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CAR-T Cell Therapy: An Advancement in Conventional Cancer Treatment Methods

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ABSTRACT

Neoplastic tissues, often known as cancerous malignant tumors, are essentially just collections of aberrant cells that can invade nearby tissues, travel to lymph nodes, and even reach other organs. CAR-T cells, or genetically engineered T cells, express the CAR protein on their surface. A recognition region on the exterior and a signaling region on the inside make up the CAR protein. The antigen plays a crucial role in the development of CAR-T cells. Targeting tumor-specific or tumor-associated antigens that are either exclusively present on tumor cells or increased on the surface of cancer cells is the usual goal of CAR-T cell therapy. The FDA and EMA have approved the commercial sale of CAR T cell treatment out of all the different types of ACTs. Six different drugs have been approved in the US and Europe to treat seven different types of B cell cancer. Patients must be screened by their physicians before considering CAR-T cell therapy. Patients may need to go through therapeutic preparatory procedures including lymphodepletion and bridging therapy before receiving CAR-T cell treatment. CAR T cell therapy's remarkable outcomes have altered dire prognoses and prevented several fatalities from severe cancers. However, an increasing amount of clinical experience is exposing their limitations. Currently, CAR-T cell therapy is mostly used to treat certain blood cancer types. Nevertheless, fresh research is being planned to determine how to improve the effectiveness, lower the cost, and expand the potential applications of therapies. Research is now being done to increase the safety and efficacy of CAR-T cell treatment.

Keywords: Chimeric antigen receptor, Major Histocompatibility Complex, Tumor-associated antigen, Cytokine storm syndrome, Cluster of differentiation.

INTRODUCTION

Neoplastic tissues, often known as cancerous malignant tumors, are essentially just collections of aberrant cells that can invade nearby tissues, travel to lymph nodes, and even reach other organs [1]. Additionally, they may grow excessively quickly. A disease may be distinguished from many other types of illnesses by its origin in an organ or tissue as well as the kind and shape of its cells [2]. It now ranks as the leading cause of disability-adjusted life years and the leading cause of death worldwide. In the upcoming decades, it is also anticipated to overtake ischemic heart disease as the primary cause of death [3]. As they multiply out of control, many cancer cells in tumors develop a variety of mutations that give the tumor cells the ability to grow more quickly, avoid cell death, and elude immune monitoring, among other traits [4,5]. Major Histocompatibility Complex (MHC) molecule expression is downregulated in

cancer cells, which frequently results in aberrant antigen processing and loading. These changes result in a reduced immune response because neoantigens (or neoantigens) are less apparent to the immune system [5,6]. Increased mutagenesis is another effect of cancer cells' innate genomic instability, and this leads to the development of several subclonal heterogeneous lineages with varying capacities, including invasion, drug resistance, enhanced proliferation, and neoantigen/ neoantigen presentation [4,7,8]. Many strategies for fighting malignant cancers have emerged in recent years. There is no question that while the death rate from cancer has decreased, the success rate of cancer therapies such as radiation therapy, chemotherapy, and surgery has grown. The most effective treatment for the majority of cancers, including blood tumors, is chemotherapy. In addition to their consistent lack of efficacy and case-to-case variability, these so-called standard medications are linked to significant adverse effects, including collateral harm to healthy tissues and, occasionally, protracted recovery periods [9, 10]. As a result, efforts have been undertaken to identify and implement novel therapeutic strategies that will lower systemic toxicity, increase overall survival, and lower the incidence of cancer. One of these techniques was immunotherapy, which modifies the host's immune response to cancer by using antibodies, cytokines, and immune cells [9,11,12].

