LEUKEMIA AND ITS TREATMENT

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ABSTRACT

In a world where so many people are suffering from different types of diseases, among them one that exists is LEUKEMIA. Leukemia is the cancer of the blood-forming tissues including the bone marrow and lymphatic tissue. Although the cancer affects both red blood cells (RBCs) and platelets, it is usually known as cancer of the white blood cells (WBCs). WBCs are a vital part of the immune system as they help in fighting infections in the body. The patient's bone marrow produces abnormal white blood cells that crowd out other cells hence disrupting their normal function. The treatment process for leukemia is very complex, and out of the many possible options, one has come to light called as the Chimeric Antigen Receptor (CAR) T-cell therapy. The treatment, which uses genetic engineering to cure cancer was approved by FDA in 2017 and has been successful in many cases.

INTRODUCTION

Cancer is a dangerous illness which has been commanding the clinical world for an extensive stretch at this point. It has transformed into a way of life as opposed to only an illness. The increasing statistics of cancer patients around the world are quite shocking. Cancer basically means uncontrollable growth of abnormal cells in the body.(1)

Cancer has existed in human beings since the start of civilization. There were 200 types of cancer known but as research in this field continued, today we know about 400 types of cancers ranging from breast cancers, lung cancers, leukemia, stomach cancers, etc. (2)

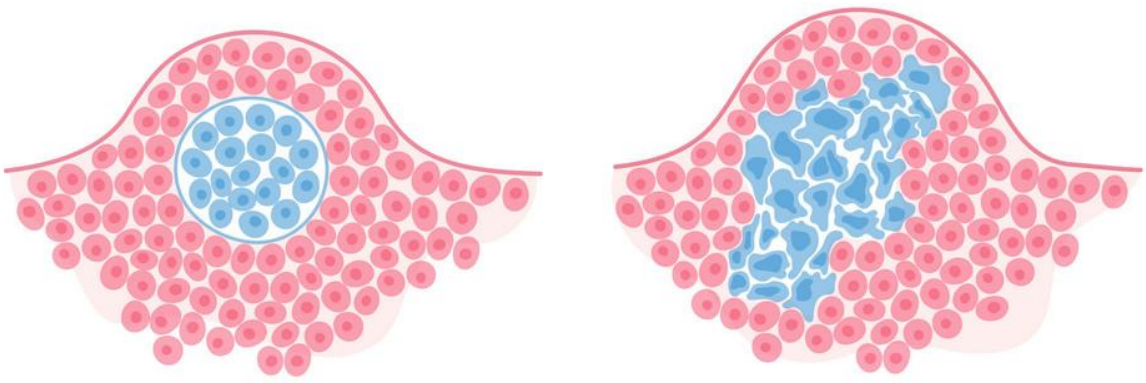
The study of cancer and its treatment has been an area of research for many scientists today.

As we are all aware, a cell divides and has a definite life cycle. All cells have a property which controls uncontrolled growth of the cell called as 'contact inhibition'. However, a cancer cell loses the property of contact inhibition, doesn't have a complete life cycle and keeps on over-piling in the same place giving rise to a 'tumor' or a mass of cells. This occurs when the immune system of the body is not functioning properly as it wipes off these cells on its own. (1)

There are two kinds of tumors- Benign tumor and malignant tumor.

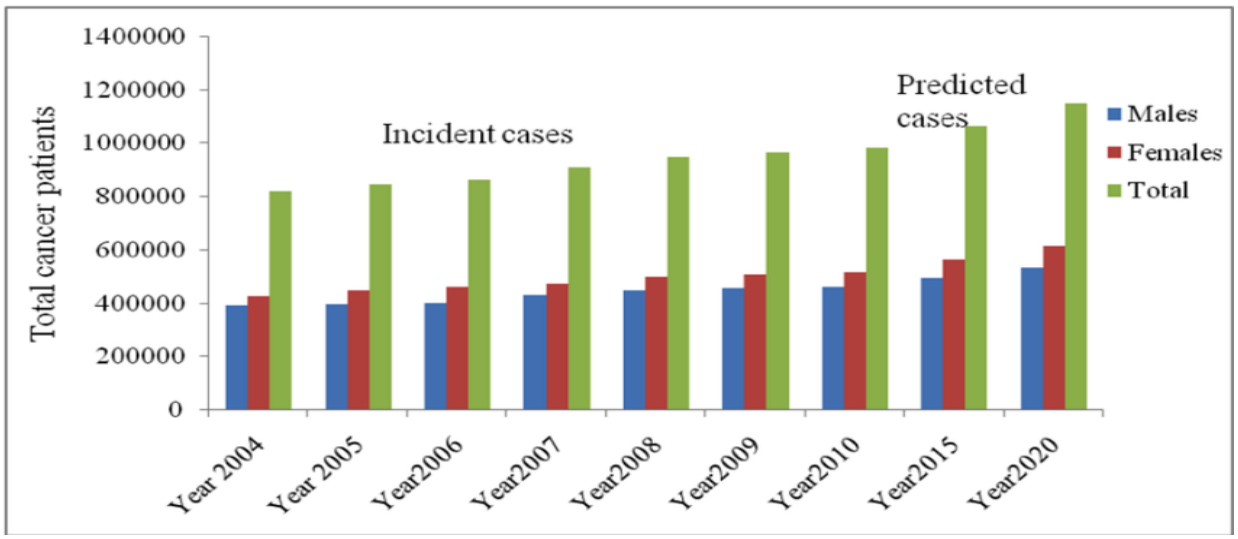
Benign tumor aren't carcinogenic. They are confined to one place and do not invade surrounding tissue. However, they can grow at a place where they press a nerve or block a blood vessel or near a vital organ causing a problem but they can be easily removed by surgeries.

Malignant tumor do not confine to one place and invade surrounding tissue. These are carcinogenic and can travel through the circulatory system including the lymphatic mechanisms to other parts of the body. (3)



Benign tumor Malignant tumor

According to stastics, more than 1200 Indians die everyday due to cancer. This mortality rate increased by 6% between 2012 and 2014. (4) The North-East has the highest affected patients in the country. (5) According to a report by the end of this year, 20 lakh new patients will be added to the total number of cancer patients and the cases will ascend by 25%. The deficient number of oncologists in India creates an issue for the country as there is only 1 oncologist for 700 cancer patients.



