Memory T Cells in Leukemia Immunotherapy

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Abstract:

T cells, or Thymus cells, are amongst the most important cell types in our adaptive immune system. Of these T cells, one subtype essential for long-lasting immunity is memory T cells. Memory T cells are created, along with other memory cells, in the late stages of an infection of a particular pathogen. These cells are particularly special because they play a key role in protecting individuals from reinfection. After being infected by, or vaccinated against a particular pathogen, memory T cells' job is to maintain immune memory and store pathogen information for long-lasting protection. If a re-infection occurs, memory T cells along with other memory cells would quickly clear the infection, effectively immunizing the individual. Recently, there has been much interest in using these memory cells in immunotherapy. Immunotherapy involves using the patient's own immune system to combat cancer, serving as a very promising treatment for many types of cancer, including leukemia. Examples of immunotherapies include monoclonal antibodies, CAR T therapy, and cancer vaccines. Leukemia is a very deadly cancer of the leukocytes (white blood cells) and is known to recur in patients even after otherwise successful treatment. Normally, after cancer treatment, there is still a substantial risk of leukemia returning later in life through the same pathways through which it initially emerged and others. In such cases, more treatment would be needed. However, because of the memory T cells' ability to remember antigens, perhaps memory T cells contain untapped potential to be used in a more efficient leukemia immunotherapy and prevent relapse. How could existing mechanisms of immune memory be optimized for this purpose? This review explores the current state of CAR-T immunotherapy, the role of memory lymphocytes, and how leveraging immune memory be optimized to create a more efficient leukemia immunotherapy.

Body:

Section I. Definitions and Literature

1.1: T lymphocytes

T lymphocytes are an integral component of the adaptive immune system.

They are produced in the bone marrow and mature in the thymus, a pyramid-shaped organ in the chest area. During T cell maturation, T lymphocytes develop T cell receptors (TCRs), enabling T lymphocytes to recognize antigens on pathogens or abnormal cells.

There are many subsets of T lymphocytes, but in a traditional immune response model, CD8+ cytotoxic T lymphocytes are activated by CD4+ helper T lymphocytes, macrophages, or dendritic cells, and commonly become active when IL-12 or type 1 IFNs drive activation to fully functional effectors. [7] Other T cell types include T regulatory cells and memory T cells. (Figure 1)

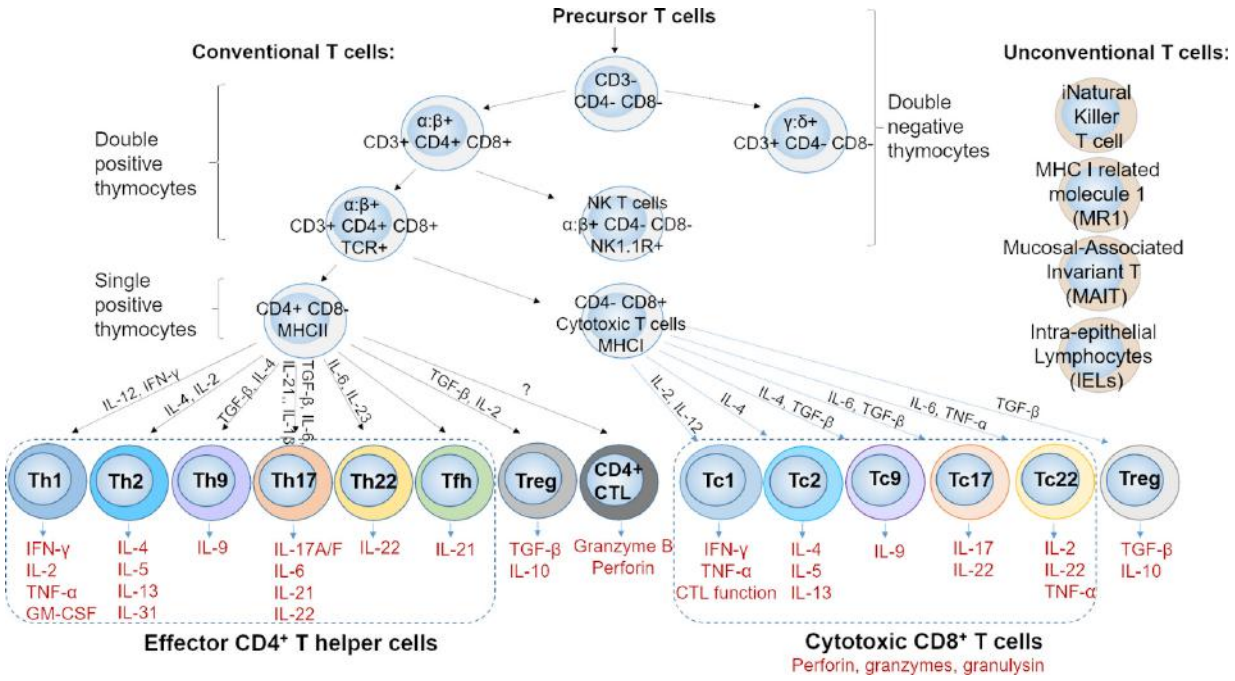

Figure 1. *A schema of T cell subsets. Figure adapted from* [21]

