International Journal of Pharmaceutics & Drug Research
ISSN: 2347-6346
Available online at http://ijpdr.com

REVIEW ARTICLE

CAR-T CELL THERAPY: A REVOLUTIONARY TREATMENT IN CANCER

Vekariya Mansi Rameshbhai*, Shukla Maitri, Bhakhar Dharmik, Patel Dipen, Mr. Bhavin Bhimani, Mr. Ghanshyam Patel, Mr Jaymin Patel

Arihant School of Pharmacy and Bioresearch Institute, Uvarsad Square, Sarkhej-Gandhinagar Highway, Post Adalaj, Gandhinagar, Gujarat 382421

***Correspondence Info:**
Mansi Vekariya,
Arihant School of Pharmacy and Bioresearch Institute, Uvarsad Square, Sarkhej-Gandhinagar Highway, Post Adalaj, Gandhinagar, Gujarat 382421
Email:
Maitrishukla1234@gmail.com

***Article History:**

Received: 14/08/2020
Revised: 26/09/2020
Accepted: 03/12/2020

ABSTRACT

The global burden of cancer continues to increase largely because of the aging and growth of the world population alongside an increasing adoption of cancer. Mainly, there are many types of cancer and with therapies for treatment to fight or slow down the carcinogenic cells. The immune cell (T- lymphocytes) plays a crucial role in fighting against the infected carcinogenic cells. Car t- cell therapy is widely used with immune cells, succeeding to fight against cancer. Chimeric antigen receptor T cells are T cells that have been genetically engineered to produce an artificial T-cell receptor for use in immunotherapy. The FDA-approved CAR T-cell therapy products are used only for patients with adult B-cell non-Hodgkin's lymphoma or childhood acute lymphoblastic leukemia who have already been through two unsuccessful standard treatments. Chimeric antigen receptors (CAR) are receptor proteins that have been engineered to give T cells the new ability to target a specific protein. CAR is potentially engineered in the patient's immune cells in the laboratory and injected back into the patient's body. Where, they bind to specific receptor, present on the surface of the tumour cells to fight cancer. It's important for the specification of the manufacturing immune cells for the successful efficacy against the cancer. There could be succeeding future aspects, amidst the review on the car t-cell therapy.

Key words: Cancer, CAR-T cell therapy, targeted approach, Non hodgkins lymphoma.

INTRODUCTION:

Cancer is the second leading cause of death worldwide after cardiovascular diseases. Cancer is not a new disease and has afflicted people throughout the world. The word cancer came from a Greek words Karakinos to describe carcinoma tumors by a physician Hippocrates (460–370 B.C), but he was not the first to discover this disease. Some of the earliest evidence of human bone cancer was found in mummies in ancient Egypt and in ancient manuscripts dates about 1600 B.C (American Cancer Society, 2019).

LYMPHOMA

"Lymphoma" is a general term for a group of blood cancers that start in the lymphatic system, which is part of the body's immune system. The two major types of lymphoma are Hodgkin lymphoma (HL) and non-Hodgkin lymphoma.

Both Hodgkin and non-Hodgkin lymphoma are further classified into subtypes.

NON HODGKIN'S LYMPHOMA AND LYMPHOBLASTIC LEUKEMIA

"Non-Hodgkin lymphoma" (NHL) is the term used for a diverse group of blood cancers that share a single characteristic—they arise from lymphocytes. Lymphocytes are white blood cells that are part of our immune system. A lymphocyte undergoes a malignant change and multiplies, eventually crowding out healthy cells and creating tumors. These tumors generally develop in the lymph nodes or in lymphatic tissue found in organs such as the stomach, intestines or skin (Jain *et al*., 2013).

Lymphocytic or lymphoblastic leukemias are closely related. A cancer that originates in the lymphatic tissue within the marrow is designated "lymphoblastic" or "lymphocytic" leukemia. Acute lymphoblastic leukemia and chronic lymphocytic leukemia are the two major examples of this type of blood cancer (Roschewski *et al*., 2014).

NHL can grow and spread at different rates and is grouped into two subtypes: aggressive or indolent. Aggressive lymphomas are rapidly progressing or high-grade NHL subtypes and account for about 60 percent of all NHL cases. Diffuse large B-cell lymphoma (DLBCL) is the most common aggressive NHL subtype (Appelbaum, 2014).

Stages

Stage I: Involvement of one lymph node group

Stage II: Involvement of two or more lymph node groups on the same side of the diaphragm (a thin muscle below the lungs)

Stage III: Involvement of lymph node groups on both sides of the diaphragm

Stage IV: Involvement of one or more organs other than the lymph nodes and possible involvement of the lymph nodes (Freedman *et al*., 2015).

Categories A, B, X and E. The four stages of NHL can be divided into categories.

- The A category indicates that patients have not experienced fever, exaggerated sweating and weight loss.
- The B category indicates that patients have fever, excessive sweating and weight loss.
- The X category indicates bulky disease (large masses of lymphocytes).
- The E category indicates areas or an organ involved other than the lymph nodes or has spread to tissues beyond, but near, the major lymphatic areas (American Joint Committee on Cancer, 2017).

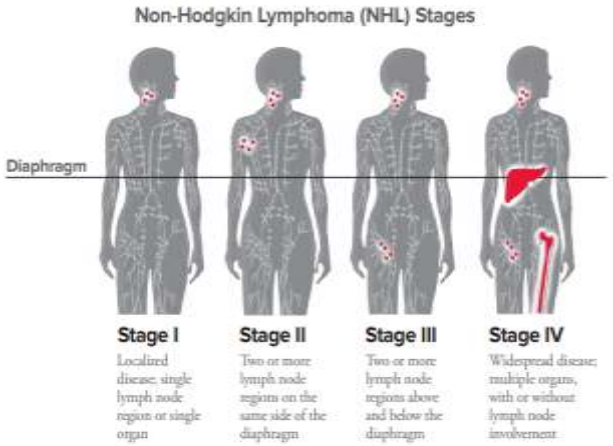

Figure 1-NHL stages

Treatment

In general, chemotherapy and radiation therapy are the two principal forms of treatment for NHL. Although radiation therapy is not often the sole or principal curative therapy, it is an important additional treatment in some cases. Stem cell transplantation and a watch-and-wait strategy are also used to treat some NHL subtypes. Other forms of treatment are emerging, and some are already approved for specific forms of NHL (Cheson *et al*., 2014).