The common cancers among men, women and children are:

MEN	WOMEN	CHILDREN
PHARYNX	**BREAST**	**LEUKEMIA**
ORAL CAVITY	**ORAL CAVITY**	**BRAIN AND CNS**
LEUKEMIA	**OESOPHAGUS**	**NEUROBLASTOMA**
OESOPHAGUS	**OVARY**	**WILMS TUMOR**
LUNG	**LEUKEMIA**	**LYMPHOMA**
STOMACH	**STOMACH**	**RETINOBLASTOMA**

(6)

HISTORY OF CANCER

The father of medicine, a Greek physician, Hippocrates gave the word ‘cancer’. He used Greek words ‘carcinos’ and ‘carcinomas’ for tumors and ‘karkinos’ for cancer. The words are actually used to describe a crab, the animal to which a tumor resembles. Hippocrates may have named the disease but was not the first scientist to discover cancer, its history dates long back. (7)

The world’s oldest documented case dates back to 1500 BC. It was recorded by Edwin Smith Papyrus about eight tumors in the bosom. The tumors were treated by a process called ‘cauterization’ which used a hot instrument called the “fire drill” to obliterate the tissue. It was clearly mentioned that there was no treatment for the tumors except for palliative treatment. (8)

These suggestions represented 1000 years.

For 3 centuries starting from 13th century doctors were allowed to anatomize the body and analyze the cause of disease.

Wilhelm Fabrey, a scientist from Germany believed that the breast tumor was due to a coagulation in the mammary gland.

De La Boe Sylvius, a Dutch scientist believed cancer was caused by chemical imbalance in the body.

In 1761, John Hill, a renowned physician discovered cause of nose cancer was tobacco sniff. Scrotum was common in fireplace cleaners during that time.

In the 18th century as microscopy came to light it lead to deeper research in cancer. An English surgeon Campbell De Morgan was the first person to formulate the disease between 1871 to 1874. (9)

The U.S. General Surgeon’s 1964 warning that smoking causes lung cancer was based on the book published that linked tobacco to lung cancer

In 1970s, scientists discovered oncogenes and tumor repressing genes.

In 2012, after 200 years of research, IARC identified 100 different types of chemical, biological and physical carcinogens.

Over the years, the research on cancer grew and so did its treatment process. New technologies have been developing in biotechnology for the process.

TYPES OF CANCER

There are many types of cancer that the medicine industry deals with everyday. The cancers are named for the area from where they begin, regardless of the body part they spread to. For example, if a cancer begins in the ovary and spread to surrounding pelvic region, it will still be called ovarian cancer.

There are five terms essential for characterization of cancer:

- CARCINOMA is a cancer that starts in the skin or epidermal tissues
- SARCOMA is the disease of perineurium tissues
- LEUKEMIA is cancer of the blood-forming tissue, including bone marrow and lymphatic system
- LYMPHOMA AND MYELOMA are tumors of the immune system
- CENTRAL NERVOUS SYSTEM CANCERS is a cancer that begins from brain and spinal cord (2)

Some cancers are very common and affect millions of people every day, howver some are very ararely found in humans.

Few common cancers include :

1. Lung
2. Breast
3. Prostate
4. Skin
5. Liver
6. Stomach
7. Colorectal

A few rare cancers include :

1. Bartholin gland carcinoma
2. Insulinoma
3. NUT midline carcinoma
4. Heart cancer
5. Glucagonoma
6. Sertoli cell tumor

CAUSES OF CANCER

The causes can be broadly divided into four categories :

- Biological or internal factors
- Environmental exposure
- Occupational risk factors
- Lifestyle related factors

- BIOLOGICAL OR INTERNAL FACTORS: These include age, heredity, gender and type of skin. Less than 0.3% of the world's population are carriers of cancer-related genetic mutations caused due to some genetic defect in the body. This makes up about 3-10% of the world's cancer cases. However, it is not necessary that this gene would express itself in the person's lifetime.

- LIFESTYLE RELATED FACTORS: With the changing lifestyle in today's world it becomes one of the major risk factors for cancer. With the unhealthy lifestyle that we have adapted, it is responsible for 30-35% deaths all over the world. There are 3 components to our lifestyle-

    - Alcohol and tobacco: Alcohol contains carcinogens which are responsible for 3.7% of cancer cases and 3.6% of deaths in the world. Tobacco is the major cause of mouth cancer all over the world. It contains 66 known potential carcinogenic chemicals.
    - Diet: This is a very important component of our lifestyle and has come under risk factors over the past few years. Scientists have discovered red meat (such as pork, beef and lamb), processed meat (such as salted, smoked or preserved) and barbecued meat to be high in carcinogens. For instance, excessive salt in the diet can cause gastric cancer.
    - Exposure to sunlight: The UV radiations in sunlight can cause skin cancer, hence highly strong SPF creams are recommended by doctors all over the world.

- Occupational risk factors: Exposure to various chemical and toxins or radiations in daily work life containing highly carcinogenic chemicals can be a cause for cancer.

- Chemicals and toxins: A recent study shows that exposure to asbestos fibres, benzene, aflatoxin, polynuclear hydrocarbons like benzoprene or metal compounds like nickel, cadmium can lead to cancer. Other risk factors include tar and pitch and plastic chemicals like vinyl chloride.
- Radiations: 10% of cancer cases are caused by radiations. There can be high-level radiations and low-level radiations which can also affect the cells depending upon the duration of exposure. Mainly there are 2 types- Ionizing and Non-ionizing radiations. Ionizing radiations include radon, uranium, ultra violet from sunlight, alpha, beta, gama rays and radiations from X-ray sources. Non-ionizing radiations consists of all types of electromagnetic radiations.

- Environmental exposure: Various pathogens in the environment are studied to be carcinogenic. Human Papilloma Virus (HPV), Epstein-Barr Virus (EBV), Hepatitis B and C virus, and Helicobacter pylori are some of the species of pathogens known to cause cancer. (10)

In the 21st century, many other risk factors have come to light. Hormones, obesity, radiations from cell phones, chronic inflammation and lack of exercise are included in the list of cancer causing substances. However, proving whether or not a substance causes cancer is very difficult. The more a person is exposed to carcinogens the chances of cancer increase. (2)

TREATMENT OF CANCER

The approach towards treatment of cancer has been difficult for scientists around the world due to different types of cancers. The treatment process for cancer had started from Ancient Egypt and new modern technologies developed in the 19th and 20th century. As advancements in medicine industry improve, cancer treatments are becoming more specialized with better prognosis.