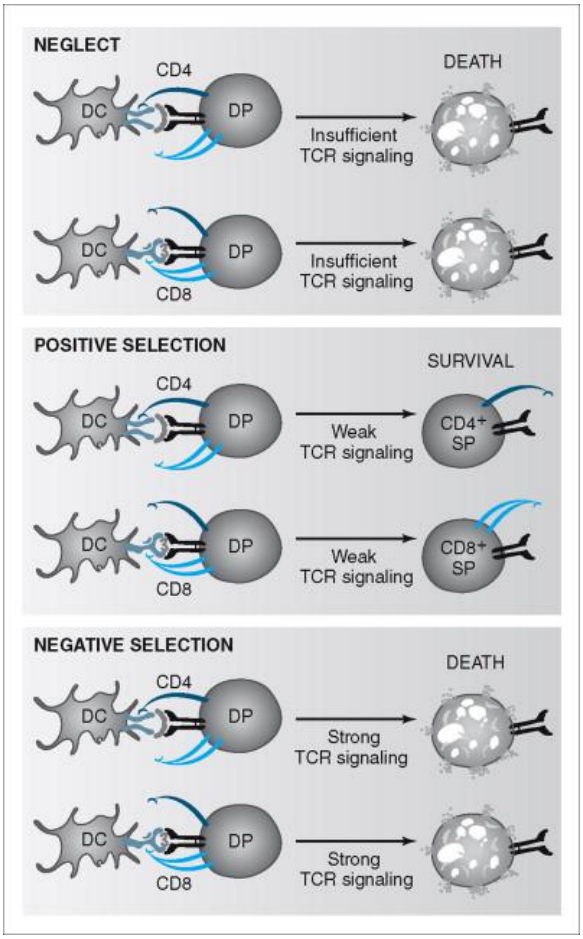

Figure 2. *T cell selection in the thymus. Figure adapted from* [35]

1.2: Memory T lymphocytes:

After the clearance of an infection, about 10% of the remaining, short-lived effector T lymphocytes become memory T lymphocytes [15]. Memory T lymphocytes are crucial for long-lasting immunity to pathogens and a rapid immune response in case of occurrence.

Memory subsets include:

Tissue Resident Memory

Tissue resident memory T cells (T_{RM}) are a subset of memory T cells that persist long term in peripheral tissues. After infection, they differentiate and accumulate in tissues, and most likely evolved to be in areas where contact with pathogens is higher- for example, barrier defenses such as the skin, gut, and tracts of the body [32]

Populations of T_{RM} have been observed to persist in tissues long after the initial infection. The highest amount of T_{RM} is usually found in areas where the infection last persisted.

Studies in mice and humans have shown T_{RM} expresses CD69 and a subset expresses CD103. A distinct differentiation pathway shows that T_{RM} is most similar to each other and contrasts to effector memory T cells. [34]

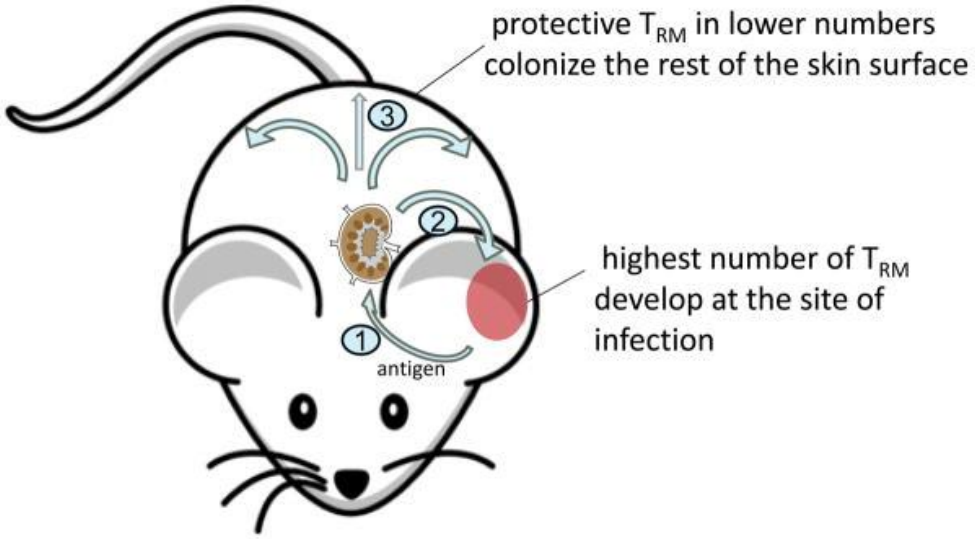

Figure 3. T_{RM} mostly populates in the site of infection. Figure adapted from [6]

Effector Memory

Effector memory T cells (T_{EM}) are a subset of memory T lymphocytes that circulate the blood, lymphoid organs, and sometimes non-lymphoid tissues. T_{EM} lacks lymph node homing receptors such as CCR7 and L-selectin, which allows them to circulate into non-lymphoid tissues. T_{EM} is important because it allows a body wide immune defense against a familiar pathogen. [18]

Central Memory

Central memory T cells (T_{CM}) are a subset of memory T lymphocytes that patrol lymph nodes and are similar to stem cells in that they can self-renew. In mice it was shown that it allows more powerful immunity against bacteria, viruses, and cancer versus T_{EM}. They have intermediate to high expression of CD44 and express CCR7 and L-selectin. [20,40]

