WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 14, 1444-1476. Research Article ISSN 2277– 7105

IMMUNOTHERAPY OF CANCER USING CAR – T CELL THERAPY AND ITS TOXICITY AND MANAGEMENT

***Sameer Kumar Chinari**

School of Pharmaceutical Sciences, Delhi Pharmaceutical Sciences and Research University, New Delhi.

Article Received on
21 Sept. 2020,

Revised on 11 Oct. 2020,
Accepted on 31 Oct. 2020

DOI: 10.20959/wjpr202014-19192

***Corresponding Author**
Sameer Kumar Chinari
School of Pharmaceutical Sciences, Delhi Pharmaceutical Sciences and Research University, New Delhi.

ABSTRACT

Cancer has caused About 9.6 million estimated deaths in 2018. Medications developed in past years have helped in overcoming the disease, but not all patients are fortunate enough and suffer a relapse. In recent times, a newer therapy has emerged, which involves the use of the patient's immunity and adapt it to identify and kill the tumour cells in the body. This is known as Adoptive Cell Therapy/Transfer, where some specific cells of the diseased organism are transferred into a healthy organism to impart immunity against the disease. This idea, with time developed into a new treatment method named Chimeric Antigen Receptor (CAR) – T cell therapy. The therapy involves the isolation of the T – cell lymphocytes from the patient who is suffering from cancer, specifically Large B-cell Lymphoma (LBCL) or Acute Lymphoblastic Leukaemia (ALL). specific genes are added to the cells that will code for cancer-specific receptor named Chimeric Antigen Receptor. Bulk volume of these cells is developed in cell cultures. Before transfusion, the patient is administered some chemotherapy to decrease the risk of inflammation due to the therapy. After the transfusion, patients are kept in observation for up to 2 weeks in case there is any adverse effect due to the therapy. The most usual side effect is Cytokine Release Syndrome which causes disorders like fever, fatigue, nausea, rashes, headache, low blood pressure and other inflammatory responses. The side effects are largely managed by some medications. Currently, the US-FDA has approved three treatment therapies for ALL and BLCL.

KEYWORDS

- Chimeric Antigen Receptor – T cell Therapy (CAR-T cell therapy)

- Immunotherapy
- Cancer therapy
- Adoptive Immunotherapy
- Leukaemia
- Lymphoma
- Personalized therapy

Review of literature

Introduction to Adoptive Cell Therapy

Adoptive cell therapy is an immunotherapy approach which involves the identification of antitumor lymphocytes that are then multiplied ex vivo and then introduced into the cancer patients, usually complimentary with the growth factors that promotes the in vivo impact of the transferred cells.[1] Given that, ACT requires the infusion of ex vivo cultured, a sheer volume of the antitumour lymphocytes can be synthesised and transferred. The identification of highly selective synthesised cells towards the tumour antigens can be credited to the fact that they can be tested prior to infusion. The naturally synthesised antitumour lymphocytes are usually tolerized in vivo, this allows these cells to be cultured ex vivo without the negative influence of the tumour antigen that occurs in vivo and then administering the highly activated cells to the patients which can produce multiple antitumour functions. Therefore, the primary determinant for the success of the ACT is the ability to transform the host before the infusion to provide an optimal environment for the transferred cells.[2]

In recent years ACT has emerged as one of the most fruitful treatments for metastatic melanoma. The newer studies have indicated that normal human lymphocytes can be genetically modified and re-engineered to recognize the cancer antigens. This has resulted in the suppression of cancer *in vivo* and has broadened the spectrum of ACT-based therapies for patients with various types of cancer.

The immunotherapy for cancer can be classified under three categories:

1. **Non-specific immunomodulation:** The introduction of the T – cell growth factor Interleukin 2 (IL2) can mediate the immunomodulation. This can activate the endogenous tumour reactive cells and can suppress the growth of some human cancers.[3] This method was first approved for the treatment of Renal cancer in 1992 by the United States Food and Drug Administration (US-FDA) and further for metastatic melanoma in 1998.