The treatment varies from individual to individual as it depends broadly on type of cancer and its stage. There is a sequenced protocol for each patient that has to be followed during treatment, however each treatment incorporates mainly the following segments: surgery, chemotherapy, radiation therapy or combination treatment (combination of two to three treatments).

- SURGERY: It is one of the early medicines which still stand in practice. Non-hematological malignant growths are totally treatable and can be evacuated through medical procedure. Be that as it may, on the off chance that the malignant growth is metastasized before the medical procedure, at that point in some cases relieving and totally taking out disease turns out to be about unthinkable. This is appropriate for the little malignant growths present in the body.

Surgery isn't the best way to expel malignant growth yet is a stage to take a gander at the arranging of disease. Surgery is possible at any point of time during different types of treatment. It is one of the subsidiary treatments. In this way, the effective pace of surgery is distinctive for various tumors for example breast malignancy has a superior achievement pace of medical procedures when contrasted with lung disease.

- RADIATION THERAPY: Radiation treatment is the treatment which utilizes non-ionizing radiations and ionizing radiations. This treatment is increasingly restricted and confined in the specific area. The radiations are for the most part centered around the malignancy cells, however it harms both disease and typical cells. Typical cells can adapt up to the harm yet the malignancy cells are influenced and it meddles with the existence cycle and crushed it. Its primary reason for existing is to limit the harm of typical cells and annihilate the disease cells, with this treatment malignant growth cells DNA is harmed. It is utilized for each strong disease.

- CHEMOTHERAPY: Chemotherapy is the treatment which utilizes hostile to malignancy medications to treat disease. Chemotherapy quickly decreases the division of the phones;

it fundamentally meddles in the duplication of DNA or in the meddling of the recently shaped chromosomes. It influences both the ordinary cells and the dangerous cells, however typical cells have the capacity to fix themselves after the chemotherapy. Treatment of malignant growth, for example, leukemia and lymphomas require high-portion chemotherapy.

- IMMUNOTHERAPY: This treatment is significantly structured by the remedial procedures of the patient's insusceptible framework to battle malignant growth. This method of treatment is given along with the other piece of the treatment. A bone marrow transplant can be viewed as a type of immunotherapy.

- DIRECTED THERAPIES: This treatment become accessible in the late 1900s, it has indicated a huge impact on a portion of the tumor while this is one of the fundamental dynamic locales of research. In this treatment, there is an utilization of a specialist which deregulates the protein present in the disease cells.

- HORMONE THERAPY: Some malignant growths can be forestalled by blocking or inhabitation crafted by the specific hormones present in the body of the patient.

- RESEARCH IN THE FIELD OF TREATMENT: Clinical preliminaries have been taken a stab at a portion of the individuals with malignant growth. This aides in the advancement of the past medications and furthermore helps in the check of the new medicines, for example, quality treatment.

- EXOSOME RESEARCH: An exosome is a vesicle with lipid covering which is shed by the strong tumor in the blood and urine of the infected. These can be utilized later on for the checking and discovery of an assortment of diseases.

- BLOOD TEST HOPE TO DETECT CANCER BEFORE THE SYMPTOMS (TTOI): A fresh blood test has been created which helps in distinguishing 10 sorts of malignant growth before disease shoes its side effects this has been an exact test for pancreatic, ovarian, gallbladder and liver tumors. Around 1,600 individuals have just utilized the blood test which has indicated 90% of exactness in the cases. This test is a 'fluid biopsy' which is better from the customary biopsy which is utilized. (11)

LEUKEMIA

Leukemia is the malignancy of the blood cells including the bone marrow and the lymphatic tissues. The white blood cells assume a significant job in the resistant framework for battling

infections. Leukemia prompts creation of abnormal white blood cells. The white blood cells that are not able to fully grow and develop are called blasts or leukemia cells. These abnormal cells cannot completely develop and isolate, they lose their property of ‘contact inhibition’ and overcrowd the area. Subsequently, the other blood cells get crowded out and lose their function to carry oxygen, battle diseases and control excessive bleeding in a wound. In the U.S., consistently there are more than 50,000 cases of leukemia and around 30,000 deaths. Leukemia cases makes up about 3.7% of all cancer cases.

TYPES OF LEUKEMIA

There are two ways to divide leukemia. One mode of classification depends upon how quickly the disease develops and the type of cells produced.

- Acute leukemia: In this type of leukemia, large number of cells develop rapidly in the blood and bone marrow. This requires urgent treatment and aggressive treatment. It causes symptoms like tiredness, easy bruising or easily catching infections
- Chronic leukemia: It develops slowly over time and may not show symptoms in the early stages but if left untreated, the cells may continue to grow and divide just like acute leukemia.

The second method of characterization is based on the kind of white platelets that make up the leukemia cells. Typical blood cells develop from immature hematopoietic cells which have the potential to become any cell type.

- Myeloid or myelogenous leukemia: Myeloid stem cells develop in the bone marrow to become immature cells also called as myeloid blasts. These myeloid cells further mature to become red blood cells, white blood cells or platelets. Myeloid leukemia arises from myeloid stem cells.
- Lymphoid leukemia: Lymphoid stem cells develop in the bone marrow to become lymphoid blasts. These lymphoid blasts mature to become B and T lymphocytes, which are special kinds of white blood cells. Lymphoid leukemia arises from lymphoid cells.

There are 4 common types of leukemia-

- Acute lymphocytic leukemia (ALL): This type of leukemia is responsible for around 10,000 deaths every year in the U.S. It is recognized by its rapid growth of immature lymphoid cells in the blood. ALL mostly affects children but sometimes it is found in adults too.
- Acute myeloid leukemia (AML): This involves the rapid growth of myeloid cells in the blood. The infection can be in both children and adults.
- Chronic lymphocytic leukemia (CLL): This a slow growing cancer of lymphoid cells. It can only affect people in their mid 50's.
- Chronic myeloid leukemia (CML): This is a chronic myelo-proliferative leukemia which can affect people of all age group. (12)

Some types of leukemia are rarely found but can cause many deaths around the world. A few not so common leukemia include-

- Hairy cell leukemia
- Large granular lymphocytic leukemia
- Chronic myelomonocytic leukemia
- Acute promyelocyitic leukemia

SIGNS AND SYMPTOMS

The signs and symptoms of each leukemia depends on its type. While acute leukemia can show symptoms at a very early stage, chronic leukemia do not show any symptoms for a long time. Some common symptoms of leukemia include:

- Fevers
- Frequent infections

- Fatigue
- Sweat at night
- Recurring nose bleeds
- Easy bleeding on any wound
- Bone or joint pain
- Abdomen pain due to enlargement of spleen and liver
- Irregular weight loss
- Red spots on skin (13)

DIAGNOSIS OF LEUKEMIA

Hematologist-oncologists are specialized doctors to treat blood related cancers including leukemia.