Stem Cell Like Memory

Just like central memory T cells, T memory stem cells (T_{SCM}) have the ability to self-renew, but they also have the capability to differentiate into the entire spectrum of memory and effector T subtypes. T_{SCM} present promising opportunities for cancer therapy due to their longevity and high proliferation capacity. [6,14]

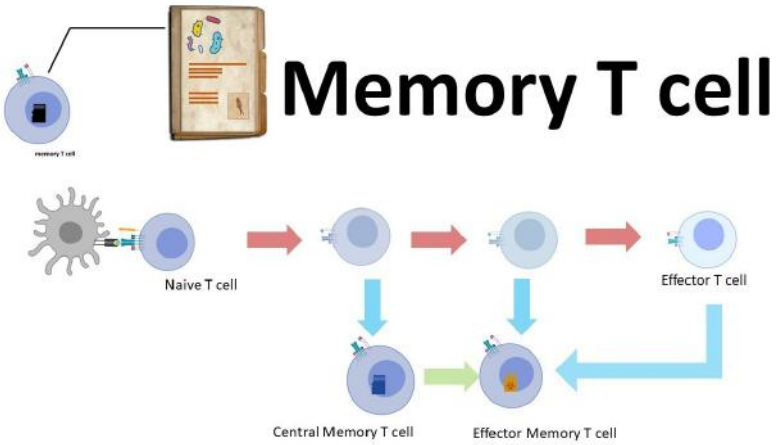

Figure 4. *Basic memory T schema.* [1]

1.3: Lifespan and Exhaustion

Effector T lymphocytes are short lived, meaning after the initial infection is over, they will rapidly apoptose to reduce inflammation and save resources. However, memory T lymphocytes have a lifespan that is much longer.

However, it must be clarified that T cell memory is not mediated through individual cells with long lifespans, but instead with long lived clones. Although a typical human T memory lymphocyte has a lifespan of 30-160 days, it is the creation of clones of T memory lymphocytes that confer immunological memory.

It is important for immunotherapies to take into account this factor of T cell memory; therapies must be able to create and sustain memory T cell populations that will survive for the rest of the patient's life. [23]

T cell exhaustion is described as a phenomenon where T cells lose killing capacity, due to being active for a long time. This can be caused by cancer, infectious disease, or other conditions. Typically an exhausted T cell would have numerous suppressive checkpoint proteins, and it is important to take this into account for T cell immunotherapy. It would not be productive to have exhausted T cells, especially in response to cancer. [10]

1.4: Leukemia

Leukemia is a deadly cancer of the leukocytes (white blood cells). Although there are many different types of leukemia, they all involve the abnormal growth of leukocytes. Although this cancer could be treated with chemotherapy or radiation therapy, there remains the possibility of the cancer returning later in the patient's life, called relapse or recurrence.

The relapse rates are as follows: [39]

- ALL (Acute Lymphoblastic Leukemia): 10-20%.
- AML (Acute Myeloid Leukemia): 50%.
- CLL (Chronic Lymphoblastic Leukemia) ≈100%. It is expected that there will always be a relapse.
- CML (Chronic Myeloid Leukemia): 60%.

Because of relapse, many leukemia patients may have to undergo chemotherapy or radiation therapy once more. It is a very difficult process and there is no guarantee that the patient will survive a second time.

However, immunotherapy serves as a promising remedy for relapse. Because your immune system can be improved to fight leukemia, memory cells could be activated. Memory T and B cells would serve as a promising sentinel against leukemia. If leukemia were ever to relapse, the immune system could easily clear it and protect the patient for long periods of time.

Specifically, because cancer causes millions of deaths worldwide, a promising treatment such as immunotherapy must be researched further to protect against cancer victims.

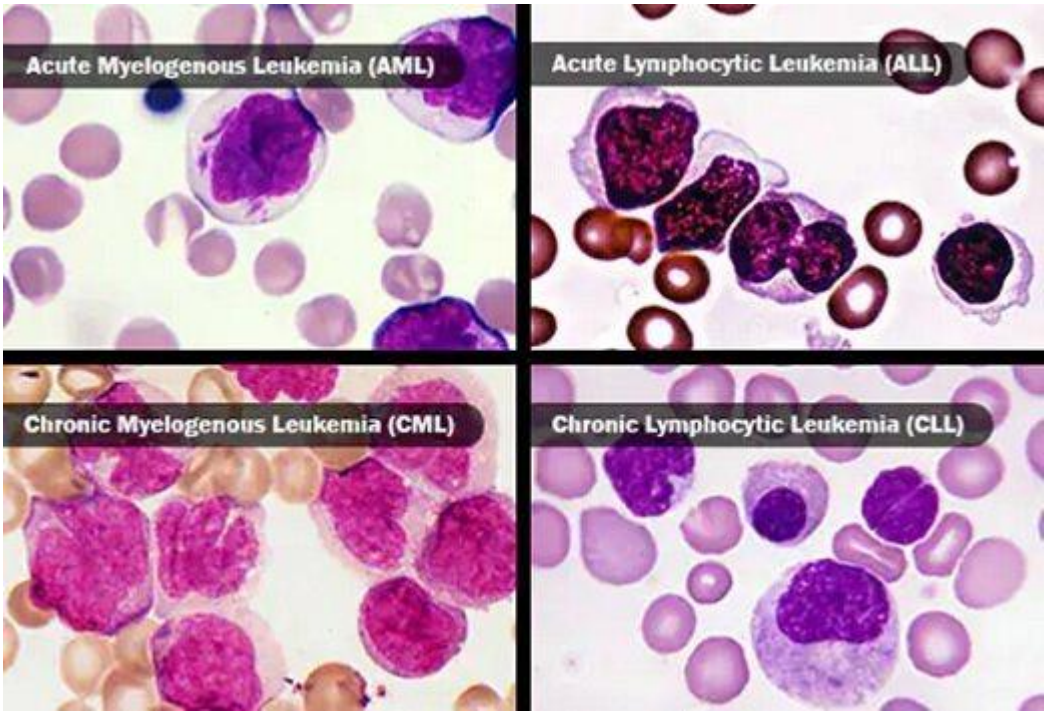

Figure 5. *Types of leukemia.* Figure adapted from [39]