ACTs, which expand on the advantages of immunological and targeted therapy, have been increasingly popular in the cancer area in recent years [13]. These techniques use lymphocytes—typically autologous T cells—to combat illnesses. The cells are grown and reinjected into the patient after being selected or modified to be more specific to a given antigen. Here, they assist the immune system in establishing long-term protection against the antigen while also killing the target cells [14]. Autologous T cells are extracted from the patient's peripheral blood, altered to more precisely target cancer cells, and then reintroduced into the body to help remove tumors as part of chimeric antigen receptor (CAR) T cell therapy. T cells are genetically modified to create a receptor known as CAR to do this. This receptor has been specially designed to identify a particular antigen found in the cancer cells of the patient. CAR T cells can multiply and provide cytotoxicity after they are identified [15,16]. Despite the relatively well-defined basic structure of the CAR, the design of a chimeric receptor is highly customizable since it is made up of many protein segments fused to form a single fusion protein, commonly known as a "chimera" [17, 18]. Among other things, the CAR chooses a suitable and effective recognition domain to customize the T cells to target a particular antigen, such as a tumor-associated antigen (TAA). These restrictions do not limit CAR T cells and may identify any expressed surface antigen, whether or not it is MHC-presented, in contrast to conventional T cells, which depend on MHC expression for antigen presentation [14]. A larger pool of targets, including more "invisible" cells that suppress antigen presentation, may be found, increasing the overall killing efficacy.

The CAR-T Cell Structure: -

CAR-T cells, or genetically engineered T cells, express the CAR protein on their surface [19]. A recognition region on the exterior and a signaling region on the inside make up the CAR protein [20]. A single-chain antibody (scFv) or antigen-binding domain is a typical element of the external recognition region. Its function is to identify and bind to specific antigens present on the surface of the afflicted cancer cells [21]. It may be possible to use genetic engineering to successfully attach the target antigen to this recognition area [22]. The signaling molecules and modules that activate T lymphocytes are frequently found in the internal signaling area [20]. The internal signaling region of the CAR stimulates CAR-T cells, particularly for their target antigen, which leads to their proliferation when they attach to it [23]. The antigen-recognition

domain [24], sometimes referred to as the exterior recognition area, is one characteristic that sets CAR-T cells apart. A scFv that binds and identifies the target antigen is often found in this domain [25]. An antigen-binding region and structural domains linked to CD3ζ or other signaling domains including CD28, 4-1BB, CD19, and OX40 make up the scFv, according to [26]. Combining changeable heavy and light chain segments with good specificity and affinity results in a single-chain antibody [27]. By attaching itself to the antigen and presenting it to T cells, the scFv initiates its antitumor response [28]. CAR-T cells' ability to recognize and eradicate certain antigens is ensured by the careful selection of scFv [28]. The internal signaling area or activation domain of CAR-T cells [29] is another name for the transduction region, which is found inside the cell and aids in the transfer of antigen recognition signals from the outside to the inside. T cells are then activated and an immunological response is initiated. The CD3ζ domain is the most often used transduction domain and is involved in the signaling pathway that activates T-cells [30]. Co-stimulatory domains are employed to enhance the impact and proliferation of CAR-T cell activation [31]. Co-stimulatory molecules such as OX40, 4-1BB (CD137), and CD28 are frequently used to enhance the antitumor response, activity, and survival of CAR-T cells [32, 33]. Furthermore, the CAR gene may be introduced into T cells via viral vectors such as lentiviruses and retroviruses, while non-viral techniques like transfection are also feasible [34]. Most first-generation CARs typically feature both a CD3ζ transduction domain and an antigen recognition domain [35]. For low-level and heterogeneously-expressed antigens, this basic structure has little influence and poor therapeutic efficacy [37], and it merely offers signals for T-cell activation and early antigen recognition [36]. Researchers have developed second and third-generation CARs to improve therapeutic efficacy and extend CAR-T cell activation and durability [38, 39]. One or more co-stimulatory factor domains, such as CD28 or 4-1BB, are added to the first generation of CARs to produce the second generation. This increases cell survival, proliferation, and T-cell activation [38]. Compared to the second generation, the third generation of CARs has more co-stimulatory factor domains [39]. Stimulatory secretion cassettes or polyclonal antibody secretion systems are added to fourth-generation CARs to optimize their CAR structure [40,41]. When CAR-T cells attach to antigens, these extra secretion components may release certain cytokines, such as IL-12 and IL-18, to improve T-cell activation, the immune response, and anticancer effects [42,43]. To enhance the early activation state of CAR-T cells, preactivation domains such as CD28 or CD137 have been included in many CAR designs [44, 45]. Before antigen binding, these regions stabilize CAR-T cell activation, which is a favorable step [46,50]. To prevent cross-reactivity with normal tissues that have the same antigenic characteristics, CAR-T cells with limited antigen recognition capabilities have started to be developed [51,52]. Building restricted antigen recognition domains from tumor-specific neoantigens or fragile tumor-specific antigens increases therapeutic efficacy while reducing side effects [53]. These new CAR designs seek to improve antitumor response and cell-killing capabilities, increase selective and specific identification, decrease undesired toxicity, and improve CAR-T-cell persistence [54]. Furthermore, improved designs can increase survival and cell proliferation signals, reduce activation-induced inhibitory signals, and stimulate memory T-cell expansion [55].