LYMPHOBLASTIC LEUKEMIA

Acute lymphocytic leukemia (ALL) is also called acute lymphoblastic leukemia. “Acute” means that the leukemia can progress quickly, and if not treated, would probably be fatal within a few months. "Lymphocytic" means it develops from early (immature) forms of lymphocytes, a type of white blood cell (Hoelzer, 2011).

ALL mainly affect the bone marrow and the blood. Sometimes it can be hard to tell if a cancer of lymphocytes is a leukemia or a lymphoma. Usually, if at least 20% of the bo.ne marrow is made up of cancerous lymphocytes (called lymphoblasts, or just blasts), the disease is considered leukemia (2012).

Treatment

- **Induction therapy.** The purpose of the first phase of treatment is to kill most of the leukemia cells in the blood and bone marrow and to restore normal blood cell production (Pui, 2010).
- **Consolidation therapy.** Also called post-remission therapy, this phase of treatment is aimed at destroying any remaining leukemia in the body, such as in the brain or spinal cord.
- **Maintenance therapy.** The third phase of treatment prevents leukemia cells from regrowing. The treatments used in this stage are often given at much lower doses over a long period of time, often years (Pui *et al*., 2012).
- **Preventive treatment to the spinal cord.** During each phase of therapy, people with acute lymphocytic leukemia may receive additional treatment to kill leukemia cells located in the central nervous system. In this type of treatment, chemotherapy drugs are often injected directly into the fluid that covers the spinal cord (Vardiman et al., 2009).

DIAGNOSIS

Table 2: Common Laboratory Test for Diagnosis of all

Test	Description	Clinical applications
Bone marrow aspirate or biopsy	Examination of a greater concentration of hematopoietic cells	Identification of blast cells in acute myelogenous leukemia and acute lymphoblastic leukemia Extent of marrow involvement correlates with prognosis in chronic lymphocytic leukemia
Cytogenetic testing	Examination of whole chromosomes through karyotyping or fluorescence in situ hybridization analysis	Detection of the Philadelphia chromosome (BCR-ABL1 fusion gene) for the diagnosis of chronic myelogenous leukemia Identifying chromosomal abnormalities to diagnose leukemia subtypes Can be used to guide treatment and determine prognosis
Flow cytometry with immunophenotyping	Sorting and counting cells from peripheral blood or bone marrow sample) by specific cell surface markers	Counting cloned cells of lymphoid lineage for the diagnosis of chronic lymphocytic leukemia Identifying certain cell surface markers to diagnose leukemia subtypes
Molecular testing	Testing for specific mutations at the DNA level through polymerase chain reaction testing	Detection of the Philadelphia chromosome (BCR-ABL1 fusion gene) for the diagnosis of chronic myelogenous leukemia Aids in the diagnosis of leukemia subtypes; can also be used to guide treatment and determine prognosis
Peripheral smear	Examination of whole blood specimen under the	Identification of Auer rods in acute myelogenous leukemia, and blast cells in acute myelogenous leukemia and acute lymphoblastic leukemia

CAR T-Cell

CAR T-cell therapy is a cancer treatment that uses a patient's own immune system cells, known as T cells, after these cells have been modified to better recognize and kill the patient's cancer. The T cells are engineered in the laboratory & then expanded to large numbers and infused back into the patient. This type of treatment transfers an immune system into the patient that is capable of immediately killing the cancer. CAR stands for chimeric antigen receptor, which represents the genetically engineered portion of the T cell. The CAR part of the T cell contains proteins that allow the T cells to recognize the specific cancer cells that become highly activated to kill the cancer cells. Once in the body, the CAR T cells can further grow to large numbers, persist for long periods of time, and provide ongoing tumor control & possible protection against recurrence (John *et al.*, 2017).

Chimeric antigen receptor T cells are engineered constructs composed of synthetic receptors that direct T cells to surface antigens for subsequent elimination. Many CAR constructs are also manufactured with elements that augment T-cell persistence & activity (Newick *et al.*, 2016). T cell engineering allows to rapid generation of T cells of any desired specificity. The rationale for this approach to cancer Immune therapy is to bypass the barriers to active immunization to establish T cell-mediated tumor immunity. CARs are recombinant receptors for antigen (Zhu and Paul, 2008), which in a single molecule, redirect T cell specificity and eventually increased anti-tumor potency (Zhao et al., 2015).

CARs T-cell therapy ushers in a new dawn in the age of cancer immunotherapy. The ability of genetic engineering to change the genome and induce specificity of T-cells against cancer cells in the form of chimeric antigen receptor is a revolutionary step in the rapidly evolving cancer treatment. It is a form of adoptive cell transfer (ACT) (Hasan et al., 2015).

Recent U.S. Food and Drug Administration "FDA" approval of "CAR-T" cell therapy for relapsed refractory (R/R) acute lymphoblastic lymphoma and for B cell NHL (Diffuse large B cell lymphoma) is encouraging and underscores the need for continued engagement in developing this novel therapy (Mchayleh et al., 2019). CAR T cells have demonstrated tremendous success in eradicating hematological malignancies (e.g., CD19 CARs in leukemias). This success is not yet extrapolated to solid tumors, and the reasons for that are being actively investigated. Among patients with relapsed and refractory malignancies, chimeric antigen receptor T cell

therapy is a novel immunotherapy that has shown promise in both preclinical and early clinical studies. This therapy allows for CARs directed against tumour cells - associated antigens (eg, CD19, HER-2) to be introduced into a patient's T-cells; this serves to reprogram these cells to target the patient's tumour cells.