Though this treatment has a drawback of toxicity due to the administration of IL2 which leads to capillary leak syndrome, the co-administration of cytokine has significantly decreased the treatment-related mortality rate by up to <1%.[4] The more recent development of the non-specific immunotherapy using surface inhibitory antibody molecule, cytotoxic T – lymphocyte-associated 4(CTLA4), the outcomes were promising in objective clinical responses in around 10-12% patients, but limited only to patients those suffered renal cancer or metastatic melanoma. This demonstrates that the aforementioned types of tumours are exceptional in their ability to produce endogenous antitumour cells of sufficient strength and volume to cease cancer progression when stimulated appropriately in vivo.[5],[6]

2. **Active immunization approach (cancer vaccine):** This method is based upon the process of immunization of the cancer patients against corresponding cancer by utilizing either the entire cells, proteins, peptides or an array of immunizing vectors. Presently, only rare and highly sporadic regression of solid tumours has been successful by using this method.[7] Although immunization of patients with melanoma can be induced by 30% of the circulating anti-melanoma CD8+ T cells, the tumour progression can still occur, proving that low avidity cells are introduced. This is due to endogenous factors.[8] There have been studies where antitumour T cells from rare tumours were isolated and grown, again indicating that T cell precursors reacting against non – melanoma antigens are currently at low frequency.[7]

3. **ACT:** In this method of treatment, the autologous or allogeneic lymphocytes are identified ex vivo that have antitumour activity, which are then transferred into the cancer patients, usually along with the required growth factors to provide an appropriate environment for their survival and multiplication in vivo. Compared to the other methods of treatment mentioned previously, ACT possesses substantial advantages both practical and theoretical. Only a few antitumour cells are required to be identified that have the desired properties which can be expanded to large volumes ex vivo for the treatment. The required functions for the cancer regression can be identified by in vivo tests. The identified cells can then be activated in the laboratory in the absence of the inhibitory factor and can further be promoted to demonstrate the desired antitumour actions, the treatment using this method in cancer experiments in animals and cancer patients have been proven highly beneficial.[1] The ACT therapy can be classified into three categories.

I. **Tumour Infiltrating Lymphocytes (TILs):** These are lymphocytes that have the potential to infiltrate the tumour cells, and once the IL – 2 has been activated, they pose a stronger antitumor effect. TILs have shown remarkable results in patients with melanoma but were not effective in other types of tumours.[9]

II. **T – Cell Receptor (TCR) T cells:** These are heterodimeric proteins which are formed of two-structural domains namely TCRα and TCRβ. These cells inhibit the multiplication of cancer cells or kill them by particularly identifying the assembly, modification and processing of the cancer cell-specific proteins via cancer-specific Major Histocompatibility Complex (MHC) molecules by activating cytotoxicity and releasing cytokines. These cells have the ability to target most of the tumour specific antigen, especially those which can identify the tumour cell antigen. Therefore they have a broader range of antigen recognition than the tumour antibody-drug.[10]

III. **Chimeric Antigen Receptor (CAR) T – Cell therapy:** This therapy is one of the methods of ACT therapy used for the treatment of a few types of leukaemia and lymphoma. It consists of a genetically engineered domain to attach to the antigen and promote anti-tumour activity.

The advancement in the methods to cultivate large numbers of antitumour lymphocytes has facilitated the development of different cell transfer therapies. These therapies have been a few of the most successful treatments of various cancers.[2]

Chimeric Antigen Receptor (CAR) T – Cell Therapy

Chimeric Antigen Receptor (CAR) T – Cell therapy is one of the methods of ACT therapy used for the treatment of a few types of leukaemia and lymphoma. The treatment involves the collection of blood from the patient, focusing primarily on the T – cells. A gene that codes for a special receptor that binds the cancer protein in the patient is attached synthetically in the laboratory. This special receptor is referred to as the Chimeric Antigen Receptor. These newly synthesis CAR – T cells are then grown in large numbers in the laboratory. Once a sufficient amount of these cells are synthesised, they are infused back into the patient.[11] The first iteration of CAR –T cell can be traced back in 1987 when it was first developed by Yoshikazu Kuwana et al.[12] It was then further developed by and Zelig Eshhar in 1989.[13] In the past few decades, the CAR – T cell have been engineered for the most desirable outcomes in the cancer treatment.