The CAR-T Cell's Targeted Killing Mechanism:

The antigen plays a crucial role in the development of CAR-T cells [56]. Targeting tumor-specific or tumor-associated antigens that are either exclusively present on tumor cells or increased on the surface of cancer cells is the usual goal of CAR-T cell therapy [49,56]. A single-chain variant antibody (scFv) on the CAR protein is one method

CAR-T cells locate and bind to the target antigen, which is often a particular protein or glycoprotein that is overexpressed on the surfaces of cancer cells [57]. The robust attachment of scFv to a target antigen enables accurate identification of the antigen by a CAR-T cell [58]. CAR-T cells will activate CAR signaling domains like CD3ζ to start an intracellular signaling cascade after identifying the target antigen [59]. The activation of a typical T-cell receptor that binds antigens is comparable to this procedure [60]. The activated CAR-T cells then target tumor cells that express the target antigen for death through a variety of mechanisms, including i) Direct cytotoxic release, in which the tumor cells directly undergo lysis and apoptosis due to the release of cytotoxins by the activated CAR-T cells, such as perforin; ii) cytokine release, in which the cells release activated CAR-T cells secrete cytokines, such as interferon γ and tumor necrosis factor α, to further stimulate immune cell activation and inflammatory response [47,61,62]; and iii] immune cell alliance, in which activated CAR-T cells can recruit and activate other immune cells, such as macrophages and natural killer (NK) cells, to form an immune cell alliance to jointly attack tumor cells [48,63,64].

Presently Offered CAR-T Cell Treatments: -

1) The FDA and EMA have approved the commercial sale of CAR T cell treatment out of all the different types of ACTs. Six different drugs have been approved in the US and Europe to treat seven different types of B cell cancer. referring to Table 1. Anti-CD19 CAR T cells, designed to selectively target and kill cells that express the Pan-B cell antigen, are used in the great majority of approved medications. The following two elements are largely responsible for this target treatment's outstanding success:

2.) Hypogammaglobulinemia (low serum antibody levels) and B cell aplasia (enlarged B cells) are frequent adverse effects of the therapy because the anti-CD19 CAR T cells want to eradicate all of the patient's B cells [66]. Immunoglobulin infusions can allow people to live a relatively normal life despite the severity of these illnesses [67].

Along with HER2, IL13Rα2, and other antigens, antigens like CD20 and CD22, which have shown some promise in immunotherapies, are also being studied for potential CAR T development [68].

It's important to note that compared to solid tumors, hematological malignancies have demonstrated a significantly higher response to CAR T cell therapy. This is partly due to the notable variations in tumor stroma permissiveness. In conclusion, because the extracellular matrix (ECM) is thinner in blood malignancies than in other tumor types, CAR T cells may more easily access the tumor cells.

Even though there are currently few CAR T cell therapies available on the market and the majority of them are merely well-defined formulas, one can envision a time when precision medicine and personalized therapy will enable each patient's treatment to be customized to their unique condition [65,69]. Nonetheless, studies that aim to create standardized allogeneic products that might be marketed commercially are gaining attention. However, at the time, it appears that worries about poor persistence and graft-versus-host disease (GVHD) are impeding any significant advancements [70].

Table 1: A list of the CAR T cell products that are available in the USA under the FDA or Europe under the EMA

Product Name	Manufacturer	Application	Approval
Axicabtagene ciloleucel (anti-CD19)	Kite Pharma, Inc. (Los Angeles, CA, USA)	LBCL HGBCL PMBCL FL	EMA and FDA FDA EMA and FDA EMA and FDA