A number of small clinical trials using anti-CD19 CAR-T cells in haematological malignancies have demonstrated sustained responses in patients with advanced disease. CAR-T cell therapy for solid malignancies has also identified a number of potential cancer-specific targets & previous preclinical studies investigating efficacy & feasibility show promise (Grigor et al., 2017).

T CELL ACTIVATION:

Signal One:

T cells are generated in the Thymus and are programmed to be specific for one particular foreign particle eg.antigen. Once they leave the thymus, they circulate in the body until they recognise their antigen on the surface of antigen presenting cells (APCs). The T cell receptor on both CD4+ helper T cells and CD8+ cytotoxic T cells binds with the antigen as it is held in a structure called the MHC complex, on the surface of the APC. This triggers initial activation of the T cells. The CD4 and CD8 molecules then bind to the MHC molecule too, stabilising the whole structure. This initial binding between a T cell specific for one antigen and the antigen-MHC it matches sets the whole response in the motion. This normally takes place in the secondary lymphoid organs.

Figure 3- interaction between T-Cell & dendritic cell

Signal Two

In addition to T cell receptor (TCR) binding to antigen-loaded MHC, both helper T cells and cytotoxic T cells require a number of secondary signals that become activated and respond the threat. This molecule on the T cell binds with one of two molecules on the APC – B7.1 (CD80) or B7.2 (CD86) – and initiates T-cell proliferation. This process leads to the production of millions of T cells that recognise the antigen. In order to control the response, stimulation of CD28 by B7 induces the production of CTLA-4 (CD152). This molecule competes with CD28 for B7 and so reduces activation signals to T cell and winds down the immune response. Cytotoxic T cells are less reliant on CD28 for activation but it requires signals from other co-stimulatory molecules such as CD70 and 4-1BB (CD137).

T cells must recognise foreign antigen strongly and specifically to mount an effective immune response and those which are given survival signals by several molecules, including ICOS, 4-1BB and OX40. These molecules are found on the T-cell surface and stimulated by their respective ligands which are typically found on APCs. Unlike CD28 and the TCR, ICOS, OX40 and 41BB are not constitutively expressed on T cells. Their respective ligands are only expressed on APCs following pathogen recognition. This is important because it ensures T cells are only activated by APCs which have encountered a pathogen and responded. Interaction of the TCR with

peptide-MHC in the absence of co-stimulation switches the T cells off, so that they do not respond inappropriately (Findlay, 2019).

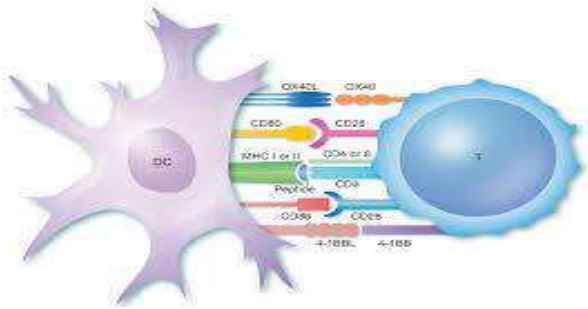

Figure 4- Schematic of early T cell activation.

The T cell encounters a dendritic cell (DC) bearing its cognate peptide in an MHC molecule, and binds the peptide-MHC though CD3 and CD4 or 8. Subsequently, co-stimulation occurs through DC-bound CD86, CD80, OX40L and 4-1BBL. This induces full activation and effector function in the T cell.

Signal Three

Once the T cell has received a specific antigen signal and a general signal two, it receives more instructions in the form of cytokines. These determine which type of responder the cell will become – in the case of helper T cells, it will push them into Th1 type (cells exposed to cytokine IL-12), Th2 (IL-4), or IL-17 (IL-6, IL-23). Each one of these cells performs a specific task in the tissue and in developing further immune responses. The resulting cell population moves out to the site of the infection or inflammation in order to it deal with the pathogen. Other cells that present at the tissue site of inflammation– such as neutrophils, mast cells, and epithelial cells – can also release cytokines, short peptides and other molecules which induce further activation and proliferation of the T cells (Luckheeram et al., 2012).

CARs combine with both antibody-like recognition with T-cell activating function. They are composed of an antigen-binding region, that typically derived from an antibody (Eshhar et al., 1993), a transmembrane domain to anchor the CAR to the T cell (Bridgeman et al., 2010), and one or more intracellular signaling domains that induce persistence, trafficking and it gives effector functions in transduced T cells (Finney et al., 1998; Krause et al,1998) .