Structure of CAR – T cell

The Chimeric Antigen Receptor – T cells are formed of three parts namely.[14]

- **Extracellular domain**

The extracellular domain also called the ectodomain is the domain that resides outside the membrane protein and is exposed to the extracellular space. This domain comprises of a signal peptide, antigen recognition region and spacer.[15] The function of the signalling peptide is to signal the nascent protein into the endoplasmic reticulum (ER). There is an scFv domain formed of the variable parts of the heavy and light chains of the immunoglobulin attached through a flexible linker. The scFv serves as an antigen recognition region which can recognise the tumour – associated antigens with specificity and affinity. The antigen recognition domain and the transmembrane domains are attached through a spacer. The hinge region of the IgG1 forms the simplest form of the spacer and is optimal for most of the scFv – based construct.

- **Transmembrane domain**

The transmembrane domain is formed of a hydrophobic alpha helix that stretches of the membrane. The stability of the receptor depends upon the transmembrane domain. The incorporation of artificial TCR into the native TCR is caused due to the presence of the native CD3ζ transmembrane. Currently, CD28 transmembrane domain is considered as the most stable receptor.

- **Intracellular domain**

The intracellular domain also referred to as endodomain is formed of the immunoreceptor tyrosine – based activation motif (ITAM) of the TCR complex CD3ζ. This activates the co-stimulatory signals. Once the antigen is recognised, the receptor cluster and signals are activated. The signals are then transmitted to the T – cell. A co – stimulatory signalling is required during the progression.

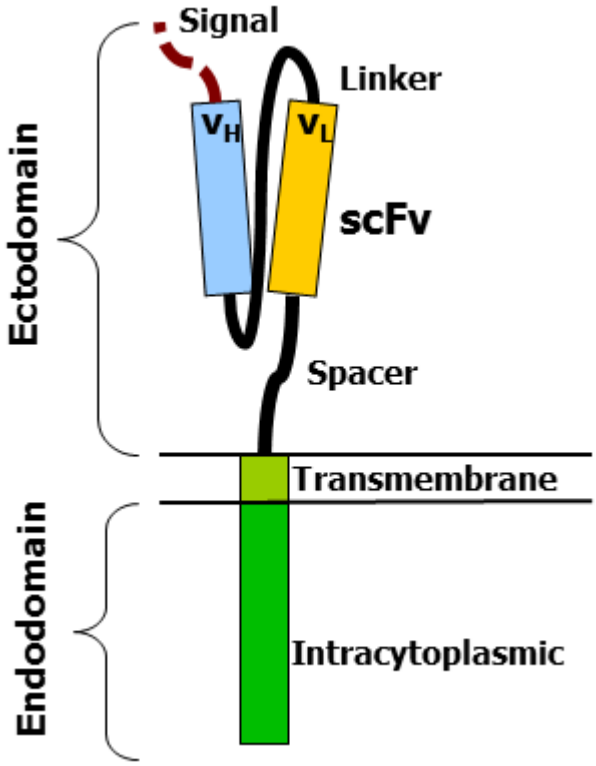

Fig. 1: Structure of chimeric antigen receptor (CAR). The CAR includes ectodomain, transmembrane domain and endodomain.
(Source: Engineering CAR-T cells - Scientific Figure on ResearchGate)

Development of Chimeric Antigen Receptor (CAR) – T cell Therapy

Since 1989, when the first CAR – T cell was developed, they can be divided into four generations based on their structures of the Intracellular domain.

- **First-Generation CAR – T cells:** The first-generation CAR – T cell consists of an svFv antigen-binding epitope with a single signalling domain. These are activated by the CD3ζ chain. This CD3ζ then further provides the signal for the activation of the T cells, lysis of target tumour cells, IL-2 secretion regulation and antitumour immunoregulatory activity.[16] Although these CAR – T cells produced IL-2, it was necessary to administer IL-2 from outside, as the amount produced was not sufficient to kill the tumour cells. Thus it was necessary to administer exogenous cytokines alongside the first-generation CAR – T cells to obtain a substantial benefit. Despite that, the anti-tumour activity of the

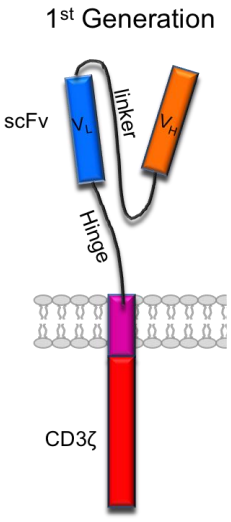

Fig 2: First Generation CAR - T Cell (Source: CellCultureDish Inc.)