Tisagenlecleucel (anti-CD19)	Novartis Pharmaceutical Corporation (Basel, Switzerland)	LBCL HGBCL FL B-ALL	EMA and FDA FDA EMA and FDA EMA and FDA
Lisocabtagene maraleucel (anti-CD19)	Juno Therapeutics, Inc. (Bristol-Meyers Squibb) (Seattle, WA, USA)	LBCL HGBCL PMBCL FL(3B)	EMA and FDA FDA EMA and FDA EMA and FDA
Brexucabtagene autoleucel (anti-CD19)	Kite Pharma, Inc. (Los Angeles, CA, USA)	MCL B-ALL	EMA and FDA FDA
Idecabtagene vicleucel (anti-BCMA)	Celgene Corporation (Bristol-Meyers Squibb) (Summit, NJ, USA)	MM	EMA and FDA
Ciltacabtagene autoleucel (anti-BCMA)	Janssen Biotech, Inc. (Beerse, Belgium)	MM	EMA and FDA

Abbreviations:

The following acronyms stand for their respective diseases: follicular lymphoma (FL)(3B), multiple myeloma (MM), B-ALL (B cell acute lymphocytic leukemia), MCL (mantle cell lymphoma), BCMA (B cell maturation antigen), EMA (European Medicines Agency), FDA (Food and Drug Administration), HGBCL (high-grade B cell lymphoma), PMBCL (primary mediastinal large B cell lymphoma), and FL(3B). Data taken from the official websites of the FDA (https://www.fda.gov/ (accessed on October 28, 2022)) and the European Medicines Agency (https://www.ema.europa.eu/en (accessed on October 28, 2022))

Treatment Procedure For CAR-T Cell Therapy: -

Patients must be screened by their physicians before considering CAR-T cell therapy [71]. This method often includes evaluating the patient's vitals, immune system, kind of sickness, and stage of progression [72]. Following blood collection, the T cells are separated from the patient's peripheral blood using centrifugation and the immunomagnetic bead test. The CAR gene is inserted into T cells by genetic modification in the lab, giving T cells the ability to identify and target certain tumor cells. It is feasible to generate a sufficient number of CAR-T cells for therapeutic use by growing and dividing the modified T cells in a regulated setting [73]. Patients may need to go through therapeutic preparatory procedures including lymphodepletion and bridging therapy before receiving CAR-T cell treatment. To improve the survival and therapeutic effectiveness of CAR-T cells, patients must first undergo lymphodepletion, a conditioning process that lowers the patient's competing cell populations, such as macrophages, natural killer cells, and regular T cells [74]. Chemotherapy medications or radiation therapy are used to reduce the body's immune cell count. However, there are drawbacks to this strategy, such as the fact that it temporarily weakens the patient's immune system and raises their risk of infection. Antibiotics are therefore required to prevent bacterial infections as well as to monitor patients for symptoms of sickness and treat them appropriately [73]. To restrict tumor growth or offer temporary therapeutic advantages, bridging therapy occasionally entails the administration of additional treatments, such as radiation therapy, chemotherapy, or targeted therapy, before CAR-T cell therapy. Bridge therapy allows patients to buy themselves time while CAR-T cell treatment is being researched and

produced [74]. The physician will inject CAR-T cells into the patient's veins when sufficient numbers have developed and the patient is prepared for treatment. Patients are regularly watched following CAR-T cell treatment to ensure all is okay. This entails assessing the tumor's response, keeping an eye on the CAR-T cells' level of activity and vitality inside the body, and looking for any negative responses [75]. The proper collection and design of the patient's T cells is essential for the development of CAR-T cells, and this presents several difficulties throughout the CAR-T therapy process. Poor sample quality, insufficient cell quantity, or insufficient cell output can all lead to manufacturing errors. Additionally, some cancer patients may inhibit their immune system, which results in a decrease in T cell numbers or reduced function [76]. Cells can get contaminated during the harvesting process by bacteria, viruses, and other external pollutants. The quality of CAR-T cells may deteriorate as a result of these contaminants' detrimental effects on their survival and functioning [74,78]. Additionally, the cells may sustain chemical or physical harm while being transported, cultured, or separated. Damage to these cells that lowers their activity or even kills them might jeopardize the therapeutic efficacy of CAR-T cells [77,79]. As a result, therapy may become more challenging and unexpected, and treatment may begin later than intended. A certain amount of time is required during the production of CAR-T cells to assess their quality and multiply them. Requiring patients to receive more care while they wait may increase their workload, the demand for medical resources, and total expenses [80].