The diagnosis of leukemia involves study of blood sample. The first step of the diagnosis includes study of medical history of the patient and a physical examination to look for signs like enlargement of spleen.

Abnormal number of blood cells or abnormal type of blood cells is also one way of diagnosis. A sample of bone marrow from the hip bone can be used to diagnose leukemia. These tests are used to look for alteration in genes and certain cell surface markers by cancer cells. This test also helps in recognizing the type of leukemia.

Further, chest X-rays, MRI, CT scans and lumbar pncture are useful methods to diagnose the disease. (13)

TREATMENT OF LEUKEMIA

With the advancement in the medical world, there are a number of treatment options available for leukemia. The treatment depend upon the type of leukemia in the body, the age and health status of patient as well as whether or not the cerebrospinal fluid is affected by it.

For many chronic leukemia cases, watchful waiting is the best option. The patients is watched carefully and treatment can be started when the patient starts showing symptoms. Watchful

waiting is helpful as it eliminates risk of side effects but it puts a risk in not being able to control the disease before it becomes rapidly growing.

The treatments include chemotherapy, biological therapy, radiation therapy, stem cell transplant and targeted therapy. They can be used in various combinations according to the patient's needs. Removal of spleen can also be one method.

Acute leukemias need to be treated as soon as they are diagnosed. Inducing a remission is very important in acute leukemia and further a therapy can be given to prevent relapse of leukemia. (13)

CAR THERAPY

For many years, the treatment for cancer has been surgery, chemotherapy and radiation therapy. Over the past few decades the approach has become more targeted with drugs that target cancer cells by homing in on molecular changes seen on those cells.

But over the past several years, the “fifth pillar” of cancer treatment has emerged. This is particularly called as immunotherapy- a therapy that strengthens the immune system of the patient in order to fight the tumor in the cells. (14)

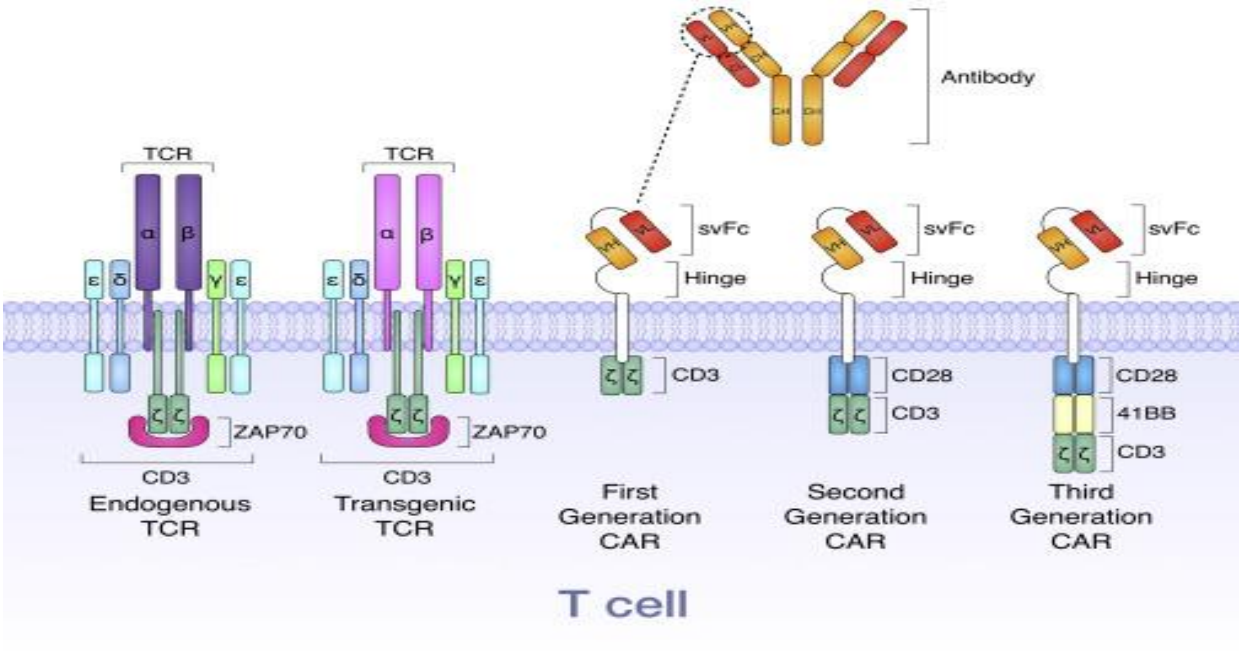
The immune system of the human body is used to defend the body from infections. Lymphocytes, a type of white blood cell makes up the major portion of the immune system. The lymphocytes have 3 categories-

- B lymphocytes (B cells): These cells make antibodies which help in fighting off infections
- T lymphocytes (T cells): They are called helper cells as they help the B-cells to produce antibodies and also directly kill the infected cells in the body
- Nature killer cells: These cells attack infected cells and other pathogens like viruses (16)

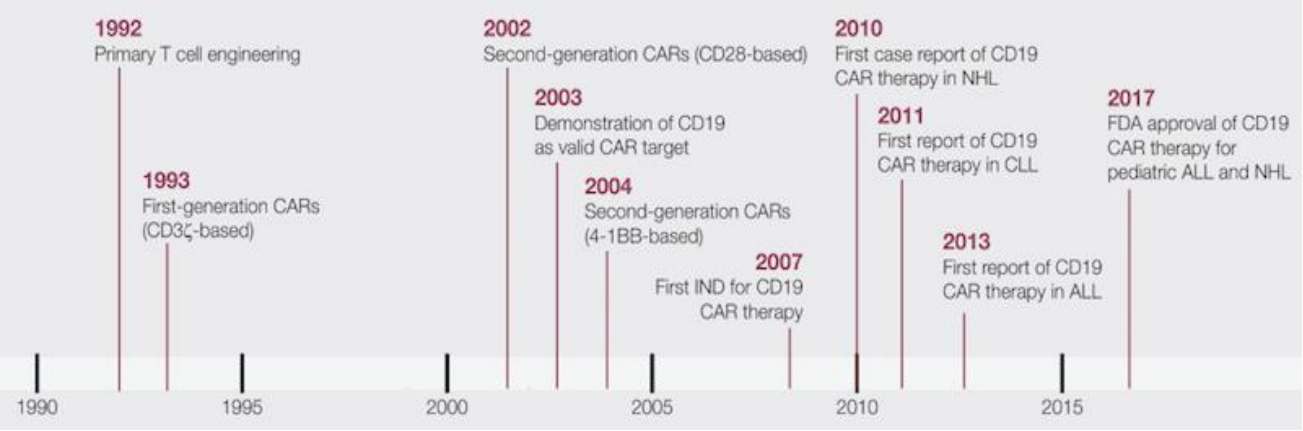
A rapidly emerging branch of immunotherapy is adoptive cell transfer (ACT). This therapy is about collecting and using the patient’s own immune cells to treat cancer. Out of the various types of ACT, one that has proven to be really effective is CAR T-cell therapy.