first-generation CAR – T cells was constrained to in vivo, and the continuous decline in the T cell proliferation eventually led to the apoptosis of the T cells.[17]

- **Second-Generation CAR – T cells:** The addition of a co-stimulatory receptor protein to the cell forms the second-generation CAR – T cells. These co-stimulatory molecules usually include the addition of either CD28 or 4-1BB receptor (CD137). The CD28 co-stimulatory molecule promotes the synthesis of IL2 which completes the activation of T cells and prevents apoptosis. The naive T cells are not able to perform their normal roles in the absence of a co-stimulatory signal and it is the same case if the T – cells are activated by antigen. Hence the inclusion of only the CD3ζ sequence in the CARs is unable to activate the CAR – T cells without the co-stimulatory signal. There have been many studies that demonstrate that the second-generation CAR – T cells do not have a specific antigen, and when compared to the previous generation, the second-generation CAR – T cells cytokine secretion, proliferation and antiapoptotic protein synthesis are increased, along with that the second-generation CAR – T cells lead to delayed antigen-induced apoptosis, this prolongs the lifespan in vivo.[18] The co-stimulation produced by CD28 is necessary for the regulation of proliferation and survival of lymphocytes and makes a significant contribution towards the establishment of the memory cells and effector cells. The other co-stimulatory receptor CD137 or 4-1BB can maintain proliferation and enhance IL2 production. Alongside that, it maintains the response signal of T cells, which is important for the survival of T cells and the memory of $CD8^+$ T cells. Both the scFvCD19-CD137-CD3-CAR – T cells and scFvCD19-CD28-CD3ζ-CAR-T were employed for the treatment of B cell malignancies and showed better results compared to the first-generation CAR – T cells.[19]

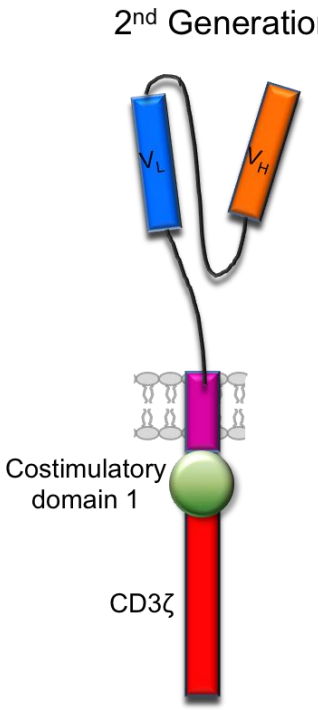

Fig3: Second Generation CAR - T Cell (Source: Cell CultureDish Inc.)

- **Third-Generation CAR – T cells:** The third-generation CAR –T cells were made by incorporating both the CD28 and 4-1BB (CD137) receptor co-stimulatory molecule making scFvCD20-CD28-CD137-CD3ζ-CAR – T cells. The CD28 receptor enhances the antitumour activity while the CD136 (4-1BB) receptor lengthen the survival of the T cells and maintains their antitumour cytotoxic activity. The third-generation CAR –T cells were used for the treatment of lymphoma and colon cancer, but the results did not show a significant difference in the antitumour cytotoxic activities from the previous generation.[20] Studies have identified that only the second-generation CAR – T cells manage to activate CD3ζ alongside they have much stronger signal transduction and anticancer activities than the third-generation CAR – T cells.[21]

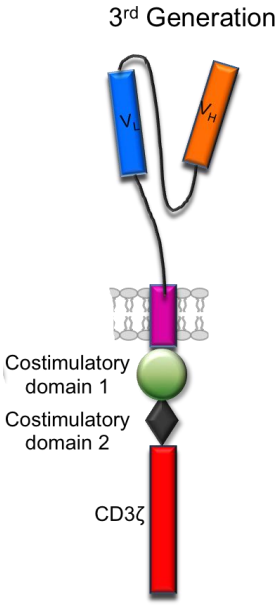

Fig4: Third Generation CAR - T Cell (Source: CellCultureDish Inc.)