Section II. Context

2.1: CAR-T Immunotherapy

Immunotherapy aims to use the patient's own immune system to more effectively fight cancer. The idea is to kill cancer cells with minimal impact to healthy cells. Other advantages of immunotherapy include:

- Personalization of immunotherapy
- Lower side effects compared to chemotherapy or radiation therapy
- Works against multiple types of cancers
- Can provide long term protection

One T cell immunotherapy is via Chimeric Antigen Receptor T cell therapy (CAR-T). CAR-T therapy occurs in several stages: [5]

1. T cell extraction
2. CAR-T engineering
3. Body preparation
4. Culturing T cells
5. Infusion of T cells

6. Monitoring patient

T cell extraction phase

Apheresis is a process where blood is drawn from the body to extract T lymphocytes. Typically patients will receive citrate to reduce clotting. Leukocytes and stem cells are extracted with centrifugation while the rest of the blood is returned via IV. [27]

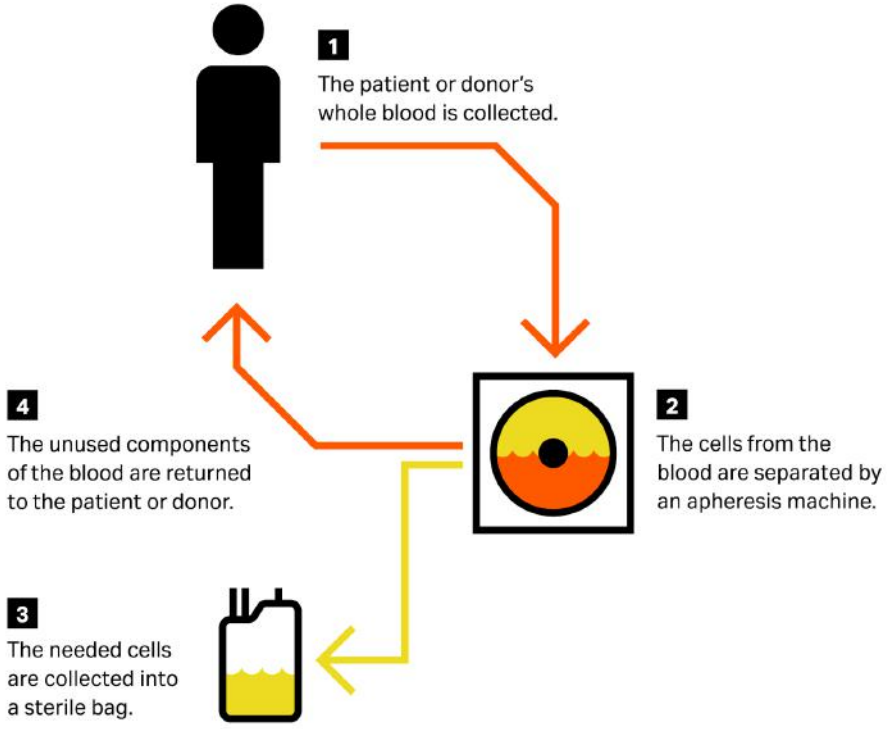

Figure 6. *Apheresis. Figure* adapted from [2]

CAR-T Engineering

CAR-T engineering of T cells can occur via a variety of ways. After T lymphocytes are washed and separated from leukocytes, CD4 and CD8 subsets can be identified with antibody bead conjugation. With the help of viral vectors, DNA can be integrated into the T lymphocyte to express CARs. Other methods include mRNA transfection and CRISPR. [15,41]