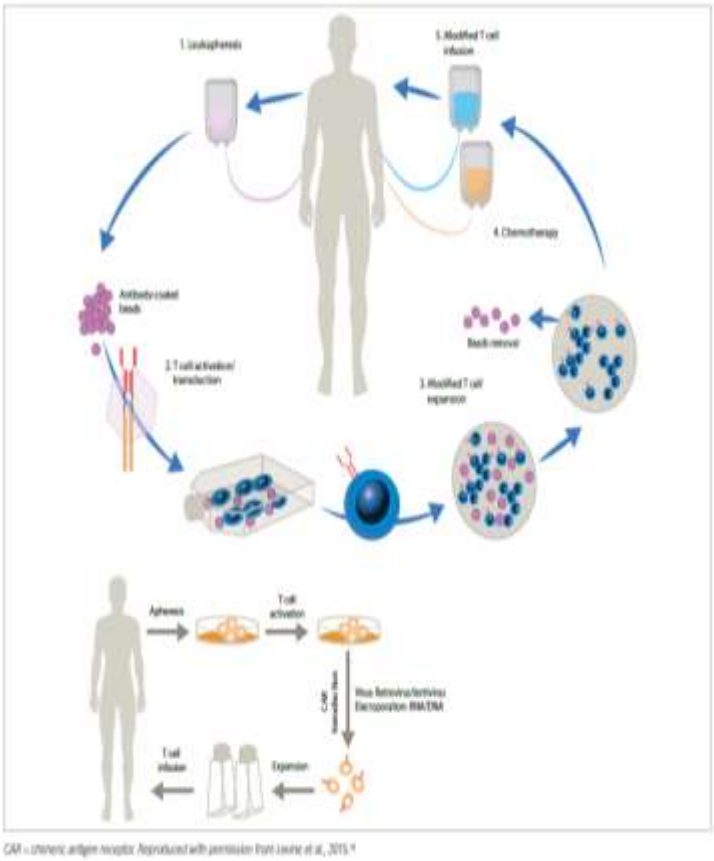

Figure 5- Generation of CAR-T cells

Sequences used to define the antigen-targeting motif for a CARs are typically derived from a monoclonal antibody, but ligands (Muniappan et al., 2000) & other receptors (Zhang et al., 2012) it can also be used. CAR-expressing T cells (CAR T cells) recognise a variety of types of antigen, not only protein but also carbohydrate and glycolipid structures typically expressed on the cell surface of a tumour. Unlike for TCR recognition, the antigen does not need to be in the process and presented by MHC and therefore the same

CARs -based approach can be used in all patients who express the same tumour antigen regardless of HLA type (Sharpe and Mount, 2015).

GENERATION OF CAR T CELLS

CAR T cell manufacture start with collection of mononuclear cells (MNCs) from the patient's blood via leukapheresis (Leukapheresis is a laboratory procedure in which WBC are separated from a sample of blood. It is a specific type of apheresis, the more general term for separating out one particular constituent of blood and returning the remainder to the circulation.) (Figure 1).

CD3+ T cells may be further enriched ex vivo and then it is modified to express a transgene encoding a tumor-specific CAR. The CAR-modified (Zhang et al., 2012), T cells undergo ex vivo expansion (Sharpe and Mount, 2015) in optimised T cell culture conditions. The final product is harvested and formulated at a specified dose.

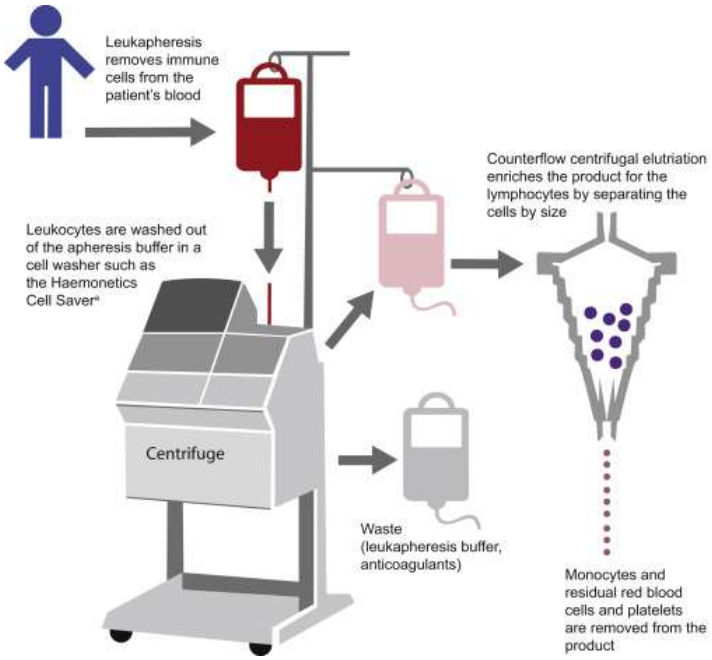

Figure 6- The lentiviral vector manufactured by Oxford BioMedica Ltd

HARVESTING AND ISOLATION T CELLS FOR CAR T CELL MANUFACTURING

Leukapheresis is the most efficient way to obtain significant numbers of T lymphocytes to initiate CAR T cell culture. Apheresis, which is derived from an ancient Greek word meaning "to take away", describes the process by which whole blood is removed from an individual to be separated into components by centrifugation; one or more components are selectively removed and the remainder of the blood is returned to the circulation.

- For the purpose of CAR T cell manufacture, the mononuclear cells layer containing lymphocytes and monocytes is the target cell layer to be collected by the apheresis device. Leukapheresis remains the method of choice for T cell collection given that semi-continuous or continuous flow of the blood allows for processing of large blood volumes providing large T cell isolation.
- It may be challenging to collect mononuclear cells (MNC) products with adequate purity.
- The mononuclear cells layer itself also contains monocytes and non-T lymphocytes including B cells and NK cells in addition to T lymphocytes. Accordingly, MNC products include these cell types to varying degrees. In addition, MNC products may be contaminated with RBCs, granulocytes and circulating tumour cells or leukemic cells, further complicating downstream manufacturing. Using leukapheresis, it is possible to enrich MNCs from several non-target cells (red blood cells, platelets and granulocytes). In addition, leukapheresis devices such as the Spectra Optia Apheresis® System

(Terumo BCT, Lakewood, Colorado, US) offer the ability to further optimise collection technique. Ex vivo, a variety of techniques and devices can be used to further separate or enrich a cellular population from the MNC product.

- Magnetic beads bearing antibodies specific for T cell surface markers can be used to isolate the T cells from the lymphocyte fraction. MNC products with low T cell percentages may benefit from bead-based selection of the target CD3+ T cell population prior to initiation of culture. In addition, beads may be used to activate and expand the T cell population (Onea et al., 2016).
- Typically, peripheral blood comprises around 20–40% lymphocytes, although circulating lymphocytes can be suppressed in patients who being treated for underlying malignancies.
- In the addition, most collections occur at steady state, i.e., in the absence of haematopoietic stem cell mobilising agents.
- Therefore, processing large blood volumes may be necessary to obtain adequate T cell yield. In this respect, anticoagulant infusion is a most important consideration. We opt for apheresis devices where anticoagulant infusion is managed carefully, for instance as with the Optia apheresis device.
- To prevent blood clotting in the apheresis device, an anti-coagulant such as Acid Citrate Dextrose Formula A (ACD-A) is mixed with the blood as it is circulating through the machine.
- As it mixes with the whole blood in circulation , citrate binds divalent cations, including calcium, a necessary component in coagulation. The patient is exposed to citrate as the blood components are returned to patient body and may experience transient hypocalcemia.15 Heparin may also be considered as an anticoagulant; however, this drug is not taken without its own risk of adverse reaction.
    - Some studies have provided strategies to allow larger volume apheresis collections to enhance yield and manage citrate toxicity (Hirahara et al., 2016).