Challenges Of CAR-T Cell Therapy: -

CAR T cell therapy's remarkable outcomes have altered dire prognoses and prevented several fatalities from severe cancers. However, an increasing amount of clinical experience is exposing their limitations. Low CAR T cell persistence, antigen escape events, insufficient tumor-killing efficacy (particularly in the clearance of solid tumors), and high toxicity profiles, including cases of severe CRS and neurotoxic side effects, are some of the problems impeding the development of new and improved therapies [81].

Efficacy and Persistence: -

Prior studies have shown that when CAR T cells are subjected to modest levels of basal, unstimulated CAR oligomerization, they enter a tonic signaling state. As a result, they are more susceptible to energy, fatigue, a decline in reactivity, and persistence [82]. Many cancers nevertheless resist treatment or recur later, even when the patient's clinical condition is grave. This is mostly because subclone lineages that are targeted and treated differently, such as antigen-negative cell populations, either do not survive long enough or grow out of control. Nevertheless, CAR T cell therapies for solid malignancies have not yet shown the expected outcomes. The scientific community has recently focused a lot of research resources on these potential medicines since overcoming these obstacles will improve their overall efficacy and usefulness.

Tumor-Antigen Escape: -

Refractory cancer subclones frequently outnumber tumors due to the heightened selection pressure that follows the delivery of a targeted medication. Even when anti-CD19 CAR T cells have eliminated the majority of CD19+ cancer cells in patients with large B cell lymphoma, CD19-cancerous cells can still withstand the targeted treatment and continue to spread the disease [83,84].

Strategies that aid in overcoming CD19-escape would significantly increase disease- and progression-free survival rates since it is one of the primary reasons for recurrence in hematological malignancies following CAR T cell treatment [85].

Solid Tumors:

Adoptive CAR T cell transfer has revolutionized the treatment of hematological cancers. However, despite

their best efforts, this remains the case for solid ones [86,87].

Many features of solid tumors explain the situation's limited CAR T cell reach and efficacy:

1) The first step is to inject CAR T cells into the bloodstream. After that, these cells have to get to where the tumor is. This mechanism varies from tumor to tumor and relies on chemokine attraction signals [86].
2) The lymphocytes must pierce the extracellular matrix (ECM) once they reach the tumor site. The buildup of heparan sulfate proteoglycan and thick, stiff collagen in tumor-associated fibroblasts might occasionally complicate this stacking process. Even worse, T cells travel less over denser matrix regions because they don't create many ECM-degrading enzymes. As a result of this barrier, CAR T cells are significantly less able to reach their target [88].
3) Moreover, oxidative, hypoxic, acidic, and nutrient-starved solid tumor microenvironments are frequently seen. The ligands CTLA-4 and PD-L1 are expressed by the tumor cells themselves, while other immunosuppressive elements include soluble substances, cells (including Tregs, tumor-associated macrophages (TAMs), and MDSCs), and cytokines. The CAR T cells enter anergic and apoptotic states when they come into contact with this immunologically harmful "cold tumor" environment [89].
4) It has been more difficult to identify TAAs that are consistently and precisely expressed in tumors than in B cell malignancies. TAAs are often expressed in greater amounts in cancerous cells. They are frequently coexpressed at low levels in non-malignant tissues, in addition to having considerable cross-reactivity and on-target off-tumor toxicity [86,89,90]. This implies that even if CAR T cell therapies were judged safe enough in preclinical testing, they frequently result in severe and even fatal toxicity, which leads to product failure [87,91]. Since solid tumors are often quite varied, antigen-loss events and broad alterations in antigen expression are relatively common, especially when dealing with TAAs that express very little in healthy cells [80].

If these barriers are removed, patients with advanced cancer of almost all types may have better chances of remission and longer-term survival.

Safety: -

cytokine storms or improper targeting of healthy organs can result in disastrous and even fatal side effects. These negative reactions are mostly brought on by either overactivation and overstimulation of CAR T cells and other immune players, or on-target off-tumor toxicity, which is brought on by CAR T cell cross-reactivity triggered by cognate antigen coexpression in normal cells. To maintain the viability and functionality of the supplied CAR T cells, clinicians frequently need to use therapeutic strategies to lessen these side effects [92].

If it were feasible to avoid these serious and frequently deadly side effects rather than cure them, the market output of CAR T cell therapies would increase.