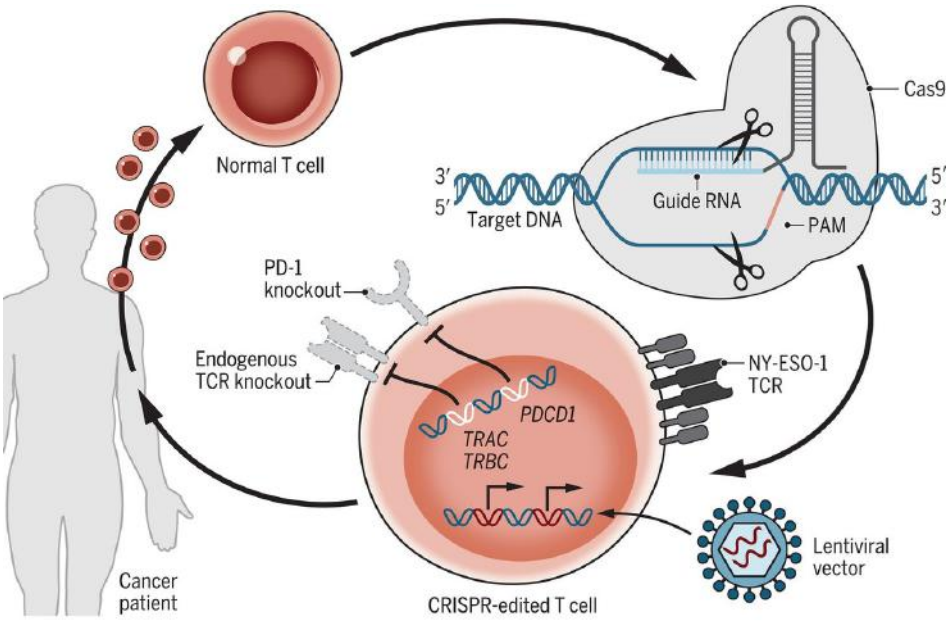

Figure 7. *CRISPR editing T cells. Figure* adapted from [8]

Body Preparation

To prepare the body for CAR-T therapy, the patient commonly undergoes chemotherapy. This is to lower the number of other immune cells to increase the CAR-T killing capacity. Some patients may also undergo radiation therapy to irradiate the bone marrow. [13]

Culturing T cells

T cells can then be cultured in the lab to grow millions for the cancer patient. This process can take weeks, and can also be analyzed with flow cytometry to see the number of each type of T lymphocyte. [12]

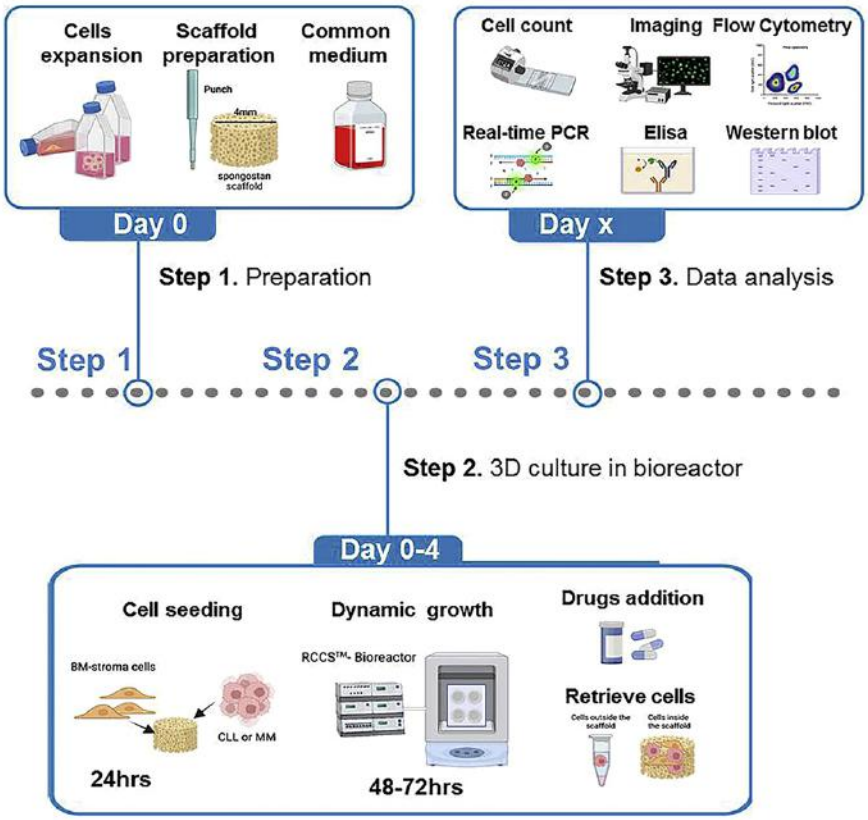

Figure 8. *T cell culture. Figure* adapted from [28]

Infusion of T cells

Similar to a blood transfusion, the new CAR-T cells are infused through a central line. The patient is then monitored. [26]

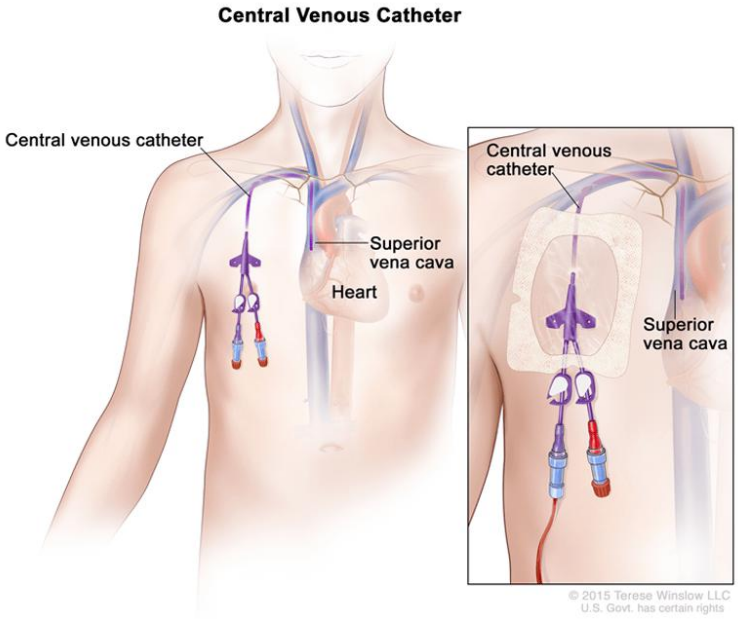

Figure 9. *Central line. Figure* adapted from [15]