ACTIVATION AND MODIFICATION OF CAR T CELLS

Once T cells have been collected, they must be activated, transduced and expanded before being re-infused into the patient. In vivo, endogenous antigen presenting cells (APCs), such as dendritic cells or B cells, may activate cognate T cells. Ex vivo, however, these endogenous APCs display inherent variability making their use impractical in a GMP setting. Robust T cell activation for CAR T cell manufacture can be done using soluble anti-CD3 monoclonal anitbodies (mAbs), anti-CD3/anti-CD28 monoclonal anitbodies (mAbs), coated paramagnetic beads or cell-based engineered bead-based methods concurrently provide a method to positively select T cells, whereas synthetic cell based artificial APCs allow for customisation of stimulatory conditions (Golubovskaya and Wu, 2016). Commercial and technical limitations of bead and APCs respective may make widely available GMP-grade, soluble monoclonal antibody (mab) stimulation preferable in some situations. After

stimulation, T cells are then genetically modified to express the CAR.

Currently, viral transduction with either gamma retroviral or lentiviral vectors are the most common method of gene transfer owing to the high efficiency of gene delivery and the persistence of integrating vectors use in the modified T cells. Lentiviral vectors may be preferable to retroviral vectors due to their ability to transduce non-dividing as well as dividing cells is more (Salas-Perdomo et al., 2018).

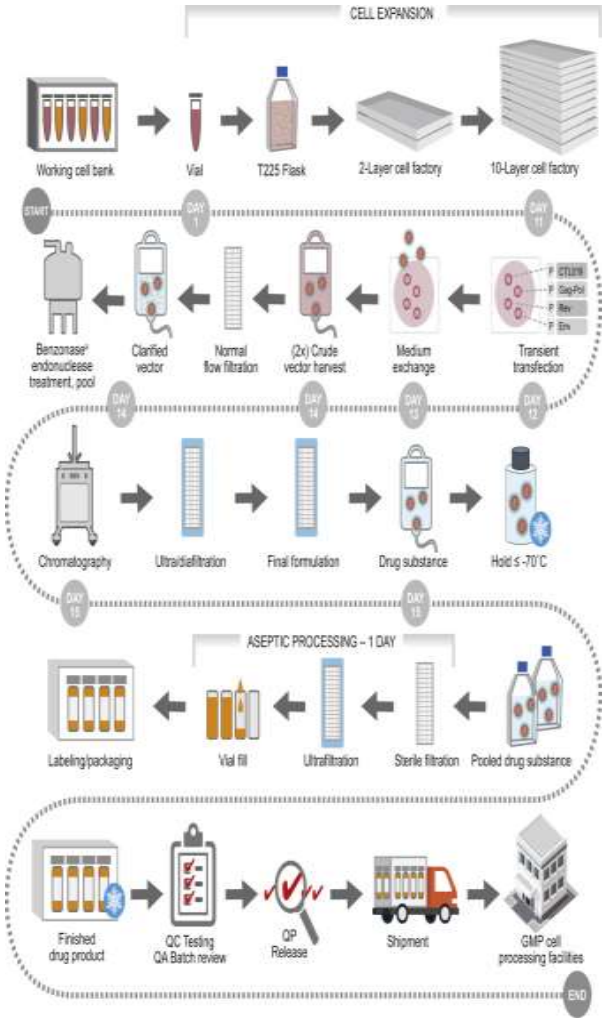

Figure 7- actual mechanism for lentiviral vector manufacturing

It undergoes several rounds of filtration and testing to ensure that a high-quality product with minimal variability is produced. The process has been designed and optimized to manufacture vector in a series of multiple sub-batches. This approach relies upon a purification and formulation step prior to cryopreservation and a subsequent 'hold' at ≤ -70º C. Specific sub-batches are then selected for final aseptic processing, which is completed in a single day. QA, quality assurance; QC, quality control; QP, qualified person. a Merck KGaA.

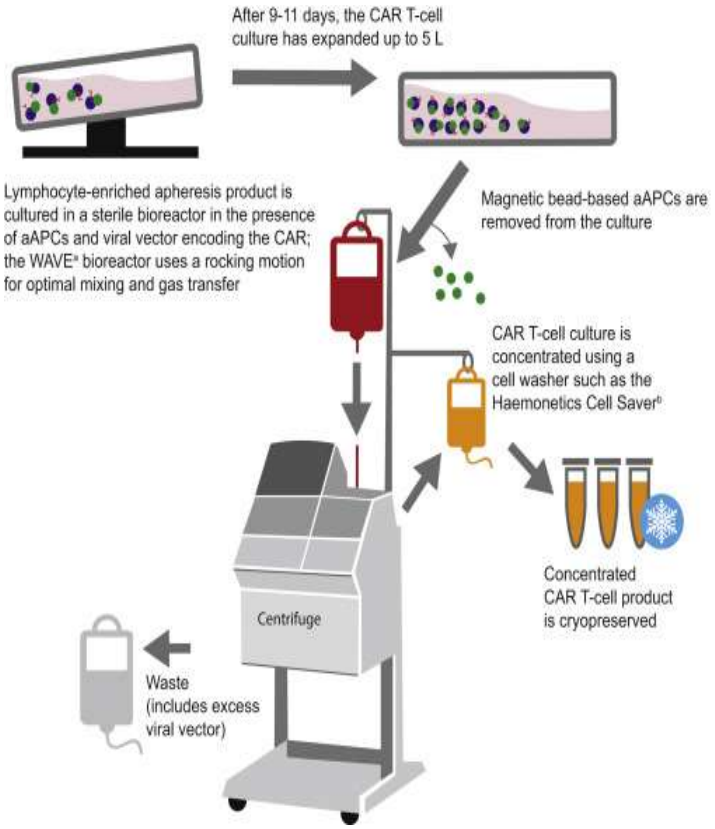

Figure 8- Transition to commercial manufacturing

As CAR T-cell therapies transition from flexible processes at single academic institutions to highly controlled processes that can be implemented across many collection, manufacturing, and treatment sites, the coordination between these sites will be crucial to ensure that the material is handled correctly and patients are scheduled properly or appropriately.