- **Fourth-Generation CAR – T cells:** The fourth-generation CAR – T cells were made by an activated T cell nuclear factor transcriptional counterpart to the base of the second-generation CAR – T cells, which enables it to secrete specific cytokines such as IL-12 in the tumour cells, thus it modifies the microenvironment of the tumour and employing and activating various other immune cells to produce an immune response. The fourth-generation CAR –T cells are known as T cell Redirected for Universal Cytokine-Mediated Killing (TRUCKs). In contrast to the previous generations of CAR – T cells, these cells have the ability to identify and eliminate some of the antigens that are unable to be recognised accurately by the T cells.[22] Thus able to remove the antigen-negative cancer cells.[16] The ability of these TRUCK T cells to modify the tumour microenvironment by the secretion of the transgene immune modifiers enables them to be used for the treatment of auto-immune disorders, viral infections and metabolic disorders in the near future.[23]

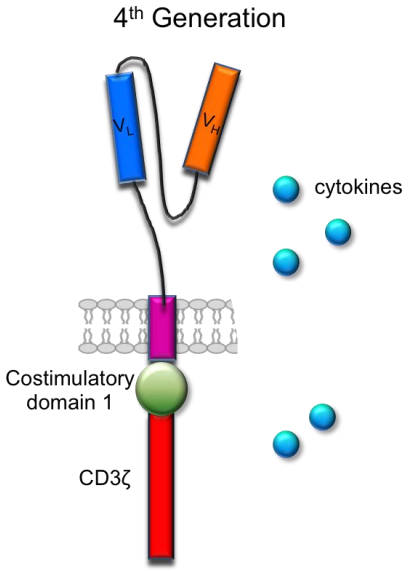

Fig 5: Fourth Generation CAR – T Cell (Source: CellCultureDish Inc.)

Synthesis of CAR – T cells

Multiple steps go into the production of CAR – T cells. One of the most important aspects of manufacturing the CAR – T cells, is maintaining the integrity of the product by doing in-process and quality control release testing throughout the entire protocol. Even though there are various designs of the CAR – T cells for different cancer treatment in the form of tumour specific scFvs, the basic methodology of manufacturing these cells remains the same. The first step is the collection of the leukocytes either from the patient or the donor by a process called leukapheresis. Next step is to separate the T cells from the leukocytes by enriching and washing it. The third step involves the separation of the T cell subsets based on CD4/CCCD8 composition by employing specific bead conjugates or markers. These T cells are cultured to allow them to activate. Once activated, CARs are then encoded with viral vectors, by which DNA is encoded from the RNA by reverse transcription and amalgamate into the genome of the patient permanently.[22] The final step is to have a large scale expansion and the making of the end-of-process formulation.

1. T cell source

The manufacturing of CAR – T cells starts from the collection of blood mononuclear from the patient or the donor, usually done by leukapheresis. After studying the treatment regimens of the patient the doctor identifies a suitable window of opportunity to extract the T lymphocytes while they are available in large amounts. Various method of processing the separated blood components are employed depending upon the downstream process. There are different devices that are used to perform apheresis. Haemonetics Cell Saver 5+, COBE2991, and Fresenius Kabi LOVO are some of the devices that can separate the gross red blood cells and platelets contaminants and remove them. Devices like the Terumo Elutra and Biosafe Sepax systems are used to separate the components based on the size of the cells for the exhaustion of the monocytes and separation of lymphocytes. CliniMACS Plus and Prodigy systems are some instrumental devices that are tasked to enrich specific sub-set of the T cells, which include $CD4^+$, $CD8^+$, $CD25^+$ and $CD62L^+$T cells. The method of separating specific sub-sets of T cells for the production of CAR – T cells is one of most appealing strategy, but the selection of the optimal T cell sub-set which is both of high therapeutic value and a low toxicity level and can easily be multiplied at large scale is still needed to be identified.[24]

Once the T cells source material is processed it can be utilized either in the further downstream process or it can be cryopreserved for future use. Though there are both pros and cons of both the methods, cryopreserving the processed cells gives time to conduct various tests for product release and provides appropriate time to plan out the downstream process.

