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CAR T Cell Therapy: Breakthroughs, Challenges, And Future Prospects.

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1. ABSTRACT:

Cancers are a diverse group of diseases characterized by uncontrolled cell growth, which can invade surrounding tissues or spread throughout the body. They are classified as neoplasms or tumors, formed by cells that undergo unregulated growth, often resulting in masses or lumps. Cancers are categorized based on the type of cell that the tumor cells resemble. Key types include:

Carcinoma: Cancers arising from epithelial cells, including common forms in the breast, prostate, lung, pancreas, and colon. Most are adenocarcinomas, which have gland-like characteristics.
Sarcoma: These originate from connective tissues like bone, cartilage, fat, and nerve.
Lymphoma and leukemia: These arise from blood-forming cells, with lymphomas maturing in lymph nodes and leukemias in the blood.
Germ cell tumors: Derived from pluripotent cells, usually found in the testis or ovary.
Blastoma: Arising from immature precursor cells or embryonic tissue.

Before CAR T-cell therapy, standard cancer treatments included surgery, radiation, chemotherapy, immunotherapy, and targeted therapy. While effective for many, these treatments often have limitations, highlighting the need for innovative approaches like CAR T- cell therapy. CAR T-cell therapy provides distinct advantages, utilizing a patient's reprogrammed T cells to specifically target cancer cell antigens. This personalized method leads to more effective outcomes and fewer side effects compared to conventional therapies. It has demonstrated significant efficacy in blood cancers and shows promise in solid tumors as well. CAR T-cell therapy involves the creation of chimeric antigen receptors (CARs), which include an antigen-binding domain, a hinge region, a transmembrane domain, and intracellular signaling domains. There are three generations of CARs, each offering enhancements over the previous one. Recent innovations, like ROR1 CAR T-cell therapy, have demonstrated promise in effectively inducing tumor cell death, representing a significant advancement in cancer treatment.

Keywords: Cancer, Carcinoma, Uncontrolled Cell Growth, Tumor, Neoplasm.

2. INTRODUCTION:

2.1. Cancer:

Cancer encompasses a wide range of diseases that share a common factor: normal cells transform into cancerous cells that grow uncontrollably and spread [62]. Cancer is the second leading cause of death in the U.S., yet mortality rates have decreased over the past 20 years. Advances in early detection and innovative treatments are curing cancer and enabling patients to live longer [61].

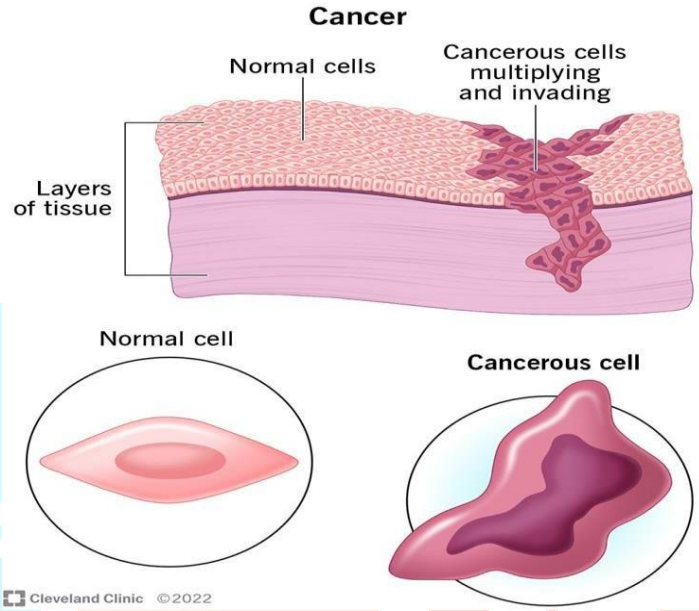

Figure 1: Normal cell and cancer cells [61].

2.2 Types of cancer:

There are over 100 types of cancer, classified by their origin in the body and the type of tissue they affect. The three main categories are:

- Solid cancers: Comprising about 80% to 90% of all cases, these include carcinomas that develop in epithelial tissues (such as skin, breast, colon, and lungs) and sarcomas that originate in bone and connective tissues.
- Blood cancers: These cancers began in blood cells or the lymphatic system, with examples including leukemia, lymphoma, and multiple myeloma.
- Mixed cancers: These involve two classifications or subtypes, such as carcinosarcoma and adenosquamous carcinoma [61].

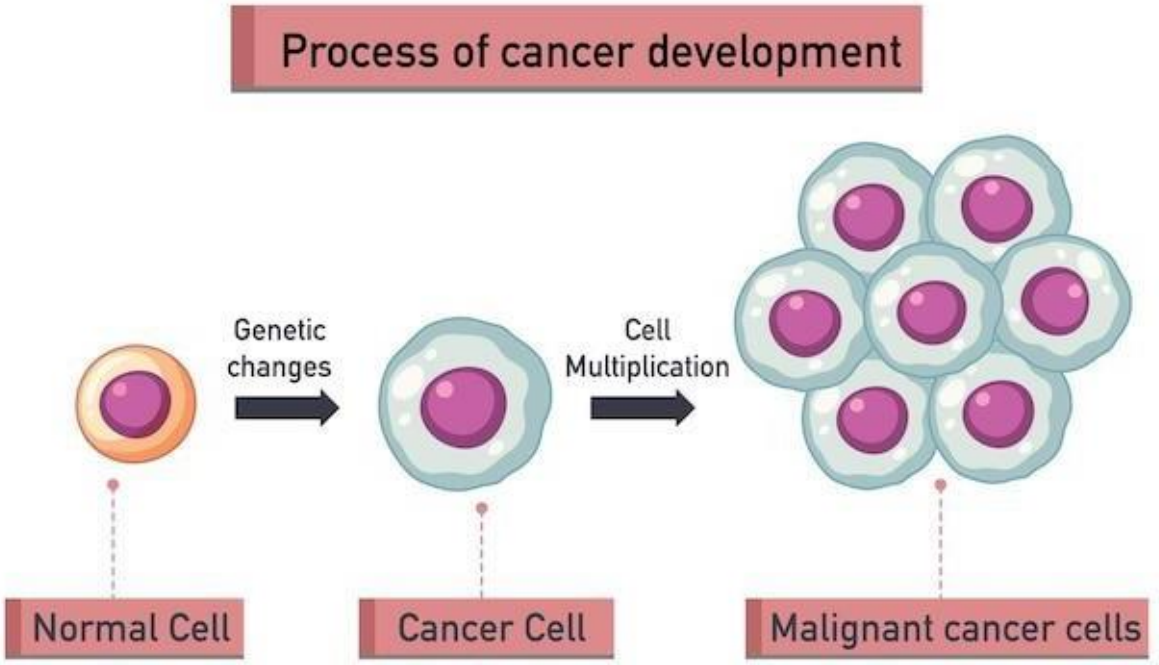

Figure 2: Cancer Cell development [5]

2.3. Symptoms of cancer:

Common symptoms of cancer include:

- Fatigue
- Nighttime fever
- Loss of appetite
- Night sweats
- Ongoing pain
- Changes in the skin, especially moles that alter in shape or size, or the appearance of new moles
- Unexplained weight loss

Additionally, cancer may lead to specific symptoms related to affected organs, which can include:

- Blood in urine or stools
- Changes in the shape, color, or size of skin moles
- Coughing up blood
- New lumps or bumps [61].