CAR T-cell therapy stands for chimeric antigen receptor T-cell therapy. Scientists call this as equivalent to ‘giving patient a living drug’.

As the name suggests, T-cells are the backbone of this therapy. First, the patient’s blood is drawn from the body and T cells are separated out. Then, a disarmed virus is used to genetically modify these T-cells into chimeric antigen receptors of CARs. These T-cells are activated and injected into the body to kill targeted cells in the immune system. The CAR’s ensure that the patient have their own T-cells which recognize and bind with antigens on the tumor cells. (14)



The CAR cells get activated when the extracellular domains bind with the tumor cells. This activates the T-cell cytotoxic to destruct the tumor cells. The CAR extracellular domain consists of a tumor specific monoclonal antibody also called as scFv. These scFv are use in the B-malignancies which is directed against the cell surfaces protein CD19 in the human bodies. Then these scFv are spread on the surfaces of B-cell malignancies, in addition of the normal B cells. In human body the expression of the non B-cell of CD19 is very limited. Then there is a presence of intercellular position in the CAR which consist of the receptor, which become activated by the tumor antigen which is bind to the scFv and it causes T-cell activation, proliferation, and cytokinin secretion which eliminate the tumor cells in body. In body to activate the endogenous T-cell it mainly requires 2 signals, which can both replicate by the CAR. the first cell is provided by the portion of T-cell receptor(TCR). While the second cell is provided by the "co stimulatory" which consist CD28 and any others form also. The outer portion of the CAR antibody is surrounded by the membrane of linker and transmembrane sequences. CAR T-cells therapy mostly involve the directly transferring of the CAR gene inside the human body which is known as patient also which consist their own T-cells, which have been collected by the procedure of leukapheresis. As the CAR gene enters into the body it immediately generate the T-cells which recognize and kill the cancer cells immediately in the patient(Gooley TA 2016).

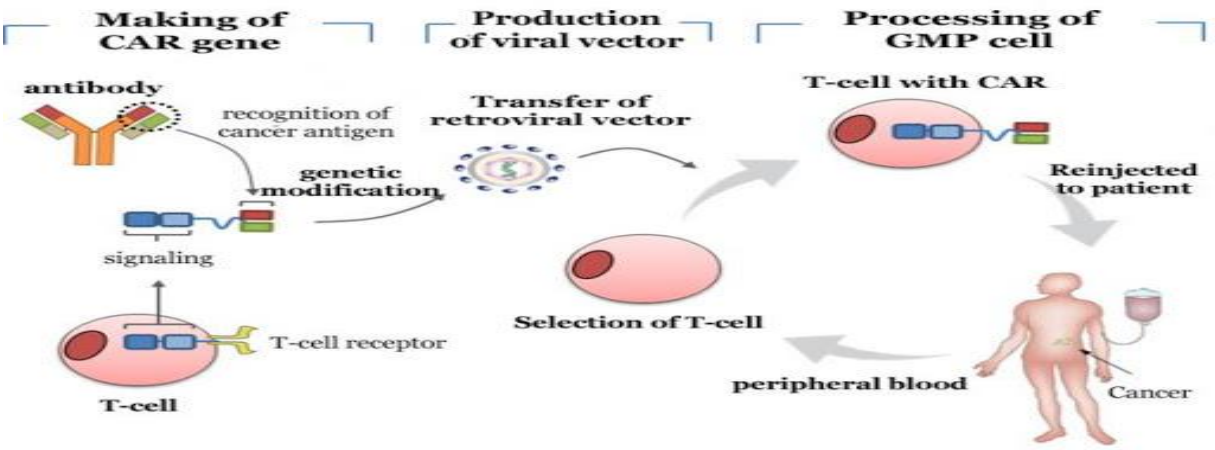


MAKING OF A CAR-T CELL

Various types of CAR T-cell therapies are being developed and tested in many clinical trials in today's world.

All these therapies share similar components although each one has its own unique sense and varies from each other. The chimeric antigen receptor or CAR present on the cell's surface comprises of fragments or domains, of sythetic antibodies. The type of domain used is important for recognizing and binding of the receptor to the affected cancerous cells. The receptors depend up on stimulation signals from inside of the cell to do their task. So all the CAR-T cells have a signaling domains inside the cell that send signals to the cell from the receptor on the surface. The type of domain used determines the cells' overall function.

Over the years, Intracellular engineering of T-cells have improved along with increasing expansion in number and persistence in the body after infusion. New technologies have also been made in the duration it takes to produce a batch of modified CAR-T cells. Initially it took several week but now most laboratories have reduced it to 7 days.



HOW CAR THERAPY WORKS

Chimeric antigen receptors (CARs) are coming into light as amazing assets for reconstructing T-cell particularity and function. CARs are hybrid receptors consisting of a ligand for a cell-surface molecule, most often consisting of a single-chain variable fragment (scFv) derived from a monoclonal antibody or an antigen-binding fragment (Fab) consolidated to flag domains that are assembled to divert T-cell function. Unlike transduced T cell receptors (TCRs), CARs endow T cells with a new specificity that is independent of HLA restriction and do so without competing with the endogenous TCR for the rate-limiting CD3 complex. First-generation CARs arbitrated limited T-cell activation, resulting in cytotoxicity but only short-term T-cell expansion. Second-generation CARs, which join initiating and co-stimulatory flagging domains, empower improved cytokine secretion, T-cell development in size and T-cell tirelessness. CARs have been produced against an enormous number of cell surface molecules, including CD19, HER2, GD2, prostate-specific membrane antigen (PSMA) and mesothelin, and a significant number of them are directly under assessment in more than 30 stage clinical trials (www.clinicaltrials.gov). Until now, the most promising clinical outcomes of this technology have been reported in patients treated with autologous CAR-modified T cells targeting CD19. CD19 is an attractive target for CAR-based therapy as it is expressed by most B-cell leukemias and lymphomas but not in tissues other than normal B lineage cells. Successful B-cell tumor eradication was eventually obtained

with different CD19-directed CARs, paving the way for multiple clinical studies and making the targeting of CD19 a pattern for evaluating CAR technology.