2. T cell Activation

The isolated T cells are then required to be expanded which requires the sustained and optimal activation. The activation of T cells requires two signals, primary and costimulatory signals. The primary specific signal is given via the T cell receptor and then the costimulatory signal which may be either CD28 or 4-1BB/CD137. Viral vectors are used in the process of transduction of the CAR cDNA which is essential for the activation of the T cells. There are various methods for activation of the T cells:

- **Cell-based T cells activation:** The dendritic cells (DCs) which are a type of APC are used as the endogenous activators of T cell responses. Even though not much is known about the therapeutic applications of DCs and is under investigation, the potency of DCs differs in different patients. Thus obstructing its use. The use of Artificial Antigen-Presenting Cells (AAPCs) is another method of cell-based activation.[25]
- **Beads based T cell activation:** Multiple ready-made clinical-grade reagents that activate T cells are made by different biotech companies. There are different types of these reagent beads based on different principles.
  - **Antibody coated magnetic beads:** Invitrogen CTS Dynabead CD3/28 and Miltenyi MACS GMP ExpAct Treg beads are two types of antibody-coated magnetic beads. The first one is super-paramagnetic beads that are covalently attached to the CD3 and CD28 antibodies. The later one is paramagnetic beads that are bound to CD3-biotin, CD28 and anti-biotin monoclonal antibodies. Both types of beads are required to be removed to complete the manufacturing process.[26]
  - **Antibody coated nanobeads:** the Miltenyi MACS GMP TransAct CD3/CD28 is an example of coated nanobeads which are polymeric nanobeads attached to either CD3 or CD28 mononuclear antibodies. The advantage of these beads over the previously mentioned beads is that these do not require washing fo removal as they are biodegradable, nevertheless, a prior upstream T cell purification is required for activation.[27]

- **Expamer technology:** An American biopharmaceutical company name Juno Therapeutics has recently developed a T cell activation reagent named Expamer. Viral-specific lymphocytes are extracted using its Streptamer technology.[28]

- **Activation with anti-CD3 antibodies:** The T cells can be activated in the presence of IL2 by the attachment of the T cell surface CD3 with the soluble anti-CD3 monoclonal antibodies.

3. Genetic Modification of T cells

Currently, viral and non-viral gene transfers systems are used primarily to deliver the gene for the stable CAR expression. The gene transfer systems that are being used are

- **γ-retroviral vectors:** These were one of the first viral vectors which have been utilized to produce a stable CD19 CAR expression.[29] At present about one-fifth of all clinical trials which require gene transfer employ the γ-retroviral vectors.[30] These vectors are used as they have a high gene expression, along with that these vectors allow the multiple stable packaging cell lines with a broad tropism.[31]
- **Lentiviral vectors:** These vectors have the ability to transduce non-dividing cells and show a much safer genomic integration profile when compared to the genetically modified hematopoietic stem cells.[32] Drawing similarities with the γ-retroviral vectors, these vectors also have a high gene transfer efficiency and are able to provide a stable level of CAR expression. Nevertheless, there have been multiple difficulties in producing a large scale of these functional vectors. Some of these problems include a lot size problem, lot-to-lot inconsistency arising due to the currently employed multi-plasmid transient transfection procedure and finally the scarcely available stable vector packaging systems.[33]
- **Transposon/Transposase systems:** This is a comparatively newer expression system based on plasmids. This system is better when compared to the aforementioned vectors which are sophisticated complex reagents and require comprehensive and costly biosafety testings, thus leading to a simpler manufacturing procedure and having a less expensive and easier release testing. The transposon/transposase systems use electroporation to introduce the CD19 CAR into the T cells.
- **mRNA (Messenger RNA) transfer-mediated gene expression:** In contrast to the other systems stable and permanent expression of the transgene provided by the previously mentioned viral transduction and plasmid DNA transfection, electroporation or endocytosis can be used to induce the in vivo transcribed mRNA. As no genomic

integration takes place in this method, thus the genotoxicity and possible generation of a replication-competent retrovirus are out of consideration.