2.4. Treatment of cancer:

Cancer treatments encompass a variety of options tailored to the specific type of cancer. These treatments can include surgery, chemotherapy, radiation therapy, hormonal therapy, and targeted therapies, which may involve small-molecule drugs or monoclonal antibodies [61].

A. Chemotherapy:

Chemotherapy is the most widely used cancer treatment today. The origins of chemotherapy can be traced back to the early 20th century. However, its application in cancer treatment started in the 1930s. During World War I and World War II, it was observed that soldiers exposed to mustard gas had reduced leukocyte counts. This observation paved the way for the use of nitrogen mustard as the first chemotherapy agent for treating
lymphomas, a treatment implemented by Gilman in 1943. In the years that followed,

researchers developed alkylating drugs like cyclophosphamide and chlorambucil to combat cancer [63-64].

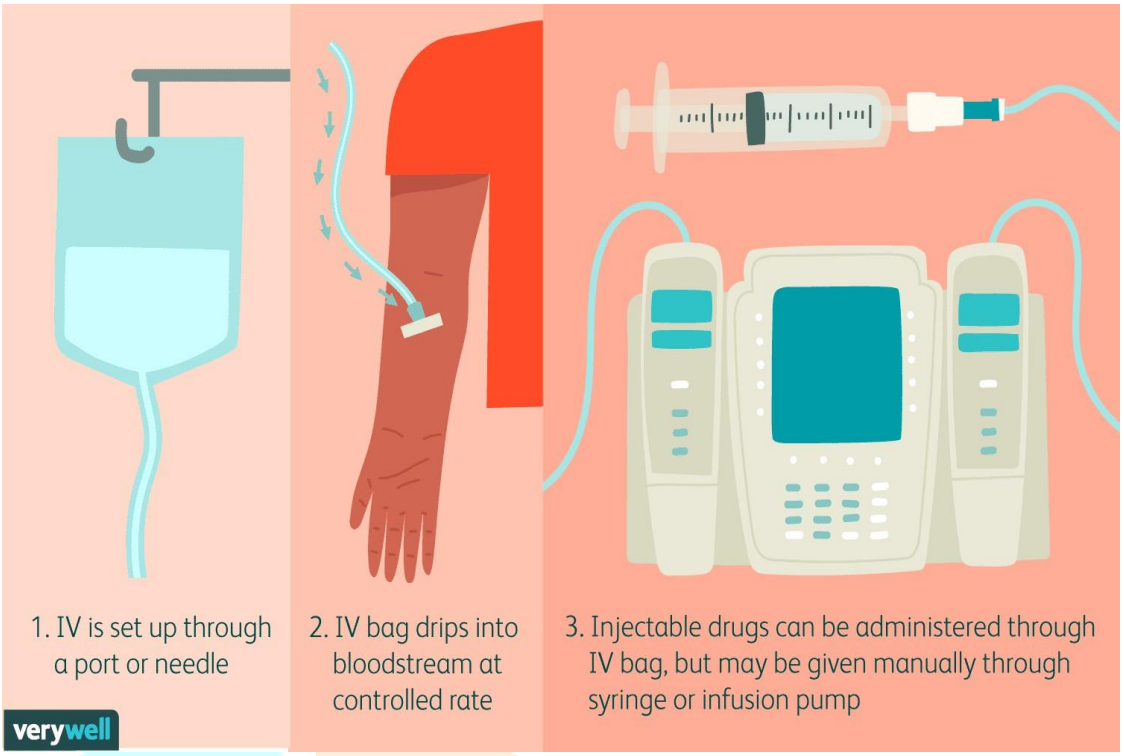

Figure 3: Chemotherapy [68].

Chemotherapy employs drugs to eliminate cancer cells and inhibit tumor growth. It can be combined with other cancer treatments, like radiation therapy or surgery. Typically administered intravenously (through a vein), chemotherapy is an effective option but may lead to side effects [65].

B. Surgery:

In 1890, Halsted conducted the first radical mastectomy, operating under the belief that more aggressive surgical techniques would improve cancer cure rates by reducing the likelihood of regional recurrences [66].