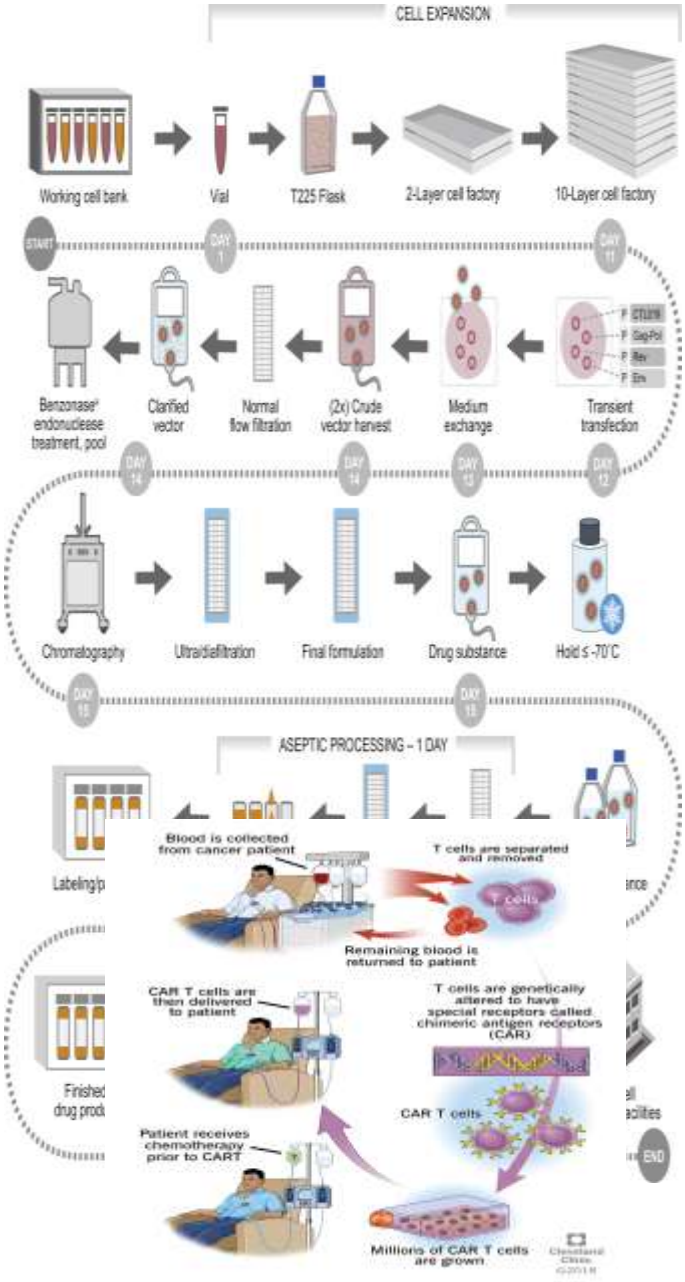

Figure 9-centres where close interaction is possible.

HOW DOES CAR T-CELL THERAPY WORK?

The process started by collecting blood from the patient with cancer. During this process, T cells are separated and removed from the blood and the remaining blood is returned to the body. This procedure is called leukapheresis or apheresis and is similar to the process of giving certain types of blood donations. T cells, which are a type of WBCs of the immune system, are the body's primary killing cells. They protect the body by destroying abnormal cells, including cancers.

Sometimes, however, T cells don't recognize cancer cells or cannot fully destroy all of them in the body. To improve the cancer-killing ability of T cells, the next step is to genetically alter them. This is done in a special laboratory condition. The altered T cells now have special receptors on their surface. These new receptors, called chimeric antigen receptors (CAR), allow the T cells to better recognize cancer cells, become activated, and kill their target cell. These altered T cells are now called a chimeric antigen receptor (CAR) T cells. The CAR T cells are then grown in a special laboratory until millions are produced.

Figure 10- working of CAR-T cell therapy

Next, the patient receives a brief course of chemotherapy, which improves the chance that the new CAR T cells will be accepted and not attacked by the immune system when returned to the body. Finally, the CAR T cells are delivered back into the patient body through an infusion into the patient's bloodstream. Once in the body, the CAR T cells continue to multiply. The CAR T cells attach to a specific structure, called an antigen (most commonly a

protein called CD19)[29], on the surface of the targeted cancer cells. Once attached, the T cells become activated and release toxins that kill the cancer cells. The CAR T cells remain in the body for a long time after the infusion, helping to fight cancer if it returns and keep the patient in remission.