4. Expansion of CAR – T cells

The expansion of the CAR – T cells is one of the important tasks in the manufacturing of the therapy as a large number of these synthesised CAR – T cells required for the treatment. The process of expansion of the CAR – T cells is dependent on the method of the genetic modification of the cells. Some of those methods are:

- **Using GE bioreactors for expansion of CAR – T cells:** One of the commonly used devices for the purpose is the GE WAVE Bioreactor system. There are multiple components in this system, which include CellBag bioreactor, temperature-enabled auto rocking base and a variety of pumps, controllers and probes. This device has the capability to expand the cell population at a high rate with cell count reaching 10^7 cells/ml and the device can hold up to 25L of cell culture in a single bioreactor.
- **Using G-Rex bioreactors for expansion of CAR – T cells:** This system is a relatively newer method for expansion of CAR – T cells. The bioreactor has a cell culture flask that attached to a gas permeable membrane present at the bottom which allows high-density cell growth without hampering the gas exchange. There are few advantages associated with this method which include one-time upfront feeding system, low seeding density, the comfort of multiplying the cells in an incubator and the feature that allows reduction of the volume at the time of harvest.[34]
- **Using Prodigy for the expansion of CAR – T cells:** Miltenyi CliniMACS Prodigy System is one the recently developed technology being utilized for the expansion of CAR – T cells. This device is a combination of multiple systems including a cell washer, a magnetic cell separation system and a cell cultivation device. This system is currently tasked for the virus-reactive T-cell preparation and stem cell enrichment.[35]
- **Using looped aACP simulation for the expansion of CAR – T cells:** The use of the transposon/transposase system for the generation of CAR – T cells depends on the selective propagation upon looped stimulation with γ-irradiated aAPCs in the availability of IL2 and IL21.

Treatment using CAR – T cell therapy

After the CAR – T cells for the treatment are prepared by the multistep process they undergo rigours quality testing before it is made available for the treatment. The prepared CAR – T

cells are packaged into blood bags tailored for every patient depending on the type of cancer and the dosage of the therapy. These bags are they either transported to the hospitals for the treatment of the patient or kept under cryopreservation for future use. Dimethyl sulfoxide is one of the components that is used in the cryopreservation of these therapies.

Once the therapy for the treatment is laid down by the physician, the patient is prepped for the course of the treatment. At first, the patient undergoes the conditioning therapy wherein the patient receives sets of chemotherapy for their cancer. This chemotherapy session called lymphodepletion helps create a room in the immune system to accommodate the infused CAR – T cell and allow them to expand and proliferate.[36] The next step is to infuse CAR – T cell preparation into the patient. The previous treatment with the low dose of chemotherapy reduces the number of cancer cells in a small number thus allowing the CAR –T cells to interact with the tumour cells. Once the CAR – T cells are delivered into the patient, they continue to multiply in numbers. The cells start to attach themselves to the specific antigens usually CD19 present on the targeted cancer cells. After they recognise the antigen and are attached to the antigen with the extracellular domain of the CAR – T cell, the T cells start producing signals using the signalling domain with the accompaniment of the co-stimulatory domain release various responses which include cytotoxins, which promote cancer cell apoptosis and interleukins which promotes immune cell development and division. The CAR – T cells remain in the body of the patient for a long period after the infusion thus reducing the chances of recurrence of cancer and keeping the patient in remission.[37]

The patient is kept under observation in the hospital for about one to two weeks after the therapy to study and treat the arising side effects of the therapy, the effects of which can be minimized in the presence and supervision of a physician. The next is the recovery phase which lasts for about 2 to 3 months depending upon the patient. This period is the risk/recovery period wherein the chances of recurrence are high, thus the patient is required to visit for regular checkups post-treatment.[36]

At present, the United States Food and Drugs Administration (US-FDA) has approved two CAR – T cell therapies for the treatment of some types of lymphomas, along with for some patients with relapse or difficult to treat leukaemia. The approved CAR – T cell therapies are:

1. **Tisagenlecleucel (Kymriah^{TM}):** It was the first-ever approved therapy for cancer-based on the CAR –T cell therapy. The therapy was developed by a team lead by Dr Carl H. June at the University of Pennsylvania and it was licensed to Novartis.[38] It was approved

by the FDA in August 2017 for the treatment of acute lymphoblastic leukaemia (ALL) for patients under the age of 25 years.[39] Along with that, it is also an approved therapy for adult patients with relapsed or refractory large B-cell lymphoma occurring after two or more lines of systemic therapy including diffuse large B-cell lymphoma (DLBCL) not otherwise specified, high-grade B-cell lymphoma and DLBCL arising from follicular lymphoma.[40] In a report conclude from a multicenter clinical trial which involved both pediatric and adult patients who had suffered from relapsed or refractory B cell precursor ALL, the overall rate of remission within three months of treatment was found to be 83 per cent.[41] The preparation is administered in a single treatment, which costs somewhere about US$475,000 and is claimed to be cheaper than other bone marrow transplants according to the company Novartis. The company also claimed not to collect any treatment cost for the patients in which the therapy did not respond.[38] Tisagenlecleucel is not prescribed for treatment in case the patient is suffering from primary central nervous system lymphoma.