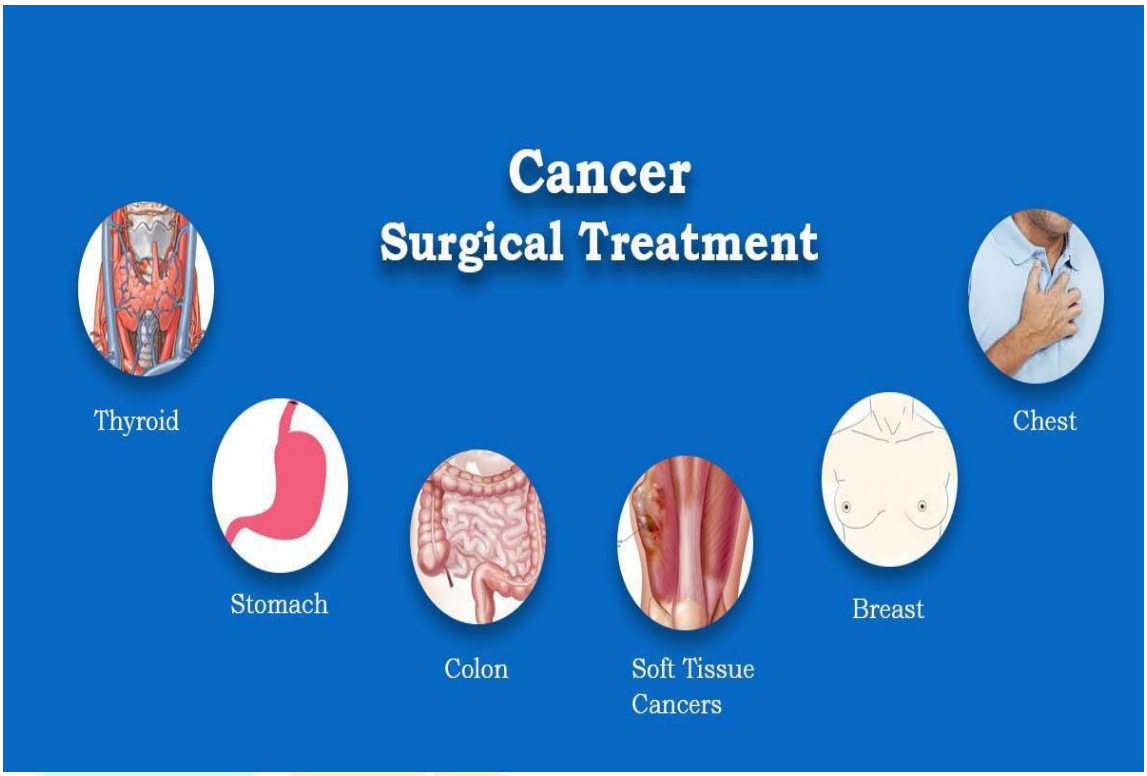

Figure 4: Cancer surgical treatments [67].

While he had many supporters at the time, advancements in chemotherapy, radiotherapy, biology, and technology have significantly changed the landscape. Today, radical surgery has largely been replaced by less invasive procedures. The early 20th century also saw the development of cancer surgery techniques, highlighted by the first abdominoperineal resection performed by Miles in 1908 [66].

Surgery for cancer involves a procedure where a surgeon excises cancerous tissue from the body. Surgeons are medical professionals who have received specialized training in surgical techniques. Surgeons typically use small, sharp instruments known as scalpels, along with other cutting tools, to make incisions during surgery. This process often involves cutting through the skin, muscles, and occasionally bone. Post-surgery, these incisions can be painful and may require time to heal properly [66].

Depending on the type and stage of your cancer, surgery may be employed for several purposes:

- Complete tumor removal: This involves excising the entire tumor from the body.
- Tumor debulking: In some cases, only a portion of the tumor is removed. This approach is used when complete removal could harm an organ or the body. By reducing the size of the tumor, this can enhance the effectiveness of other treatments.
- Symptom relief: Surgery can also be performed to eliminate tumors that are causing pain or pressure [67].

C. Radiation therapy:

The discovery of X-rays and radiation by Becquerel and Röntgen in the late 19th century marked the beginning of radiation treatment. Marie Curie's research significantly advanced the field of radiotherapy. The first case of cancer cured solely through radiation occurred in 1898 [70]. Radiation therapy, along with chemotherapy and surgery, is one of the most effective methods for treating cancer. It encompasses various approaches, with external beam radiation therapy (EBRT) being the most prevalent, as well as internal radiation therapy. There are two primary forms of radiation therapy: external beam radiation therapy (EBRT) and internal radiation therapy. Both methods function by damaging the DNA of cancer cells. When these cells lack the DNA instructions necessary for growth and reproduction, they ultimately die, leading to the reduction of tumors [70].

- **External beam radiation therapy (EBRT):**

External beam radiation therapy (EBRT) is the most common type of radiotherapy. It involves a machine directing high-energy radiation beams, such as X-rays, electrons, or protons, at the tumour. Precision is essential, and your radiation oncologist will design a plan to target the tumour while protecting healthy tissue [70].

- **Internal radiation therapy:**

Internal radiation therapy involves placing radiation directly inside your body, near cancer cells. This method is used to treat smaller tumors located in areas such as the head, neck, breast, cervix, uterus, or prostate [70].

