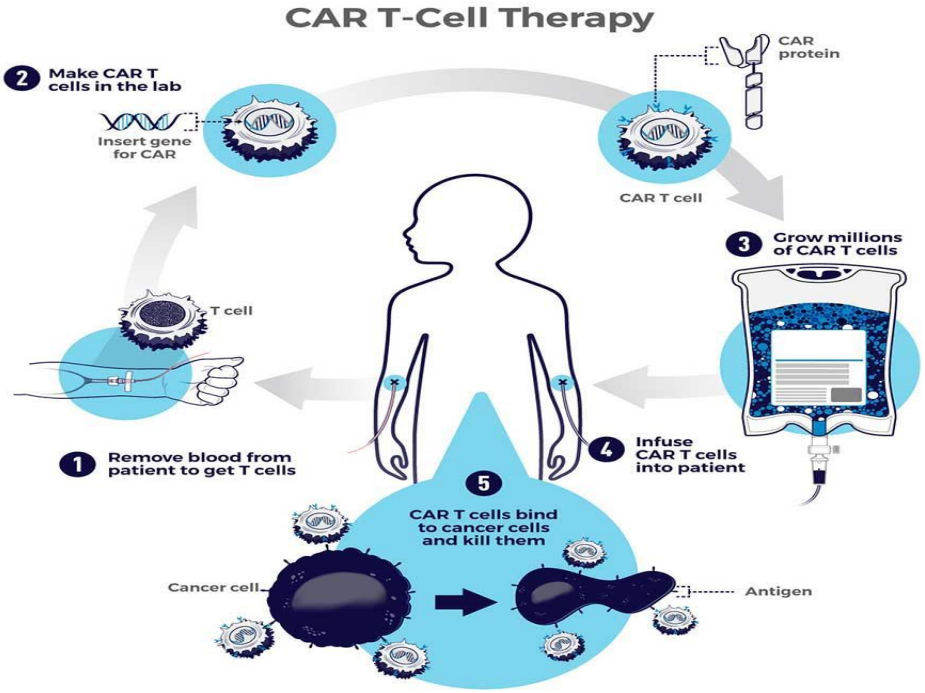

Figure 4

Fig. 4: In process the T cells of a patient are genetically modified in a lab so they will adhere to and destroy particular antigens on cancer cells.

Target Antigen for CAR-T Cell Therapy in treating CLL

CAR molecules are designed to identify the surface antigens present on the cancerous cells. These antigens can be protein, carbohydrate, or glycolipids. After binding to that specific antigen, our engeneered cells directly engage and eradicate the cancerous cells. For CAR cells to efficiently eradicate the tumors, they require a target that is abundantly present on the outer surface of the cancerous cells. Currently, target antigens with high coverage are CD19, CD20, κ or λ light chain etc (36).

CD19

CD19 is highly utilized antigenic target in treating cancer by CAR T cell therapy and it shows remarkable effectiveness and safety in treating disease characterized by abnormal growth in B cells. This target is present throughout all phases of B-cells maturation until their transformation into plasma cells (36).

CD20

CD20 CAR T-cell therapy has achieved substantial recognition in the field of cancer therapy. This therapy focuses on targeting CD20, which is a marker found on the surface of the majority of cancerous B cell in CLL. In contrast to CD19, this antigenic target is absent in the initial phases of B cell development and in fully differentiated plasma cells (37). In a recent clinical trial, three patients with CLL were enrolled to determine the effectivity of a novel treatment approach using anti-CD20 CAR T cells. The results showed the promising outcomes, with two CLL patients achieved a state of disease-free status, demonstrating a complete absence of the cancer while the remaining one exhibited significant reduction in CLL symptoms. These outcomes shows that this treatment has the potential to be a valuable strategy in preventing the emergence of CD19-negative relapses (38).

Kappa Immunoglobulin Light Chain

Researchers have explored alternative targets for CAR-T cell therapy as targeting of pan B cell markers CD19 and CD20 often results in prolonged damage to humoral immunity, causing B cell aplasia after successful treatment and hypogammaglobulinemia (39). Fully developed B lymphocytes produce monoclonal antibodies having κ- or λ-light chains, but not both of them at the same time. In patient with non-Hodgkin lymphoma and CLL, malignant B cell also maintain this clonal restriction in light chain expression. Therefore, CAR cells can be designed to specifically target the light chain manifested by the cancerous cells, while avoiding any impact on the normal B cells that shows opposite light chain. This method aid in reducing the detrimental effects on the patient`s humoral immune response, thus mitigating any potential harm to their immune system (40).