The therapy follows a few simple steps-

I. T-cells are collected from a patient: In this step, blood is taken out from the patient's body and its components such as plasma, platelets, especially white blood cells are removed. This process is called leukapheresis and is generally performed in a hospital or treatment center.

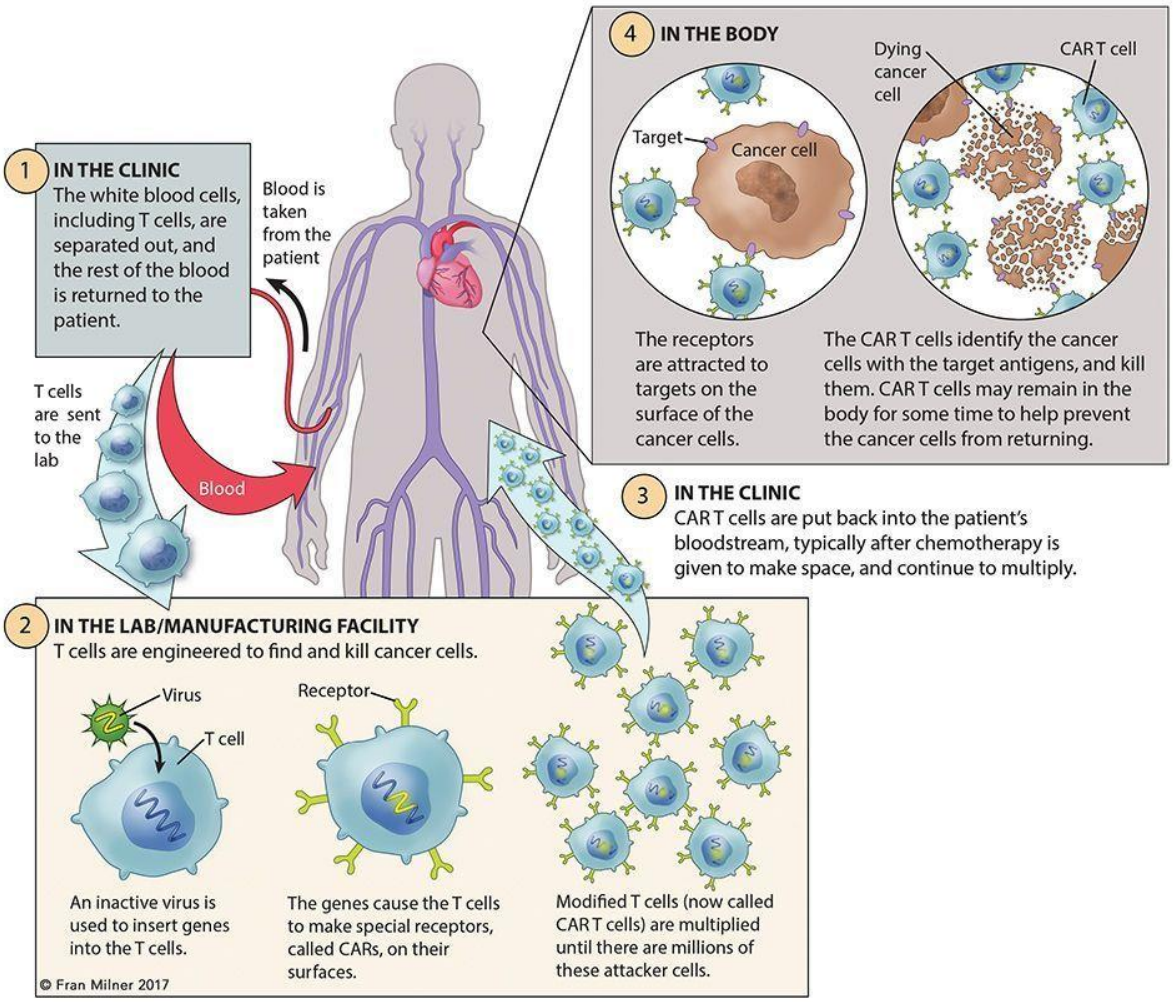
II. T-cells are reengineered in a laboratory: The second step is sending the blood sample to a laboratory or drug manufacturing facility where these T-cells are reengineered to kill the tumor. A disarmed virus is used to introduce DNA into the T-cells which are now known as Chimeric Antigen Receptors (CARs). The CAR T-cells are then produced on the cell surfaces.

III. T-cells are called as Chimeric Antigen Receptors (CARs): CARs are specialized proteins that help the T-cells to recognize a tumor.

IV. CAR T-cells are multiplied: In this step, the patient's reengineered T-cells are expanded or multiplied in number inside a laboratory. When there is an enough increase in number, the CAR T-cells are frozen and sent back to the hospital for further treatment of the patient.

V. CAR T-cells are thawed and infused into patient: Before infusing the T-cells into the patient's body, the patients is given a brief course of two-three chemotherapies, also called as lymphodepletion. This is an important step before introducing the T-cells as the doctors require a huge space to insert these CAR T-cells into the patient's bloodstream. Then, the frozen cells are heated up to convert it into a liquid form after which they multiuply in number inside the body. The second name to these cells are "attacker" cells as they recognize and attack cells with specific antigen.