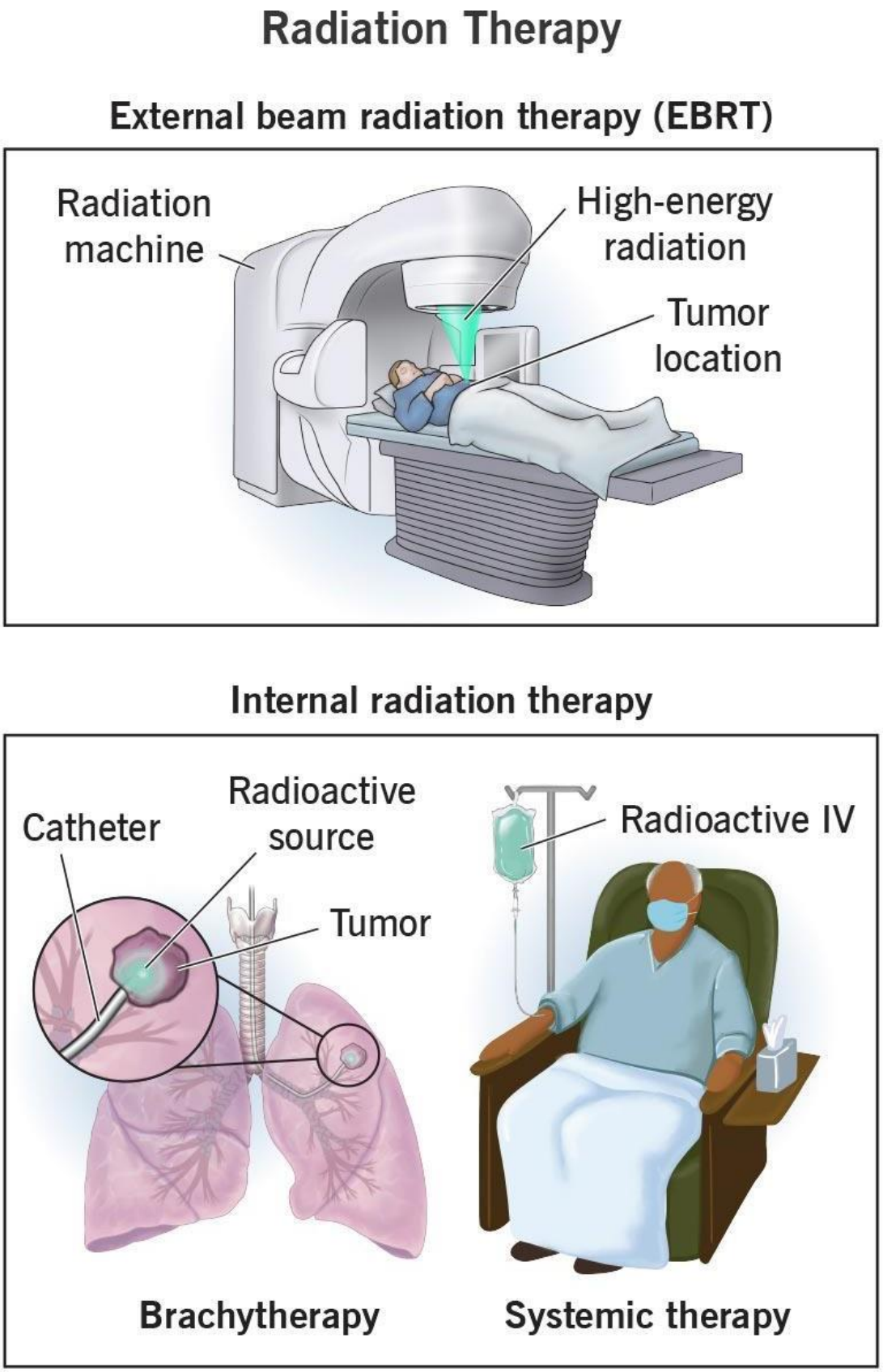

Figure 5: Radiation therapy [70].

D. Immunotherapy:

Immunotherapy is a cancer treatment that harnesses your body's immune system to locate and eliminate cancer cells. The immune system is designed to identify and attack intruders, including cancer cells. Immunotherapy enhances your immune response, enabling it to more effectively target and destroy cancer cells. This treatment has proven to be very effective for some patients, potentially extending their lives. Ongoing research is focused on developing new immunotherapy drugs to address a broader range of cancer types. Healthcare providers view immunotherapy as a first-line treatment for various types of metastatic cancer, which is cancer that has spread. It may be used in combination with chemotherapy, targeted therapy, or other cancer treatments. Different forms of immunotherapy are employed to address many cancer types, each utilizing distinct components of the immune system [72].

Types of immunotherapies include:

- Checkpoint inhibitors
- Adoptive cell therapy (also known as T-cell transfer therapy)
- Monoclonal antibodies
- Cancer vaccines
- Immune system modulators [72].

2.5. Drawbacks of other cancer therapies

I. Anemia
II. Constipation
III. Diarrhea
IV. Hair loss
V. Fatigue
VI. Infection
VII. Nausea and vomiting
VIII. Loss of appetite
IX. Dry,itchy scalp
X. Skin irritation
XI. Mouth sores
XII. Abdominal cramps
XIII. Pain [64-65-70]

2.6. CAR-T cell therapy:

Cancer poses a significant challenge to global health, and although there has been progress, conventional treatments like chemotherapy, radiotherapy, and surgery frequently come with various limitations and side effects [1]. In recent years, chimeric antigen receptor (CAR) T-cell therapy, often referred to as a "living drug," has gained prominence [2].

CAR T-cell therapy has captured significant attention in the field of cancer treatment as a groundbreaking personalized immunotherapy approach. This innovative strategy involves modifying a patient's own T cells to enhance their ability to target and destroy cancer cells. By tailoring the therapy to each individual's unique cancer profile, CAR T- cell therapy offers the potential for more effective and targeted treatment options, addressing the limitations often associated with conventional therapies. As research continues to advance, this method is paving the way for new possibilities in the fight against cancer [2-3].It operates by modifying the patient's immune system, equipping it to better recognize, target, and effectively eliminate cancer cells. This enhancement allows the immune response to be specifically directed at malignancies, increasing the likelihood of successful treatment outcomes. By reprogramming the immune cells in this way, CAR T-cell therapy empowers the body's natural defenses to actively combat cancer more efficiently [4].

Despite the notable advancements in CAR-T therapies that demonstrate substantial efficacy, particularly for patients who have undergone extensive prior treatments, several barriers can significantly impede access, even for those who are considered eligible. These obstacles can arise from various factors, including logistical challenges, healthcare provider limitations, and insurance issues. For example, a systematic review examining several CAR-T clinical trials focused on B-cell lymphomas revealed concerning statistics regarding patient access. In the JULIET trial, a striking 31% of patients who underwent leukapheresis ultimately did not receive CAR-T therapy. Similarly, in the TRASCEND-NHL-001 trial, 15% of patients faced the same fate, and in the ZUMA-1 trial, the percentage was even lower at 9%. These figures underscore a significant gap between eligibility and actual treatment delivery, highlighting the complexity of the process and the need for improved systems to ensure that eligible patients can receive these potentially life-saving therapies.

The challenges may include issues such as the availability of specialized treatment centers, the time required for the manufacturing of CAR-T cells, and logistical hurdles related to transporting patients to treatment facilities. Additionally, insurance coverage can vary widely, leading to financial barriers that prevent patients from accessing the care they need. Overall, while CAR-T therapies hold great promise, these barriers illustrate the critical need for a more streamlined approach to ensure that eligible patients can benefit from these groundbreaking treatments [6].

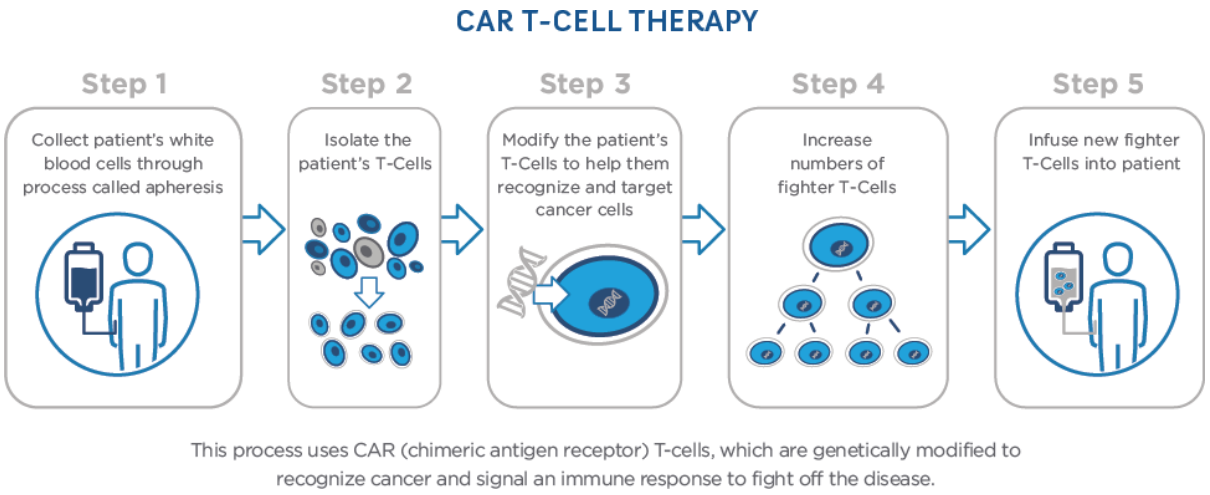

Figure 6: Formation of CAR-T cell [7]