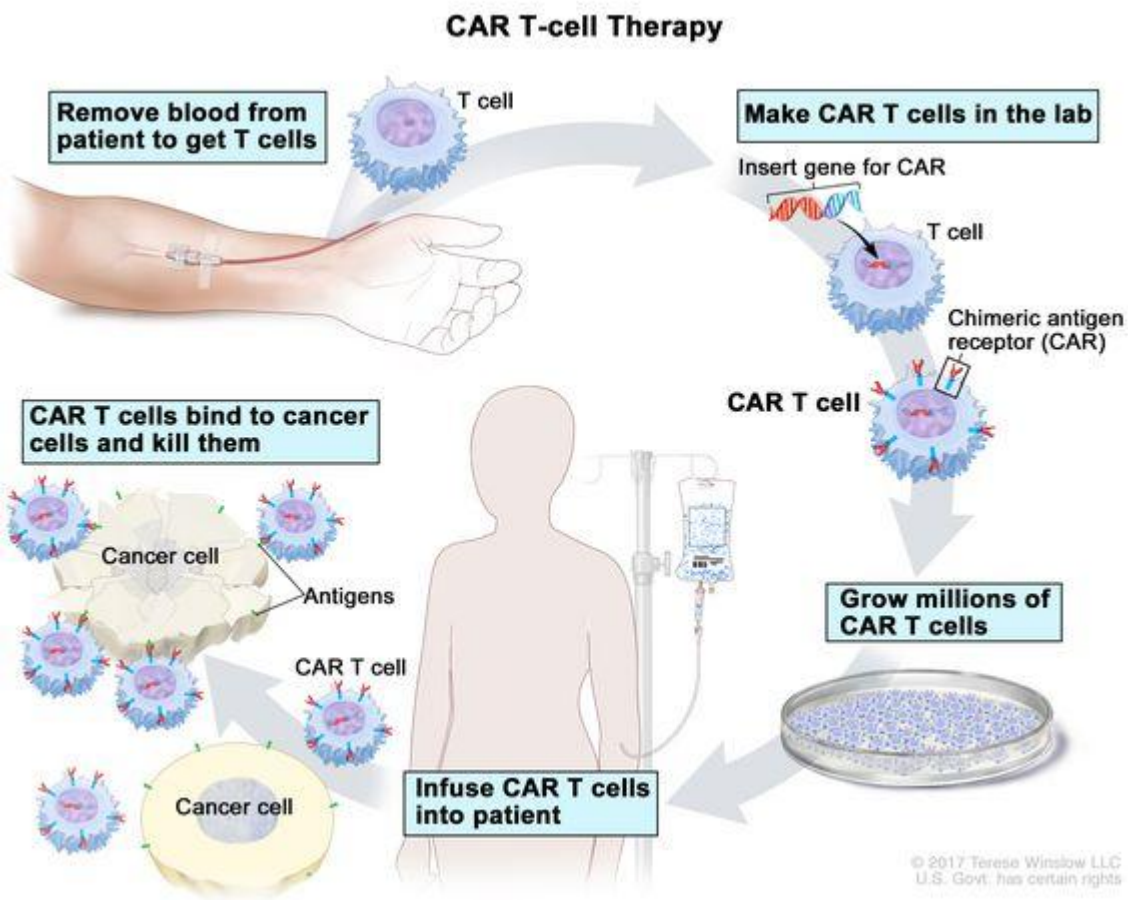

Figure 10. *CAR T-cell therapy.* Figure adapted from [9]

2.2: Role of Memory Lymphocytes

Memory Lymphocytes play a crucial role that allows the patient to reduce relapse.

Memory T lymphocytes are adaptive immune cells that can respond to previously recognized antigens as discussed earlier. The use of memory lymphocytes in immunotherapy holds promise in allowing a patient to have long term protection.

Jin et al. (2021) has established that high levels of memory T lymphocytes significantly correlated with better progression-free survival (PFS) and overall survival (OS) of cancer patients with immunotherapy. Thus, memory CD8+ T cells can be a viable indicator of immunotherapy effectiveness. [17]

It has previously been established that Trms can persist and reduce the development and spread of solid tumors, making Trms a valid candidate in cancer immunotherapy. [25] In addition, overexpression of Runx3 promotes TRM development, inhibits tumor growth, and improves mouse survival in a melanoma murine model. [24]

Memory lymphocytes have been observed to persist for years in patients with durable responses to immunotherapy. Han et al. has found that clonotypes from tumors were found in patient skin and blood up to 9 years later, showing that responses to immunotherapy allows memory lymphocytes to persist and offer long term protection. [30]

2.3: Optimization

Memory T cell subsets can be optimized in adoptive immunotherapy.

Longevity

Lymphocyte longevity is an important area to optimize because the effects must be sustained throughout time for relapse prevention. Currently, it is observed that within highly differentiated memory T cell subsets, once engrafted, it is difficult to ensure long term survival. However, a recent observation has shown that weakly differentiated memory T cells have characteristics of stem cells and memory T functions. [3]

Furthermore, studies on memory T subsets have shown that lymphodepleting chemotherapy prior to T cell infusion has led to increased levels of IL-15 and IL-7 and survival. [11]

Other studies have shown effects of metabolism on T cell longevity.