Antigens in Preclinical Development

We are examining a method to specifically target and eliminate cancerous B cells while preserving the healthy, mature B cell population. An approach involves focusing on a receptor called receptor tyrosine kinase-like orphan receptor 1, which has shown high expression in B-CLL. In laboratory setting CD8+ T cells that have been genetically modified to possess a chimeric antigen receptor targeting

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ROR1 demonstrate efficient eradication of primary B-cell CLL. Importantly, this targeted CAR-T cell therapy specifically targets cancerous cells without harming normal, mature B cells. These findings suggest that utilizing ROR1-specific T-cell therapy could serve as a promising and effective treatment option for patients diagnosed with ROR1-positve B-cell tumors (41).

CD37 also have investigated as potential target for CAR-T cell therapy in CLL. CD37 is a protein found on the surface of mature B cells in lymphoid tissues, and its expression remains even after the transformation into cancerous cells. In laboratory experiments, CAR-T cells engineered to target CD37, demonstrating cytotoxic activity against various B-cell malignancies after activation, in animal models. Furthermore, bispecific CAR cells, also been produced that target not only CD37 but also CD19 (42).

Toxicities induced by CAR-T cell therapy in patient with CLL

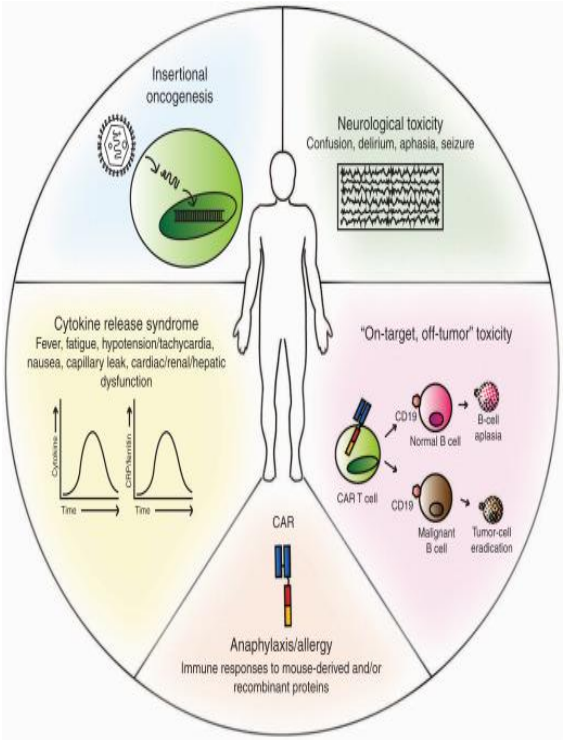

Figure 5

Fig. 5: CAR T-cell therapy linked with various reported and potential toxicities. These includes the theoretical risks of insertional oncogenesis, where the genetic modification of T cells could potentially lead to the development of cancer. Neurologically toxicity is another observed toxicity, characterized by adverse effects on the nervous system. Another phenomenon known as on target off tumor toxicity occur when CAR cells engage with the target antigen not only on cancer cells but also on healthy tissues. Anaphylaxis or allergic reactions can occur when the host`s immune system reacts negatively to the foreign antigen expressed by the CAR T cells, resulting in severe allergic response. CRS which is a systemic inflammatory response triggered by the activation of CAR T cells, leading to the release of a large quantity of cytokines.

Cytokine Release Syndrome

Cytokine release syndrome is a well-known manifestation commonly linked with CAR-T cell therapy, which is the result of the biological underlying mechanism driving this treatment approach

If we discuss anti-CD19 CAR T cell therapy then, upon recognition of CD19+ target cells, the stimulation and multiplication of anti-CD19 CAR cells trigger the initiation of cytokine release syndrome in the case of anti-CD19 CAR T cell therapy. This process entails the generation of inflammatory cytokines, including but not limited to Interleukin-6, IFN-gamma, Interleukin 10 among others which leads to the symptoms like fever, seizures and organ failure (43). Fever is the most initial symptoms observed in CRS. The timing of fever onset can vary widely, ranging from few to week after the infusion. During CRS, body temperature can exceed 104°F together with fatigue, chills, and joint and muscle pain also loss of appetite. In severe cases, CRS can escalate to life threatening conditions, including fluid leakage from capillaries, low BP, increased heart rate, decrease oxygen level, along with significant increasing in cytokine level in blood stream (44). CRS has frequently been observed in clinical trial focusing on CLL. Furthermore, patients experiencing CRS displayed higher peak level of CAR T cells in the blood stream following infusion, along with elevated peak concentration of multiple cytokines (45). Encouragingly, preliminary evidence suggests that concurrent administration of CAR-T cell therapy and ibrutinib might reduce the incidence of severe CRS, likely due to reduced levels of cytokines in patients receiving this combined treatment (46).