3. CAR (Chimeric antigen receptor) STRUCTURE:

CAR consists of three main components: an antigen recognition and binding domain, a hinge and transmembrane domain, and an intracellular signaling domain [8-9]. The extracellular domain includes a signal peptide, a tumor-associated antigen (TAA) recognition region, and a spacer. A notable feature of the extracellular domain is the single-chain variable fragment (scFv) region, which resembles the variable regions of the heavy (VH) and light (VL) chains of an antibody, connected by a flexible linker [10-11-12].

The intracellular domain of the CAR serves as its functional terminus and typically includes activation and co-stimulatory domains. The most prevalent components in the cytoplasmic domain are ITAMs (immunoreceptor tyrosine-based activation motifs), which
are commonly associated with CD3 [13]. Specifically, when an antigen recognition domain binds to an antigen, it triggers an activation signal to the T cell. Furthermore, optimal T cell function relies on co-stimulatory signaling [14]. CAR T cells have been classified into five distinct generations, each defined by the specific structure and composition of the intracellular domain. This classification reflects the evolution of CAR technology and highlights the diverse strategies employed to enhance T cell functionality. Significantly, a substantial portion of research and development efforts in the field of CAR engineering has been dedicated to understanding the effects of CAR co-stimulation. Researchers aim to identify how various co-stimulatory signals can be incorporated into the CAR design to optimize T cell activation and persistence. By fine-tuning the intracellular domain of CAR structures, scientists seek to improve the efficacy of CAR T cell therapies, enhancing their ability to target and eliminate cancer cells effectively. This ongoing investigation plays a critical role in advancing the effectiveness of CAR T cell therapies and expanding their
applications in clinical settings [15].

4. Generations of CAR-T cells.

4.1. First-generation:

The initial generation of CARs was developed by incorporating solely the CD3 chain derived from the CD3 T cell receptor or the Fc receptor chain (FcR). This design involved linking an external single-chain variable fragment (scFv) to the CD3 chain, allowing for the
activation of T cells upon recognition of a specific antigen. While this foundational approach represented a significant advancement in immunotherapy, it became clear that relying only on these components was insufficient for achieving effective T cell responses.Merely engaging the CD3 chain and the scFv does not provide the necessary signals for optimal T cell activation, nor does it support prolonged cytokine production, which is crucial for sustained immune responses against tumors. As a result, researchers recognized the need for additional signaling mechanisms that could enhance T cell functionality and durability.
This realization led to the exploration of more complex CAR designs that incorporate co- stimulatory domains, which are essential for generating robust and long-lasting immune responses. By building on the basic framework of the first-generation CARs, subsequent generations have aimed to optimize T cell activation and improve therapeutic outcomes in cancer treatment [8-16]. As a result, this generation of CARs was gradually phased out
because it demonstrated insufficient signaling capacity, which hindered effective T cell activation. Additionally, the lack of durability in the immune response and the overall
inadequacy of antitumor effectiveness observed in vivo contributed to the decision to move away from this initial design. Researchers recognized the need for advancements that would enhance these critical aspects of CAR function, paving the way for the development of more sophisticated CAR structures in subsequent generations [13].

4.2. Second-generation:

In the early 2000s, researchers began to grasp the critical role that costimulation plays in achieving durable and effective CAR-T cell therapy. This understanding prompted the innovation of second-generation CARs, which incorporated one co-stimulatory domain, such as CD28 or 4-1BB (CD137), alongside the CD3ζ intracellular domain. By adding this co- stimulatory signal, these CARs were designed to enhance T cell activation, proliferation, and longevity, ultimately leading to improved therapeutic outcomes. This advancement marked a significant step forward in the engineering of CAR-T cells, as it addressed some of the limitations observed in first-generation CARs, which only contained the CD3ζ domain and lacked the necessary signals for sustained T cell function in the tumor microenvironment.
The integration of these co-stimulatory domains has since played a vital role in the success of CAR-T therapies for various malignancies, paving the way for ongoing research and
further innovations in the field [17].

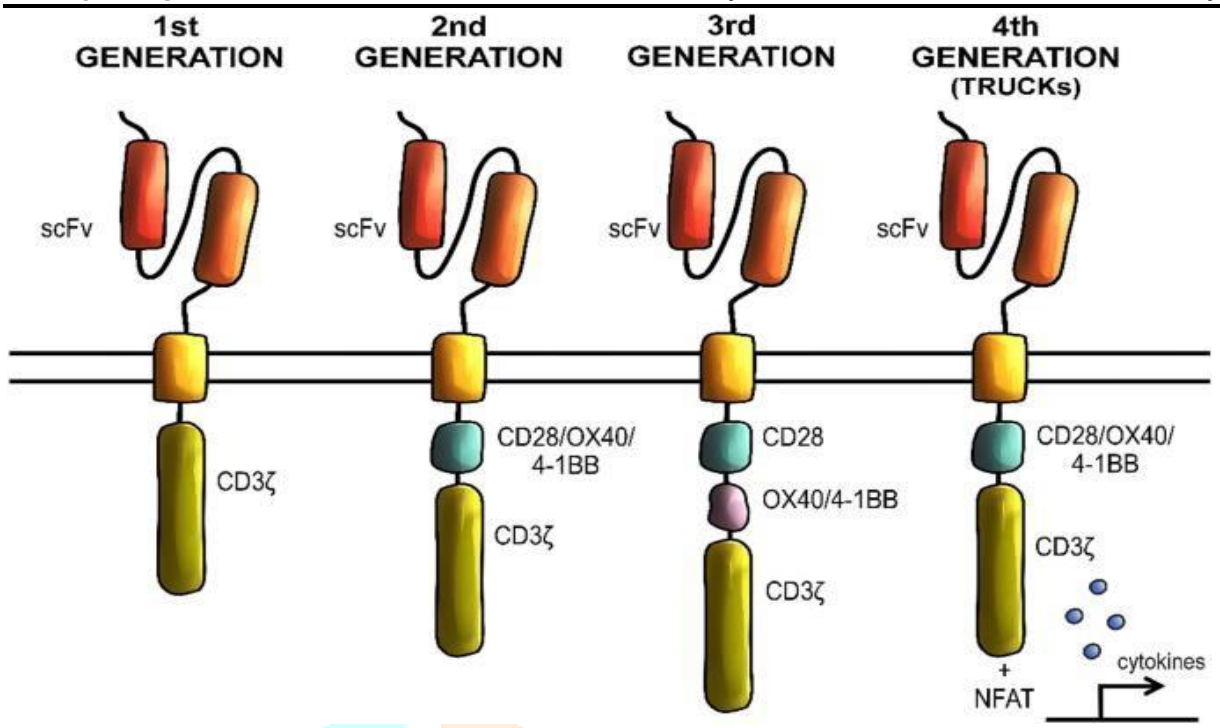

Figure 7: Generations of CAR-T cells [22]