Cytokine-Release Syndrome:

Cytokine storm syndrome (CRS), which is characterized by elevated levels of circulating inflammatory cytokines such as IL-6 and IFN-γ, is ranked from I to IV based on the severity of symptoms. Systemic inflammatory response signs, such as fever, exhaustion, and widespread discomfort, may appear even in mild instances. However, "hypotension as well as high fever and can progress to an uncontrolled systemic inflammatory response with vasopressor-requiring circulatory shock, vascular leakage, disseminated intravascular coagulation, and multi-organ system failure" [93] are extreme cases that can occasionally result in patient death. In severe situations, prompt medical attention is necessary.

Significant rates of CRS (up to 100%) and associated deaths have been consistently reported in clinical trials employing anti-CD19 CAR T cells in blood malignancies. According to research on CAR T cell treatments, there appears to be a connection between tumor load and the intensity of CRS responses [93,94]. More serious side effects linked to CAR T cell treatment include hemophagocytic lymphohistiocytosis (HLH) and macrophage activation syndrome (MAS). With "elevated serum levels of ferritin and liver enzymes, hemophagocytosis, cytopenias, renal failure, pulmonary edema, splenomegaly, and/or an absence of NK cell activity" [90], MAS is comparable to CRS.

The most widely used treatment for CRS is tocilizumab, an anti-IL-6 receptor monoclonal antibody (RoActemra). Other antibody-based techniques and corticosteroids are also used. This medication can be particularly successful against HLH/MAS, and not all instances of CRS respond to it [82,84].

Neurotoxicity:

It is anticipated that around two-thirds of leukemia and lymphoma patients who receive adoptive CAR T cell transfer have adverse CAR T cell-induced neurotoxicity, also referred to as immune effector cell-associated neurotoxicity (ICANS). Although the exact pathophysiology of CRS is still unknown, increased cytokines in serum and cerebrospinal fluid, together with worsened immunological activity, are known to play a major role in neurotoxicity and blood-brain barrier disruption [85].

Unsurprisingly, ICANS frequently occurs concurrently with or after CRS. "Expressive aphasia, tremor, dysgraphia, and lethargy; these symptoms can progress to global aphasia, seizures, obtundation, stupor, and coma" [86] are the clinical manifestations of ICANS. The preferred treatment for ICANS mitigation is corticosteroids, such as dexamethasone; nevertheless, there is conflicting evidence on the advantages and disadvantages of tocilizumab therapy for this illness.

Prospects For CAR-T Cell Therapy Developments: -

Currently, CAR-T cell therapy is mostly used to treat certain blood cancer types. Nevertheless, fresh research is being planned to determine how to improve the effectiveness, lower the cost, and expand the potential applications of therapies. Research is now being done to increase the safety and efficacy of CAR-T cell treatment. To reduce the possibility of negative responses, one strategy would be to rapidly initiate or halt CAR-T cell activity using an adjustable switching mechanism. [91] To get over immune escape, another option would be to investigate the use of bispecific CARs, which are capable of simultaneously recognizing two antigens. Last but not least, CAR-T cells might be genetically altered by gene editing to enhance their cellular activation, viability, and anticancer effects while lowering the possibility of immune escape mechanisms. There is a subpopulation of NK cells that also have anticancer effects, therefore switching to CAR-NK cells in addition to T cells would be advantageous. A family of surface-expressed receptors known as killer immunoglobulin-like receptors binds to molecules that resemble HLA-C to suppress NK cell function. To get beyond these restrictions and boost the targeted killing impact of NK cells on tumor cells, CAR-NK cells, also known as enhanced NK cells, can activate NK cells. [93] NK cells are one of the most important cells for immunity. Tumor microenvironmental factors that may impact CAR-T cell function include cytokines, immunosuppressive cells, and invading cells. Therefore, by using specific molecular approaches, such as antibodies or receptors on the surface of CAR-T cells, researchers are attempting to increase CAR-T cell survival and antitumor effects in the cancer microenvironment. With these novel strategies, we hope to improve the safety, efficacy, and resilience of CAR-T cell therapy. CAR-T cell therapy is anticipated to

play a bigger part in cancer treatment in the future due to the extensive study on CAR-T cells and ongoing technological advancements. [94]

Declaration by Authors

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