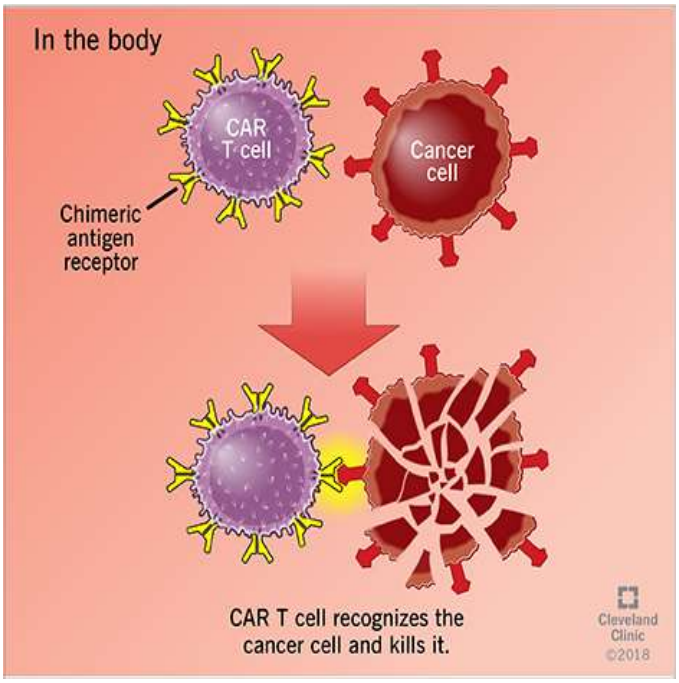

Figure11- Action of CAR-T cells inside the body

Clinical trials with CAR T cells

- The clinical evaluation of CAR therapies has grown exponentially, with the majority evaluating the treatment of B-cell cancers. Most B-cell malignancies as well as normal B cells express the CD19 antigen but this is absent from other cell types, to make it an attractive therapeutic target.
- There are slight variations in the composition of the different anti-CD19 CAR T cells in trial (Maher, 2014) and the clinical trial designs have been variable (Kershaw et al., 2013), but several trials have now reported very impressive response rates in 60-90% of patients with relapsed or refractory lymphoblastic leukaemias (Maus et al., 2014a; Lee et al., 2014; Maude et al., 2014).
- Some responding patients have been consolidated with stem cell transplantation (Lee et al., 2014), whereas others have not, & sustained remissions of up to 2 years have been reported. It is currently unclear how long anti-CD19 CAR T-cell induced remission can be sustained, but clearly this immunotherapy has the potential to be of significant clinical benefit. Following on from the great progress in B-cell malignancies, CAR T-cell therapies are also being developed that target on solid tumours. It is a field in which an earlier stage although signals of efficacy have been observed in neuroblastoma (Pule et al., 2008; Louis et al., 2011).

THE FUTURE:

Strategies to maximize outcomes of CAR T-cell therapies, minimize toxicities, broaden targets beyond receptor CD19, and target solid tumors are all areas of active investigation. And also, standards establishing how CAR T-cell therapy might best fit within current treatment paradigms have not yet been determined.

Improving CAR T-cell safety

Efforts to manage the risk of serious toxicity include:

1. Developing methods to predict individual patient risk of CRS and neurotoxicity so that the dose and timing of CAR T-cell infusion can be adjusted according to it ("risk-adapted dosing").

2. Modulation of CAR T-cell activity and persistence,
including:

- **Development of CARs** that are activated only in the presence of small molecule drugs that they can be administered

according to patient tolerability (known as "switchable" or "multi-chain" CARs).

- **"Suicide" systems**, allowing for the destruction of CAR T cells should be possible of life-threatening toxicity occur. Numerous methods are in development, including co-expressing pro-apoptotic genes under the control of inducible promoters along with the CAR gene.
- **Transient expression systems** that provide CAR expression only for approximately 7 days. Repeated CAR T-cell infusions would theoretically be required to effective disease control.

3. Improving the specificity of CARs. Efforts have included the development:

- **"Affinity-tuned" CARs**, designed with a lower affinity for the target antigen, theoretically narrowing CAR targeting only to tumor cells that greatly over express the antigen.

"Tandem" CARs, in which the 2 CAR cytoplasmic signaling domains are separated onto 2 different CAR molecules having different tumor target specificities. CAR T-cell activation results only in the presence of both targeted antigens, increasing specificity.

IMPROVING CAR T-CELL EFFICACY

Numerous efforts to improve the efficacy and persistence of CAR T cells are underway and include:

- Combining CAR T cells with other therapies, that includes PD-1 blocking antibodies or kinase inhibitors.
- Development of "armored CARs" that co-express pro-inflammatory cytokines such as IL-12 or IL-15 to allow for increased CAR T-cell proliferation & persistence in the face of tumor-mediated immunosuppression.
- Co-expression of 2 CARs that target different antigens (CD19 and CD22, for example) with the goal of reducing remissions because of escape variants.
- Incorporating human-derived rather than mouse derived scFv domains into CARs in order to avoid immune responses targeted to murine sequences in the CAR extracellular domain, which have been observed.

IMPROVING AND STREAMLINING THE MANUFACTURING PROCESS

- Efforts to streamline and standardize the manufacture of CAR T cells in a cost-effective and time-efficient manner are underway. These include the development of "universal" or "off-the-shelf" CARs, which lack endogenous T-cell markers, allowing for allogeneic CAR T-cell therapy.[27] This could be streamline production while broadening CAR T-cell therapy to those patients who lack sufficient numbers of endogenous T cells for processing.
- While CAR T-cell therapy holds great promise, emerging data from clinical trials and post-marketing surveillance of approved therapies will continue to inform to its appropriate place in the clinical management of hematological malignancies.[28]

Reference:

1. American Cancer Society (*2019*). Cancer Facts & Figures. Atlanta: American Cancer Society.
2. Jain, N. Gurbuxani, S. Rhee, C. Stock, W. (2013) Acute Lymphoblastic Leukemia in Adults. In: Hoffman, R., Benz, E.J., Silberstein, L.E., Heslop, H., Weitz, J., Anastasi, J., eds. *Hematology:* Basic Principles and Practice. 6th ed. Philadelphia, Pa: Elsevier.
3. Roschewski, M.J., Wilson, W.H. (2014) Non-Hodgkin Lymphoma. In: Niederhuber, J.E., Armitage, J.O., Doroshow, J.H., Kastan, M.B., Tepper, J.E., eds. *Abeloff's Clinical Oncology.* 5th ed. Philadelphia, Pa: Elsevier.