The inclusion of these intracellular signaling domains enhances T-cell longevity, boosts cytokine production, and increases anti-tumor effectiveness in preclinical models [18], and have been demonstrated to produce robust anti-tumor effectiveness in patients with B-cell acute lymphoblastic leukemia and Non-Hodgkin lymphoma in clinical trials. These studies have confirmed that the incorporation of these intracellular signaling domains not only enhances the therapeutic potential of CAR-T cells but also translates into tangible benefits for patients, leading to higher rates of remission and improved overall outcomes. The positive results from these clinical trials underscore the pivotal role that these engineered T cells can play in the treatment of these specific hematological malignancies, highlighting their promise as a vital component of modern cancer therapy [19-20-21].

4.3. Third-generation:

The third generation of CARs was created by combining different co-stimulatory domains, such as CD28-41BB and CD28-OX40. This combination was designed to improve the effectiveness of CAR-T cells. By enhancing their ability to produce cytokines, these CAR-T cells become more powerful in fighting tumors. Additionally, this approach boosts their ability to multiply, which further strengthens their antitumor response. Overall, the integration of multiple co-stimulatory domains helps make CAR-T cells more potent and effective in battling cancer [23]. Furthermore, various preclinical studies have shown that third-generation CARs can enhance efficacy, proliferation, and cytokine production in clinical settings when compared to second-generation CARs. These findings suggest that the advanced design of third-generation CARs may lead to improved outcomes for patients undergoing treatment. However, it's important to note that there is still some contradictory evidence regarding the overall strength of the responses generated by second- versus third- generation CAR T-cells in actual patient populations. This ongoing debate highlights the complexity of CAR T-cell therapy and indicates that more research is needed to fully understand the comparative effectiveness of these different generations in clinical practice [24].

4.4. Fourth-generation:

A fourth generation of CARs, often referred to as TRUCKs, represents an advanced form of T cell-based immunotherapy. This generation utilizes innovative transgenic genetic modifications that include transgenes for the release of cytokines and additional co- stimulatory ligands [25]. As a result, immunostimulatory cytokines such as IL-2 significantly enhance the ability of CAR-T cells to withstand the immunosuppressive conditions often found within the tumor microenvironment. This increased resistance not only improves the overall function of the CAR-T cells but also promotes their growth and proliferation, making them more effective in combating tumors. In addition to these benefits, these cytokines activate and recruit various components of the innate immune system to the tumor site, helping to create a more robust immune response. This dual action of enhancing CAR-T cell resilience while mobilizing the innate immune response underscores the critical role of cytokines in optimizing cancer immunotherapy. Moreover, the presence of these cytokines can foster a more favorable microenvironment by counteracting the effects of immunosuppressive cells and factors that tumors employ to evade detection. This recruitment of innate immune cells, such as macrophages and dendritic cells, can help to prime and activate other components of the adaptive immune system. Ultimately, the strategic use of immunostimulatory cytokines enhances CAR-T cell performance and has the potential to transform cancer treatment, offering hope for improved patient outcomes and long-lasting remissions [26].

4.5. Fifth-generation:

Fifth-generation CAR-T cells are distinguished by the addition of an extra intracellular domain, setting them apart from their predecessors. These CARs incorporate truncated intracellular domains from cytokine receptors, such as fragments of the IL-2R chain, along with specific motifs that facilitate binding to transcription factors like STAT-3 and STAT-5. This unique configuration generates signals that not only keep CAR-T cells active and promote their differentiation into memory T cells but also play a crucial role in reactivating and stimulating the broader immune system. As a result, fifth-generation CAR-T cells are designed to enhance both their longevity and effectiveness in fighting tumors while invigorating overall immune responses against cancer [27].