Neurological Side Effects

While cytokine release syndrome is a predicted side effect of this immunotherapy, unforeseen neurological complications, including cerebral edema, have been noticed in clinical study involving patients with CLL. In certain cases, these neurological complications were severe in nature. Patients treated with CD19-specific CAR T cells have experienced the emergence of neurological toxicities, such as delirium, confusion, aphasia, and seizure (19, 21). The underlying pathology responsible for these neurological toxicities remain unclear. However, based on similar occurrence observed with blinatumomad, (47, 48) it is believable to suggest that elevated levels of cytokines might contribute to the development of these neurological consequence. Alternatively, while direct toxicity of CAR cells on the CNS is theoretically possible, no evidence has been presented to support this notion.

On target/off tumor recognition

The desired target antigen is specifically limited to the tumor cells and serves as a crucial survival signal for the malignant clone. Unfortunately, many targets recognized by CAR cells are also expressed on the normal tissues to some extent, leading to toxicity. This condition manifest due to the interaction of target antigen with non-harmful tissue (49). These reported events vary in severity, ranging from controllable lineage depletion like B cell aplasia to more toxic, even resulting in loss of life.

Anaphylaxis

In most clinical trials, the majority of genetically modified T cells used contain antigen recognition domains derived from mouse monoclonal antibodies (49). As a result, it is not surprising that both cellular and humoral rejection of CAR cells have been observed due to the immunogenicity

of foreign proteins (50, 51). Ongoing efforts are being made to make the expressed proteins more human-like in order to improve the durability and strongly enhance the effectiveness of CAR cells (52). However, an immediate risk involves the recipient`s immune system recognition the injected foreign components, leading to acute anaphylaxis as observed in that patients who got the treatment in form of mesothelin specific CAR cells (53).

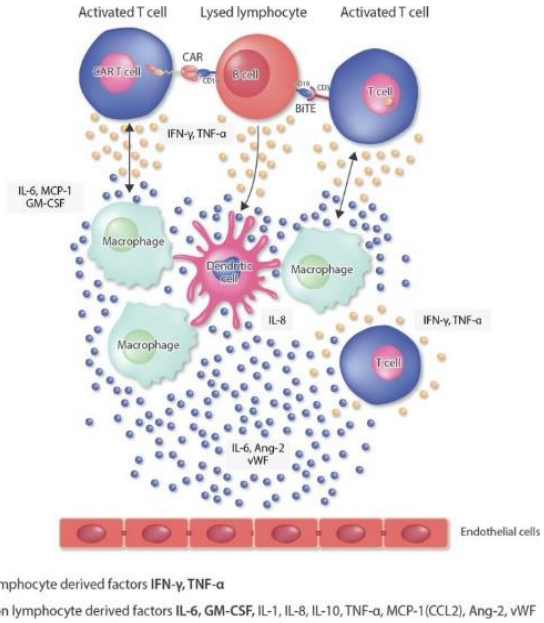

Figure 6

CAR T-Cell Therapy Beyond Cancer

CAR T-cell therapy has predominantly been focused on treating cancer, particularly lymphoma, leukemia, and neuroblastoma and has shown great potential on clinical trial and is on the path to receiving FDA approval. While these advancements are remarkable for cancer treatment, the versatility of CAR-T platforms can extend beyond malignancies. CAR cells have the potential to target antigens beyond proteins, including carbohydrates and lipids, opening up possibilities for treating diverse diseases. For instance, a recent study explored the application of CAR-T therapy in multiple sclerosis. Researchers developed a CAR that targets myelin oligodendrocyte glycoprotein (MOG), a key component in central nervous system myelination. By delivering regulatory T cells modified with a lentiviral vector and the FoxP3 gene, which regulates immune responses and promotes regulatory T cell differentiation, they observed suppressive effects in vitro and a reduction in symptoms in a murine model of the disease (54).

Inflammatory intestinal conditions like irritable bowel syndrome (IBS) have also been considered for CAR-T therapy. A study introduced a chimeric receptor into human T regulatory cells using a retroviral vector, targeting carcinoembryonic antigen, which is engaged in intestinal cell attachment and is elevated in IBS cases (55). The study showed promising results, indicating potential treatment options. Similarly, autoimmune diseases like Pemphigus vulgaris have utilized CAR technology by creating chimeric autoantibody receptor (CAR) T cells. These CAR T cells specifically targeted autoantigen Dsg3, resulting in the selective elimination of autoimmune B cells without widespread immune suppression (56).

CAR-T therapy advancements are also being explored for human immunodeficiency virus type 1 (HIV-1) treatment. A recent study tested different CARs based on broadly neutralizing antibodies, reconstructing them as single chains for improved binding. In vitro experiments demonstrated enhanced killing of infected cells and antiviral activity, paving the way for further in vivo assessments (57).