4. Appelbaum, F.R. Acute Leukemias in Adults. In: Niederhuber, J.E., Armitage, J.O., Dorshow, J.H., Kastan, M.B., Tepper, J.E., eds. Abeloff's Clinical Oncology. 5th ed. Philadelphia, Pa. Elsevier.
5. Freedman, A.S., Jacobson, C.A., Mauch, P., Aster, J.C. (2015) Non-Hodgkin's lymphoma. In: DeVita, V.T., Lawrence, T.S., Rosenberg, S.A., eds. *DeVita, Hellman, and Rosenberg's Cancer: Principles and Practice of Oncology.* 10th ed. Philadelphia, Pa: Lippincott Williams & Wilkins.
6. American Joint Committee on Cancer. (2017) Hodgkin and Non Hodgkin Lymphoma In: *AJCC Cancer Staging Manual*. 8th ed. New York, NY: Springer. 937.
7. Cheson, B.D., Fisher, R.I., Barrington, S.F., et al. (2014) Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: The Lugano classification. J Clin Oncol.32:3059-3068.
8. Hoelzer, D. Novel antibody-based therapies for acute lymphoblastic leukemia. Hematology. American Society of Hematology Education Program 2011; 2011:243-249.
9. Hunger SP, Xiaomin L., Devidas M, et al. (2012) Improved survival for children and adolescents with acute lymphoblastic leukemia between 1990 and 2005: A report from the Children's Oncology Group. Journal of Clinical Oncology. 30(14): 1663-1669.
10. Pui, CH. (2010). Acute lymphoblastic leukemia. In: Lichtman MA, Beutler E, Kaushansky K, et al. eds. Acute lymphoblastic leukemia. Williams Hematology. 8th ed. New York, NY: McGraw Hill Professional. 93.
11. Pui, C.H., Mullighan, C.G., Evans, W., et al. (2012). Pediatric acute lymphoblastic leukemia: where are we going and how do we get there? Blood. 120(6): 1165-1174.
12. Vardiman, J.W., Thiele, J., Arber, D.A., et al. (2009). The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood. 114(5):937-951.
13. John, M. Pagel, M.D. (2017). Chimeric Antigen Receptor (CAR) T-Cell Therapy; American Medical Association. 3(11) : 1595.
14. Newick, K., Moon, E., Albelda, S.M. (2016). Chimeric antigen receptor T-cell therapy for solid tumors; Official journal of the American Society of Gene & Cell Therapy.
15. Zhu, J., Paul, W.E. (2008) CD4Tcells: fates, functions, and faults; Blood. 12:1557-1569.
16. Zhao, Z., Condomines, M., Sjoukje, J.C. Stegen, V.D., Perna, F., Kloss, C.C., Gunset, G., Plotkin, J., Sadelain, M. (2015). Structural Design of Engineered Costimulation Determines Tumor Rejection Kinetics and Persistence of CAR T Cells; Elsevier Inc. 415.
17. Hasan, A.N., Selvakumar, A. O'Reilly, R.J. (2015). Artificial Antigen Presenting Cells: An Off the Shelf Approach for Generation of Desirable T-Cell Populations for Board Application of Adoptive Immunotherapy; Adv Genet Eng. 4:3.
18. Mchayleh, W., Bedi, P., Sehgal, R., Solh M. (2019). Chimeric Antigen Receptor T-Cells: The Future Is Now; journal of clinical medicine.
19. Emma, J.M. Fergusson, D.A., Haggar, F., Kekre, N., Atkins, H., Shorr, R., Holt, R.A., Hutton, B., Ramsay, T., Seftel, M., Jonker, D., Daugaa, M. (2017). Efficacy and safety of chimeric antigen receptor T-cell (CAR-T) therapy in patients with haematological and solid malignancies: protocol for a systematic review and meta-analysis; BMJ Open.

20. Findlay E.G. (2019). T- cell activation; Bitesized immunology. https://www.immunology.org/public-information/bitesized-immunology/systems-and-processes/t-cell-activation
21. Luckheeram R.V., Zhou. R., Verma, A.D., Xi, B. (2012). CD4+TCells: Differentiation and Functions; Clinical and Developmental Immunology.
22. Muniappan, A., Banapour, B., Lebkowski, J., Talib, S. (2000). Ligand-mediated cytolysis of tumor cells: use of heregulin-ζ chimeras to redirect cytotoxic T lymphocytes. Cancer Gene Ther. 7, 128–134.
23. Zhang, T., Wu, M. R., Sentman, C. L. (2012). An NKp30-based chimeric antigen receptor promotes T cell effector functions and antitumor efficacy in vivo. J. Immunol. 189, 2290–2299.
24. Sharpe, M. Mount N. (2015). Genetically modified T cells in cancer therapy: opportunities and challenges; Disease Models & Mechanisms.
25. Onea, A.S., Jazirehi, A.R. (2016) CD19 chimeric antigen receptor (CD19 CAR)-redirected adoptive T-cell immunotherapy for the treatment of relapsed or refractory B-cell Non-Hodgkin's Lymphomas; Am J Cancer Res. 6(2):403-424.
26. Hirahara, K. Nakayama T. (2016). CD4+ T-cell subsets in inflammatory diseases: beyond the Th1/Th2 paradigm; The Japanese Society for Immunology. 28 (4):163–171.
27. Golubovskaya, V. Wu, L. (2016). Different Subsets of T Cells, Memory, Effector Functions, and CAR-T Immunotherapy; Cancers (MDPI). 8: 36
28. Salas-Perdomo, A., Miró-Mur, F., Urra, X., Justicia, C., Gallizioli, M. and Zhao, Y. (2018). Planas; T Cells Prevent Hemorrhagic Transformation in Ischemic Stroke by P-Selectin Binding; Arterioscler Thromb Vasc Biol. 38:1761-1771
29. Grover N.S. and Savoldo, B. (2019). Challenges of driving CD30-directed CAR-T cells to the clinic; BMC. 19:203.