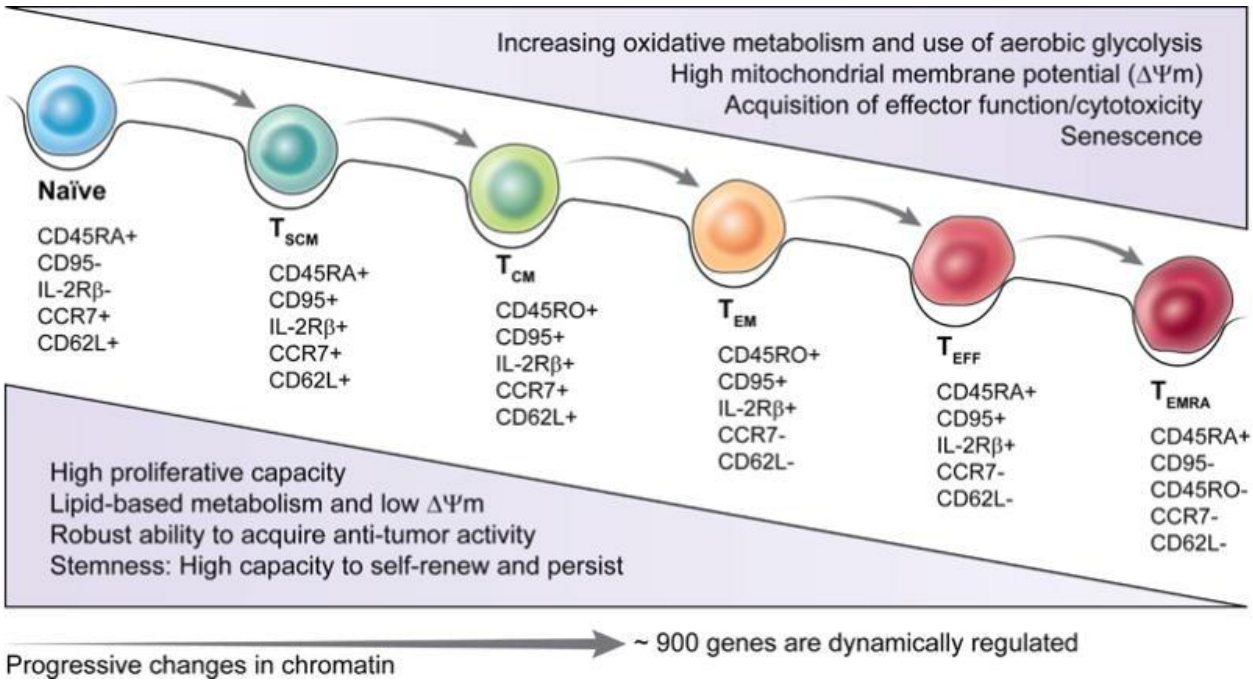

Figure 12. *T cell metabolism and differentiation.* Figure adapted from [19]

In general, T cell activation and differentiation is characterized by increased reliance on glycolysis and mitochondrial membrane potential. T cell differentiation leads to lower longevity, highlighted by shortened telomere length and repression of self renewal genes. [19]

Exhaustion

As discussed earlier, T cell exhaustion is a phenomenon where differentiated T cells have lost killing capacity due to the presence of checkpoint proteins. In the context of immunotherapy, keeping exhaustion low is an important factor to be optimized.

Lowering exhaustion can be achieved by several methods, including immune checkpoint blockades, such as inhibiting PD-1 and CTLA-4. Other methods include combating the TME, regulating transcription, and epigenetic regulation, however more extensive research is needed to evaluate the effectiveness of these methods. [42]

Section III. Evidence and Data

3.1: Effectiveness of Traditional Treatment

Chemotherapy

Chemotherapy is the most common form of leukemia treatment. Essentially, the patient is given powerful cytotoxic drugs to kill the cancerous cells. For most types of acute myeloid leukemia, the chance of remission (more or less normal blood cell levels) is 2 in 3. This means that 1 in 3 people will not have a successful treatment. [16,22]

Due to the nonspecific nature of many chemotherapy drugs, side effects include hair loss, fatigue, neurological disruption, immune disruption, urinary disruption, etc. Sometimes there are body-wide disruptions, but side effects are different across different people. [33]

Blood Transfusions

Blood transfusions do not treat leukemia, but serve to ease the patient's conditions as they are no longer producing healthy blood cells. [4]

Radiation Therapy

Radiation therapy is another common type of leukemia treatment, which involves using high energy radiation to kill cancer cells. The effectiveness is: 4 out of 10 cancer cures receive radiation therapy. However, it takes some time for radiation therapy to see results. [29]

Due to the nonspecific nature of radiation, side effects include disruptions throughout the entire body.

Radiation therapy is often used as a secondary to immunotherapy in order to irradiate the bone marrow, helping kill some cancerous leukocytes, before a transplant.