Advanced Features and Innovations

CAR T cell therapy has made significant strides in recent years, particularly in its ability to target various types of cancer cells. Despite the diverse origins and characteristics of cancer cells, they share certain antigens as target. This remarkable feature enables these engeneered cells to identify and attack cancerous cells, regardless of their specific lineage. New advancements in this field focus on identifying more accurate target antigens that are specifically expressed by cancerous cells. This approach aims to enhance the precision and effectiveness of CAR T cell therapy by directing the immune response towards cancer cells with greater accuracy (58, 59).

Bispecific CARs

Bispecific receptors are a special kind of receptor that pair two different intracellular signaling domains with two different antigen recognition domains. A notable example is the bispecific CAR CD19/CD20 designed to recognize and attach to multiple tumor antigens present on cancer cells (60, 61).

Tandem CARs

In certain situations, conventional CARs may fall short of meeting heightened expectations, particularly when targeted antigens undergo downregulation or alterations within cancer cells. This phenomenon, known as antigenic loss or escape variations, poses a challenge for CAR therapy (62). To overcome this limitation, researchers are exploring advanced technologies that involve linking two specific antigen recognition sites and placing them in tandem within a single intracellular domain. This unified CAR, called a Tan-CAR, can be expressed on the cell surface. The Tan-CAR design causes both antigens to be targeted present on a single cancer cell or within the tumor microenvironment (63). Moreover, these cells exhibit improved efficiency, also reduced toxicity, particularly in settings with a higher disease burden (64).

Inhibitory CARs

Recently, there have been significant findings related to novel immunoinhibitory receptors that play a crucial role in regulating the activation of T lymphocytes and the regulation of T cell responses. These receptors, including programmed death-1, programmed death-ligand 1, and cytotoxic T-lymphocyte-associated antigen 4, have emerged as breakthroughs in cancer immunotherapy (65). Scientist discovered that by inhibiting immune cells and using antibodies against CTLA-4, it was possible to eliminate malignant growth and prevent the formation of new tumors (66). Clinical trial happened on fourteen patients who are suffering from metastatic melanoma, and although three patients experienced relapse, the results showed promising outcomes. In 2011, the FDA approved the use of ipilimumab (antibody against CTLA4) for the treatment of melanoma (67).

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Physiological CARs

Initially, many CAR designs utilized murine-derived scFv, which posed a probability of triggering an immunological response against the engeneered cells. This immune response could lead to anaphylaxis during CAR T cell transfer, thereby limiting the durability of the injected cells. To address these limitations, scientists have developed an alternative CAR design called physiological CAR or receptor-ligand CAR. This type of CAR can embrace and attach to specific cancerous antigens, such as HER3 and HER4 (68). This engineered CAR can be introduced into blood, enhancing their ability to identify antigen expressed on cancerous cells (69).

Universal CARs

To address the limited identification sensitivity of CAR T cells, an innovative solution called uCAR (universal CAR) has been developed. Unlike traditional CARs that are specific to certain tumor-associated antigens, uCARs utilize a targeting region joined with a transmembrane domain and one or two endodomains (70). By incorporating uCAR into T cells, they gain the ability to efficiently recognize and eliminate cancer cells when stimulated by FITC-labeled or biotinylated antigen-specific monoclonal antibodies (mAbs). This recognition triggers T lymphocytes activation, multiplication, and production of cytokines, leading to effective tumor eradication (71).

Conclusion

In conclusion, CAR T cell therapy has ushered in a remarkable era in the treatment of Chronic Lymphocytic Leukemia (CLL). This innovative approach, with its intricate manufacturing process and well-defined structure, has demonstrated extraordinary potential in targeting and eliminating CLL cells with remarkable precision. Through the evolution of four generations of CAR cells, continuous refinements have been made to enhance therapy efficacy and safety. As researchers delve deeper into the mechanisms of action and target antigen selection, CAR T cell therapy has proven to be a formidable force against this challenging hematological malignancy. Despite the challenges posed by potential toxicities, the field of CAR T cell therapy for CLL continues to forge ahead with ongoing research and clinical trials. Collaborative efforts among scientists, clinicians, and pharmaceutical companies are rapidly propelling the therapy's development and paving the way for broader accessibility and increased patient benefit. With each milestone achieved, CAR T cell therapy moves closer to becoming a mainstream treatment option, bringing new hope to CLL patients who have exhausted traditional therapies. As the story of CAR T cell therapy for CLL unfolds, the promise of improved outcomes and the potential for a cure shine brightly on the horizon, illuminating a future where this groundbreaking therapy may change the lives of countless patients and their families.

Declarations

Data Availability statement

All data generated or analyzed during the study are included in the manuscript.

Ethics approval and consent to participate

Approved by the department Concerned.

Consent for publication

Approved

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The authors declared absence of conflict of interest.

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Conception of Study, Development of Research Methodology Design, Study Design, Review of manuscript, final approval of manuscript.
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