VI. CAR T-cells help guard against recurrence: After attacking the existing cancer cells, these CAR T-cells remain in the body for a long time and help to fight cancer cells for a lifetime. (16)



SIDE EFFECTS OF CAR THERAPY

Like every other therapy, the CAR T-cell therapy comes with its own pros and cons. The patient's tumor maybe treated with the therapy but the side effects can also be massive in some cases. There are many side effects of CAR therapy, such as-

- CYTOKINE RELEASE SYNDROME: Out of the many side effects seen, the most important one is cytokine release syndrome or CRS. The cytokines are called as messenger cells that aid the T-cells to perform various functions. These side effects occur when the T-cells are multiplying in the body. While multiplying, the T-cells release massive amount of chemical called cytokines also called as cytokine storm. This is potentially dangerous to the patient as this cytokine can attract to build an association with the tumor cells. Depending upon the patient and the CAR T-cells the patient may show CRS in 7 to 21 days of therapy. They symptoms of CRS may vary from mild flu like symptoms like nausea, fatigue, headache, fever, etc. Or there can also by serious symptoms like low blood pressure, cardiac arrest, capillary leakage, cardiac failure, multiple organ failure, poor lung oxygenation, etc.
- NEUROLOGIC SYMPTOMS: Neurological effects like seizures, aphasia, confusion, hallucinations, unresponsiveness have been seen in many cases. However, they have been treated successfully within a short duration of time. There can also be life-long symptoms but scientists are still researching about it.
- MACROPHAGE ACTIVATION SYNDROME: The MAS is caused by excessive multiplication of T-cells and macrophages. Generally, this side effect is shown by patients with autoimmune diseases. The MAS, being a higher version of CRS can also be treated by introducing a monoclonal antibody in the patient.
- ANAPHYLAXIS: It is the second name for a life-threatening allergic reaction. There are possible chances that the immune system reacts this way to the CAR therapy. The symptoms include face swelling, hives and respiratory distress.
- B CELL APLASIA: The absence or low number of B cells is a very common symptom of this therapy. The CAR T-cell therapy not only destroys the cancerous B-cells but also the normal B-cells resulting in low immune response to fight off various infections that might enter the body after this treatment. (16)

RESULTS OF CAR THERAPY

After the CAR T-cells are inserted into the body, they start showing great impacts in the patient's body. Trials show that almost 90% patients with relapses or did not show response to any standard treatments like radiation therapy, chemotherapy, etc. showed tremendously well results with the CAR therapy. In cases where relapses occur, there can be three reasons- inhibition of

CAR-T cells, tumor cells losing expression of cluster of differentiation antigen or little persistence of the T-cells.

The therapy is very impactful in cases of ALL, CLL and myeloma as well. It reduces chances of relapse as the CAR T-cells stay in the immune system and fight any other cancer cells that might form in the body throughout their lifetime. It shows results in patients of all age groups, from children to all adults. The therapy came into light due to all these reasons and continuous study to make it more patient-friendly is being done.

Safety during CAR therapy

For the safety measures to be understood properly, doctors take one example of a leukemia case and try to give details to make one understand fully. There was a child whose age was 15 years and was suffering from the dreadful disease leukemia. He had gone through the CAR therapy

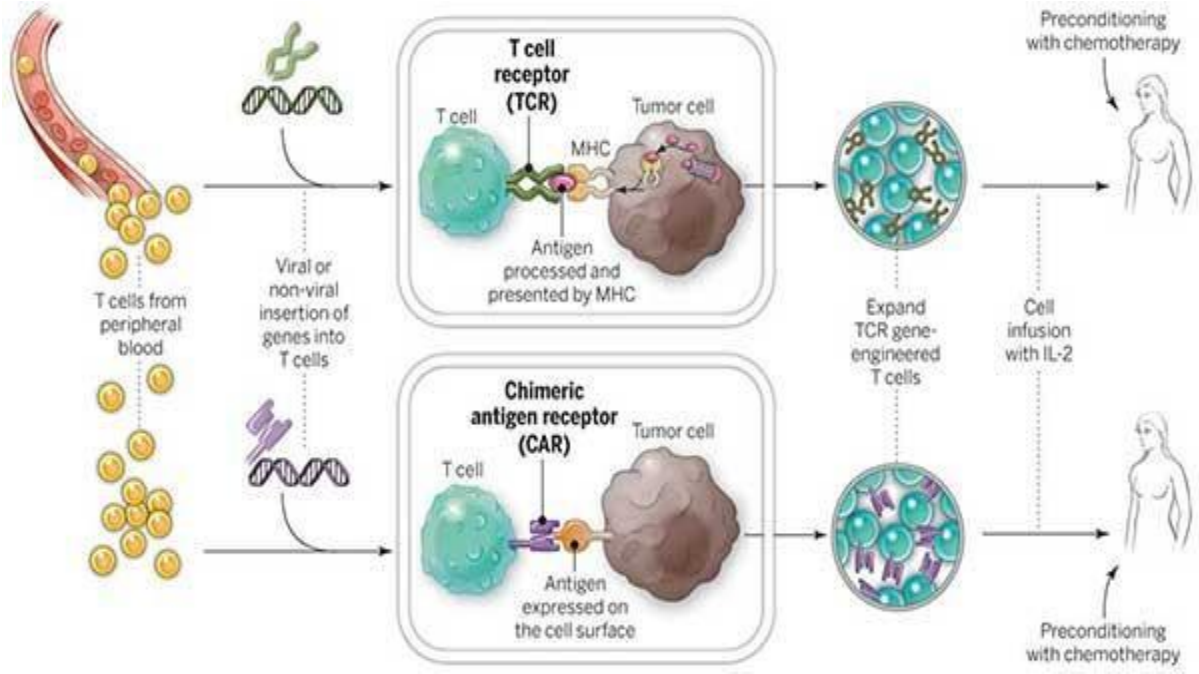
during which he also had a slight temperature of 107^{o}c. At the moment his father was beside the child and very frightened due to the condition of his son. He had thoughts about his son dying. After a few days, his fever finally broke and he became normal and he got back to his normal life as earlier. The boy had been suffering from leukemia ever since he was born and his first diagnosis was when he was 3 years old. His father thought that it was the last hope for his survival. The little boy had spent six years of his life in chemotherapy which is normally a quiet large period of time for a 3 year old child. Everything turned out well for the boy and his father but an unfortunate turn in events took place. After three years, in 2016, his cancer relapsed. At that time the boy started studying about the CAR therapy and its side effects and he and his family decided that he will go through the therapy once again as the chances of survival increase. So, they got him admitted to the Duke University Children's Hospital. The doctor took his blood sample and sepearted the T-cells. These cells were then modified and genetically engineered and after approximately 30 days the doctor infused those T-cells inside the patient body where they can get multiplied. Then by the approval of the FDA doctor gave him a technique called the kymriah procedure. These results were particularly saddening because the patients in these trials were dealing with a recurrence of cancer or had been through at least two other treatments that didn't work. But doctors also said that with advancement in technology, comes it side effects. Many patient who are gone through these CAR T-cell therapy like Connor had suffered from a low power immune system after infusion of the T-cells into their body. These cells make the immune system extra active which is not healthy for the human body because it destroys other vital organs. The second reason for the T-cells to be not marked up to safety mark is because it can cause cytokines into the bloodstream. These cytokinesis release syndrome causes high fever to the body along with effects to heart including rise in blood pressure which can further cause death.(Ruella,2016).