3.2: CAR-T Effectiveness

A study conducted by researchers in China found that CAR-T therapy in ALL was successful in 192 out of 194 (99%) of the patients. After 12 months, 74% of the patients had no symptoms of leukemia, but relapse was observed in 26%. In addition, 78 patients underwent a bone marrow transplant after receiving CAR-T therapy, and results saw that complete remission was observed in 99% of 194 patients with ALL. However, 43 patients experienced relapse. [37]

A systematic review by Wang et al. shows CAR-T clinical trials up to 2022. In summary, CAR-T treatments have shown to have very effective results and even have manageable side effects, however, the chances of relapse is high at around 50%. This illustrates that better

understanding of immunological memory functions will be extremely beneficial for the development of cell therapy. [38]

Section IV. Reflection

4.1: Additional Patient Considerations: Side Effects

T cell therapy inevitably comes with several side effects.

Cytokine Release Syndrome

The study in China observed that 88% (198) of the patients who underwent CAR-T therapy experienced cytokine release syndrome. Another 47 developed neurotoxicity, and three died from the side effects. [37]

Cytokine release syndrome is described as a systemic inflammatory response which can be triggered by a variety of factors, such as infections and drugs. CRS is a dangerous side effect that is very common for those who undergo CAR-T therapy. Hundreds of millions of infused T cells would cause a large inflammatory response by releasing cytokines in a positive feedback loop.

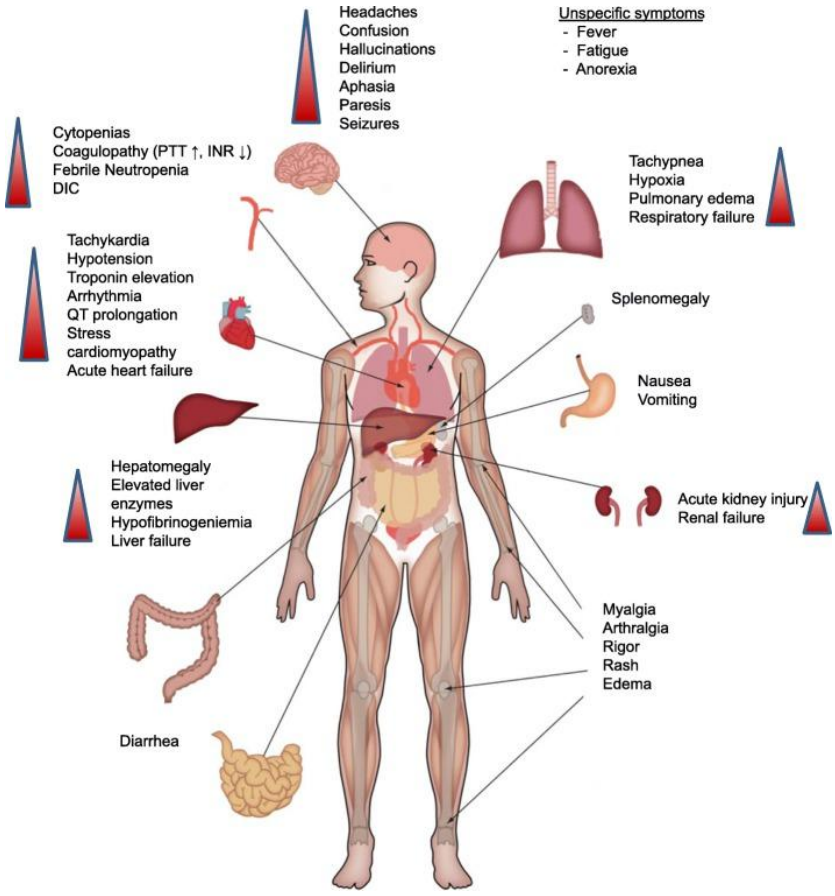

Figure 13. *Cytokine release syndrome.* Figure adapted from [31]

Other side effects of CAR-T therapy include neurological problems (immune effector cell-associated neurotoxicity syndrome) or ICANS, including seizures and balance problems. It is a possibility that the high levels of cytokines in the cerebrospinal fluid can cause disruptions in the blood brain barrier. IL-6 and other cytokines released by CAR-T can cause myeloid cells to hyperactivate and the subsequent inflammatory response can activate endothelial activation and disruptions of the BBB. [36]

Although side effects are less severe and common than traditional forms of cancer treatment, it is important to investigate to improve the therapy for all body demographics.

4.2: Future Perspectives

CAR-T is a promising treatment for many cancer patients, however, there are still many problems associated with it, including CRS and relapse. Although CRS has many countermeasures to it, relapse is less well explored.

Studies associated with memory lymphocytes show that it can be a viable optimization of CAR-T therapy. With memory lymphocytes, relapse can be prevented and allow for greater survivability. Optimization of memory lymphocytes, including the longevity, potency, and lowering exhaustion, is also important within this approach. There have been extensive clinical trials regarding CAR and its implications. As previously established, it has been mostly observed that while CAR-T has high effectiveness and lower side effects in comparison to more traditional treatments, relapse chance is high.

Furthermore, poorly developed academic and industrial collaborations could delay a CAR-T product to the market. Other considerations include regulatory and financial differences. More prioritization on the development of these therapy products and financial support could accelerate CAR development and hopefully a more efficient one in the future.

Conclusion:

Memory T cells are created, along with other memory cells, in the late stages of an infection of a particular pathogen. They are integral to allowing for long term immunity against a certain pathogen. Recently, the study of memory T cells, especially their roles in CAR-T, were observed, and found that they could pave the way for long term remission for cancer. Strategies for their optimization were also studied and found that there are many viable options to be explored. In summary, this review evaluated the current state of CAR-T, the role of memory, and how it could be optimized for a more efficient leukemia immunotherapy.

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