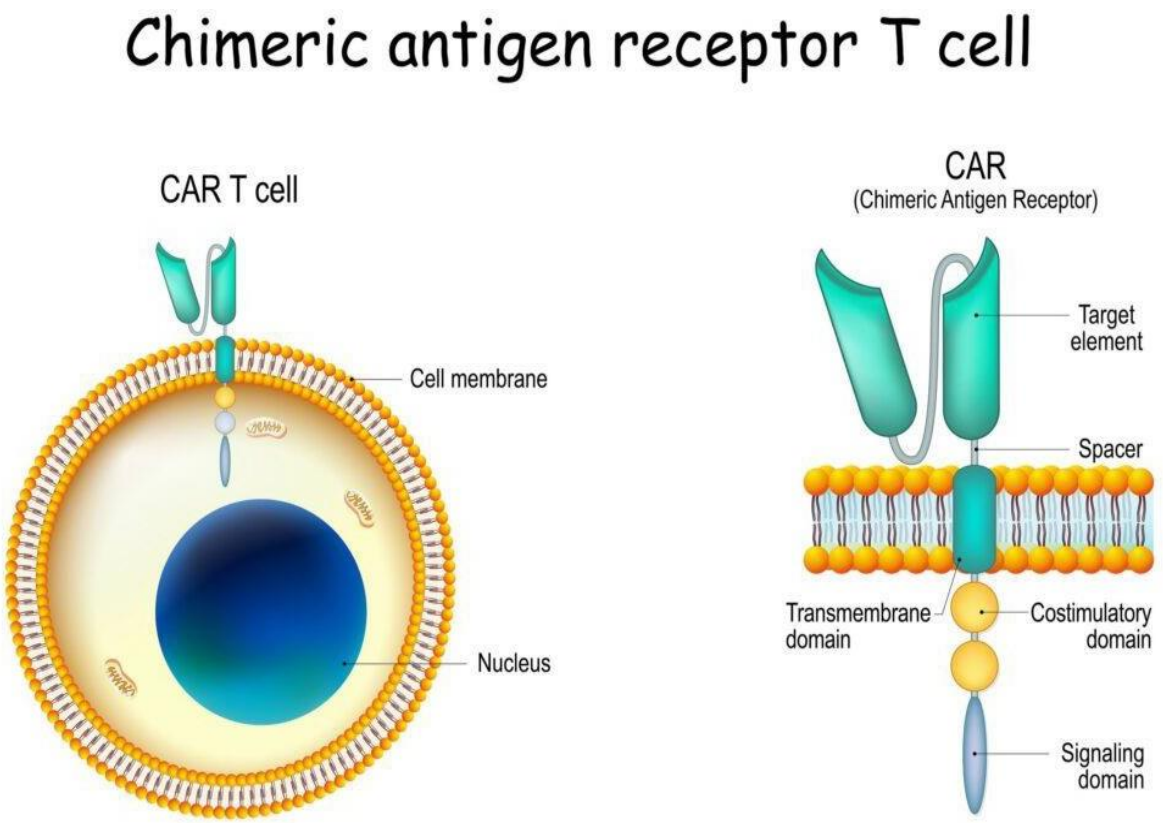

Figure 8: CAR-T cell [28].

5. How do CAR T-cell therapies eliminate cancer cells?

The mechanism by which CAR T cells activate and kill cancer cells is generally viewed as comparable to the signaling process of normal T cell receptors (TCRs). Both involve the recognition of antigens and subsequent activation of T cells to mount an immune response. However, recent studies have begun to reveal significant discrepancies that challenge this assumption. These novel findings suggest that the signaling pathways and functional outcomes of CAR T cell activation may differ in important ways from those of traditional TCR signaling. As researchers continue to explore these differences, they are uncovering new insights that could enhance our understanding of CAR T cell efficacy and improve therapeutic strategies [29]. For instance, the protein arrangement in CAR T cell immune synapses is more chaotic compared to that of physiological T cells. This disorganization speeds up lytic activity and influences the kinetics of effector-target dissociation [30].

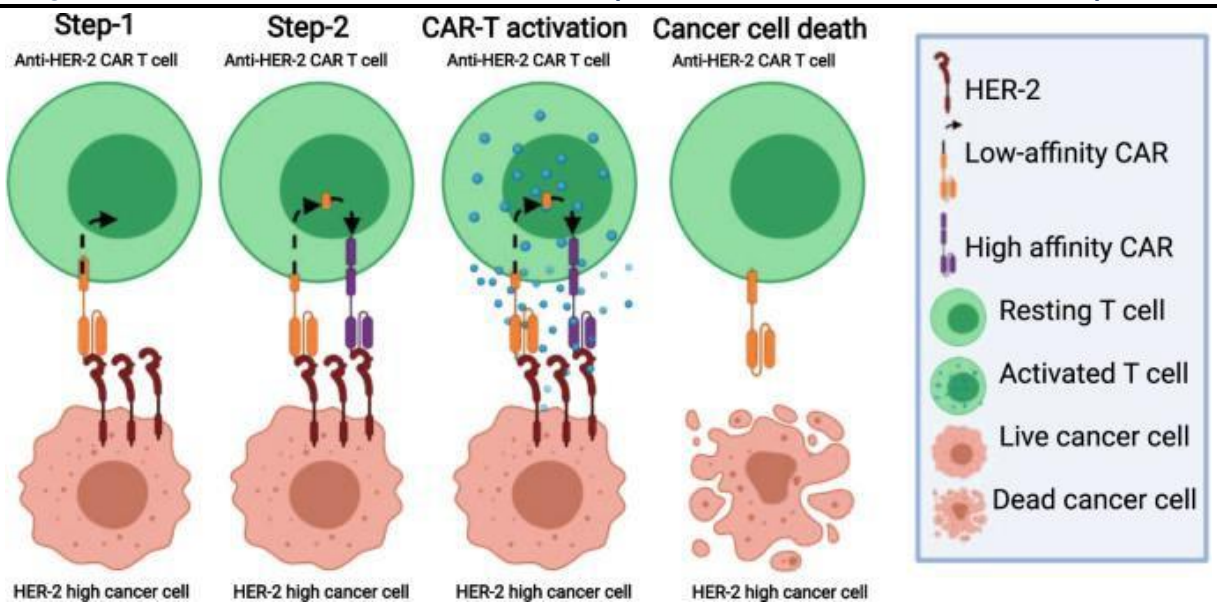

Figure 9: CAR-T cell killing Cancer cell(31)

When CAR T cells first recognize a ligand, they create a non-classical immune synapse with the target cell. This small, tight space allows multiple receptor-ligand interactions to occur close together. This clustering activates a complex response that includes clonal expansion and the destruction of the target cell.

The cytotoxic effects occur through two main pathways:

1. Slow-acting mechanisms: CAR T cells express TNF ligands that trigger apoptosis when they bind to their receptors (like FasL/FasR).

2. Fast-acting mechanisms: CAR T cells release perforin and granzyme granules, which create pores in the target cell's membrane and induce apoptosis through both caspase-dependent and caspase-independent pathways [32].

Additionally, activated CAR T cells release cytokines such as IL-2, IL-6, and IFN-γ, which help recruit and enhance the activity of other immune cells, including NK cells, macrophages, and additional T cells. This collaboration creates a stronger tumor-suppressive environment. By fostering communication among various immune components, CAR T cells amplify the overall immune response against tumors. The cytokines also help sustain T cell proliferation and survival, contributing to a more prolonged attack on cancer cells.

Furthermore, this synergistic action can lead to the activation of innate immune responses, further enhancing tumor destruction. Ultimately, the secretion of these cytokines plays a critical role in shaping the effectiveness of CAR T cell therapy in combating cancer [33].

6. Currently available CAR-T cell therapies :

Commercial Name	Product Name	Manufacturer	Application	Approval
Yescarta	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
Kymriah	Tisagenlecleucel (anti-CD19)	Novartis Pharmaceutical Corporation (Basel, Switzerland)	LBCL HGBCL FL B-ALL	EMA and FDA FDA EMA and FDA EMA and FDA
Breyanzi	Lisocabtagene maraleucel (anti-CD19)	Juno Therapeutics, Inc. (Bristol-Meyers Squibb) (Seattle, WA, USA)	LBCL HGBCL PMBCL FL3B	EMA and FDA FDA EMA and FDA EMA and FDA
Tecartus	Brexucabtagene autoleucel (anti-CD19)	Kite Pharma, Inc. (Los Angeles, CA, USA)	MCL B-ALL	EMA and FDA FDA
Abecma	Idecabtagene vicleucel (anti-BCMA)	Celgene Corporation (Bristol-Meyers Squibb) (Summit, NJ, USA)	MM	EMA and FDA
Carvykti	Ciltacabtagene autoleucel (anti-BCMA)	Janssen Biotech, Inc. (Beerse, Belgium)	MM	EMA and FDA

Table 1: Available CAR-T cell therapies [71].