Recent reports reveal that in hospitals where patients are treated with the kymriah , almost 60% patients have seen side effects. And even in some patients these syndrome were so high that some of the patient had to be under strict watch for a long time. In 2016, almost five patients died due to fatal swelling in their brain. After all these deaths, the company stopped the trails for this treatments and the doctors treating them were questioned about the cause of all the deaths that kept happening. The treatment has been specified to very limited people because of these side effects which have seen to be fatal.(Ruella,2016).

Even after going through the FDA approved treatments, these side effects have been seen in many patients. This is the only reason why only very specialized doctors have been approved to give this treatment as it requires a lot of precision and experience. For example the 'yescarta' which is also an FDA approved technique is only given in 50 hospitals of the US. From these clinical trials, research has been continuing in this field to make these genetically engineered T-cells in a different way or adding new techniques in infusion to prevent the side effects of the therapy. The doctors are studying to manage these side effects for their patients efficiently.(Ruella,2016).

Concluding from all the reports and case studies, it is seen that the medical world has been trying to improve itself with the new technologies and treatments. But after the treatments, it is only

oneself that can promote healthy and safe living for a better world. The doctors effeorts only matter if the patient puts in his own as the consequences and the reality of the situation can only be felt by him. To make this world a better and healthy place, the first step is to have the strength inside the patient to fight off everything that comes in his way. (Ruella,2016).



Future of CAR T-cell therapy

The future of CAR T-cell therapy is still very vague as it was evident from case study of Conner who had spent 18 years of his life with this disease, going from hospitals to hospitals where he could have had a bright future studying instead. So from this report we can clearly understand

that the future of CAR T-cell has a lot of problems, all from different aspects like technical and logistic, economical, ongoing innovations and future perspectives. A few of these are listed in the table below along with a solution-

Challenge	Possible Solution	Example
Immunosuppressive microenvironment	Cytokine secretion	IL-12 secretion[15]
		IL-18 secretion[23]
	Costimulatory molecule ligand expression	Coexpression of a 1928z CAR with 41BBL[24]
	Blockade of tumor derived T cell inhibitory signals	Adenosine 2A receptor blockade[25]
		Monoclonal antibody-mediated checkpoint blockade[26]
		CRISPR/Cas9-mediated PD-1 knockout[20]
		PD-1 blocking scFv secretion[27]
	Chimeric switch receptors	PD-1 receptor combined with CD28-signaling domain[28]
	Metabolic reprogramming	Review of methods targeting T cell metabolism to enhance immunotherapy[29]
		Regulation of metabolism through different CAR-signaling pathways[30]
Tumor site trafficking and infiltration	Enzyme secretion	Heparanase secretion[31]
	Chemokine receptor expression	Forced CCR2b expression increased trafficking to CCL2 expressing tumor cells[16]
	Local infusion	Regional delivery to treat breast cancer brain metastases[32]
Toxicity	Novel cytokine inhibition	GM-CSF inhibition to reduce CRS and neurotoxicity[33]
	Suicide switches	Cetuximab targeting of truncated EGFR[34]
		Inducible caspase 9[35]
	Drug induced on/off switches	Doxycycline induced Tet-On switch[36]
	Logic gates allowing greater control of on-target off-tumor effects	synNotch driven combinatorial antigen recognition ("AND" gate)[37]
		Inhibitory CAR ("NOT" gate)[38]
	Transient CAR expression	mRNA electrotransfer to generate an EGFR-specific CAR[39]
	Humanized scFv	mRNA engineered mesothelin-directed CAR[40]
		Clinical trial with a humanized CD19-directed CAR-T cell[41]
Improving efficacy and persistence	Novel tumor-associated antigen selection	A Tn-Glyoform of MUC1 as a novel tumor-associated antigen[42]
	Simultaneous infusion of T cell stimulating cytokines	IL-2 vs IL-15 in support of antigen-specific CTLs[43]
	T cell subset selection	CD4:CD8 ratio[44]
		CD4+ and CD8+ T cell subsets[45]
		Virus-specific central memory CD19-directed CAR-T[46]
		Stem-like T cells[47]
	Reduction in tonic signaling	Mechanisms of 4-1BB-related tonic signaling[48]
		Review of methods to combat tonic signaling[49]
	Locus-specific insertion	CRISPR/Cas9-mediated TRAC locus insertion[18]
Tumor heterogeneity	Multiple targets	Modular, universal CAR systems[13,14]
		APRIL-based CAR targeting multiple MM antigens[50]
Time of manufacture	Allogeneic "off-the-shelf" CARs	Endogenous TCR elimination using the Sleeping Beauty system[51]
		TALEN-mediated TCR and CD52 elimination[52]
		CRISPR-mediated universal CAR-T cell generation[53]
		iPSC derived CAR NK cells[54]
	Accelerated manufacture	Review of manufacturing techniques and process[55]
		CliniMACS Prodigy[21]
		In vivo CAR T cell manufacture[22]
T cell neoplasms	CAR-NK cells	CD3-targeting CAR engineered into an NK cell line[56]
	Target gene knockout	Genomic disruption of CD7 in a CD7-targeted CAR-T[19]

This is not an exhaustive list, but seeks to highlight several of the areas where technological developments have the potential to forward the efficacy, safety, and availability of CAR-T cell therapy. For further discussion of the challenges facing CAR-T cell development, the reader is directed toward Elahi et al's comprehensive review.[57]
CAR = chimeric antigen receptor, CRS = cytokine release syndrome.

The medical world has been bringing a lot of new advancements in this field to evade antigen-negative relapses, improve the persisting power of T-cells, make the T-cells more efficient in killing tumors and bringing CAR T-cell therapy for treatment of other diseases as well.

CONCLUSION

From the above research, we can conclude that leukemia is a life-threatening disease and CAR T-cell therapy has proven to be very efficient for its treatment. As with all good comes a single defect, the CAR T-cell therapy also shows some fatal side effects which can last for a lifetime.

But with the ongoing research on T-cells, the therapy will become more patient-friendly and also be used to treat other dreadful diseases in the medical world.

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