Limitations of CAR-T cell therapy:

The infusion of CAR-T cells is not entirely safe, and there are significant limitations associated with this treatment approach that need to be addressed. Although researchers and clinicians have made considerable efforts to improve the effectiveness of CAR-T cell therapy, the results for treating solid tumors have not been as promising as those for blood cancers, known as hematological tumor's. In fact, the mortality rate for patients with solid tumors has not shown a significant decrease, which is concerning. Additionally, most of the therapeutic benefits observed have been temporary, meaning that patients often do not experience long-lasting improvements in their condition. These challenges arise from several factors that CAR-T cell therapy faces when trying to effectively target and treat solid tumors, making it a complex issue that requires further investigation and solutions [34-35].

In this section, we will examine several key limitations of CAR-T cell therapy that affect its effectiveness and safety. One major issue is antigen escape [36-37], where tumors may lose the targeted

antigens, allowing them to avoid destruction. Another concern is the toxicity associated with CAR-T cells [38-39], which can lead to serious side effects for patients. Antigen heterogeneity [40] presents another challenge, as different cancer cells within the same tumor may express various antigens, complicating targeting.

Additionally, the ability of CAR-T cells to effectively traffic to and infiltrate tumors [42-43-44] can be problematic. Poor stability of the CAR-T cells [45] may also limit their effectiveness, as they may not survive long enough in the body. Moreover, the immunosuppressive microenvironment [46-47] often found in tumors can inhibit CAR-T cell function. Finally, there is the issue of ineffectiveness in treating B cell-associated malignancies [48], where outcomes may not be as favorable. Each of these limitations poses challenges that need to be addressed to enhance CAR-T cell therapy.

6.1. Antigen escape:

Antigen escape and downregulation have become significant issues regarding the long-term effectiveness of CAR T-cell therapy. When cancer cells present adequate tumor-specific antigens, CAR-T cells can activate and effectively target those cells for destruction. However, tumor cells may evade this immune response by producing antigens that do not have the extracellular epitopes recognized by CAR-T cells [49]. Additionally, solid tumors exhibit similar patterns of resistance to antigen escape [50]. To reduce the recurrence rate in cancer patients treated with CAR-T cells, targeting multiple antigens has gained popularity. This is achieved through the use of dual CAR constructs or tandem CARs, as well as single CAR constructs that incorporate two scFvs to simultaneously target multiple tumor antigens [51]. CAR-T cell toxicities:

Even with the great progress made in CAR T cell therapy, there are important limitations to consider. These include both expected and unexpected side effects, such as immune effector cell-associated neurotoxicity syndrome (ICANS), a neuropsychiatric condition, and cytokine release syndrome (CRS) [52]. CRS is an inflammatory condition characterized by elevated levels of pro-inflammatory cytokines, such as soluble interleukin-2 receptor alpha (IL-2Ra), IL-10, IL-6, and interferon-gamma (IFN-γ) [53].

6.2. Antigen heterogenecity:

The first crucial step for effective cell therapy is selecting the right target antigen. However, due to the high antigenic heterogeneity of tumor cells, T cells often struggle to recognize the target cells. Additionally, after CAR T cell therapy, tumor cells may reduce the expression of tumor-associated antigens (TAAs) [54-55]. One effective approach to address this challenge is the production of CAR T cells that express two or more specific CARs on their surface, allowing them to recognize multiple tumor-associated antigens (TAAs). Alternatively, using CAR T cells that specifically target cancer cells can also be a viable strategy [36-56-57-58].

6.3. Immunosuppressive tumor microenvironment:

If the challenges of CAR T cell infiltration into the tumor microenvironment are resolved, the cells will still face an immunosuppressive environment once they reach the tumor. This environment is created by four main factors: (1) Immunosuppressive soluble factors like prostaglandin E2 and cytokines such as TGF-β and IL-10; (2) Immunosuppressive cells, including regulatory T cells (Tregs) and M2 macrophages; (3) Inhibitory molecules on CAR T cells, like PD-1 and CTLA-4; and (4) Metabolic conditions caused by hypoxia and nutrient deprivation [59-60-61].

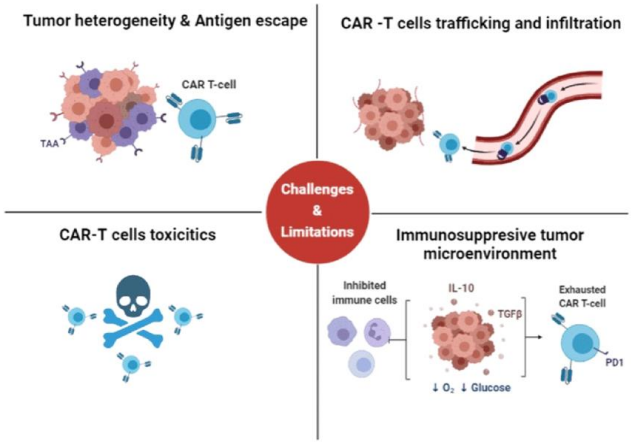

Figure 10: Limitations of CAR-T cell therapy [73].

7. Conclusion:

CAR-T cells are immune cells that can replicate themselves and have improved cancer treatment. They are currently approved only for patients who haven't responded to previous treatments or those whose cancer relapsed even after responding to the initial therapies. Their success depends on effective production and safe administration. However, CAR-T therapy can cause serious side effects like cytokine release syndrome and neurotoxicity. Learning more about these side effects of CAR-T therapy can help doctors take better care of patients. Understanding these effects can lead to improved ways to prevent or manage them, making treatment safer and more effective. While CAR-T therapy is promising for blood cancers, researchers are also looking into its use for solid tumors. Ongoing clinical trials are essential for evaluating the effectiveness of CAR-T therapy across different cancer types and refining treatment protocols. Combining CAR-T with treatments like chemotherapy or oncolytic viruses may improve results. Overall, CAR-T therapy's effectiveness varies by cancer type, and more research is needed.

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