2. **Axicabtagene ciloleucel (Yescarta™):** This was the second CAR – T cell therapy that was approved by the US-FDA on 18th of October 2017, two months after the first therapy. The therapy was developed by Kite Pharma, a California based Pharmaceutical Company. It was approved for the treatment of relapsed or refractory diffuse large B-cell lymphoma after two or more lines of systemic therapy including diffuse large B-cell lymphoma (DLBCL), transformed follicular lymphoma, and primary mediastinal B – cell lymphoma. Currently, it is available only in the USA and Canada for a treatment cost of US$ 373,000. The therapy is currently not prescribed for treatment in case the patient is suffering from primary central nervous system lymphoma.

CAR – T cell therapy toxicity and its management

There have been multiple toxicities associated with the CAR – T cell therapy that has been reported. Even though it was assumed that there would be some level of the immune response to the therapy, severe Cytokine Release Syndrome (sCRS) was observed in the patients receiving the CD-19 specific and CD-22 specific CAR – T cell therapy. One of the toxicities that were not expected and were observed in the therapy was the neurological complications which ranged from moderately severe to life-threatening. Most of the toxicities associated with the CAR – T cell therapy are either reversible or manageable with pharmacological aids.

1. Cytokine Release Syndrome (CRS)

Cytokine Release Syndrome has been one of the most common adverse effect associated with the CAR – T cell infusion. This immune activation has been observed in other cases where the patient was infused with therapeutic monoclonal antibodies (mABs), systemic interleukin – 2 (IL2) and the bispecific CD19 – CD3 T cell engaging antibody named blinatumomab.[42] The first generation of the CAR – T cell, which only had a single stimulatory molecule led to a lack of T cell proliferation and insufficient anti-tumour activity due to low cytokine production. Whereas in the case of the second generation of the CAR – T cells which included an additional co-stimulatory molecule (CD 28/41BB) showed an impressive anti-tumour response largely due to an improved T cell proliferation which led to high cytokine release. Thus, this property of high cytokine release led to the CRS following the CAR – T cell infusion.[43]

The primary identifiable key of CRS is the activation of the immune system leading to an increased inflammatory cytokine level. The different levels of CRS varied from a mild response (constitutional symptoms/ grade 2 organ toxicity) to severe response (sCRS, grade ≥ 3 organ toxicity) which is potentially fatal.[44] The various clinical features of CRS include high fever, fatigue, myalgia, malaise, nausea, anorexia, tachycardia, capillary leak, renal impairment and disseminated intravascular coagulation.[44]

Currently, C – reactive protein made by the hepatocytes in response to IL6 is being used as the laboratory marker for identifying CRS onset and severity.[45] It has been shown that the severity of CRS is proportional to the disease burden, that means the patient receiving the infusion who have high tumour burden undergo a sCRS.[46] The prevalence of sCRS in patients receiving CD19 – specific CAT – T cells for the treatment of refractory B – cell acute lymphoblastic leukaemia has varied from 19% to 43%.[47] Thus a majority of patients receiving the therapy experience at least mild CRS usually fever.

One of the major challenges following the diagnosis of CRS is to device an appropriate therapy wherein the physiological symptoms of CRS are suppressed without affecting the anti-tumour activity of the infused T cells. Systemic corticosteroids have been used for the suppression of CRS without compromising on the anti-tumour effects. But the drawback is that these corticosteroids cannot be used for long durations i.e. >14 days as the prolonged use of high dose corticosteroids lead to the removal of the engineered T cells in the body leading to the dampening of the long term anti-tumour activity.[45] Thus the US-FDA approved mAB,

Tocilizumab an alternative IL6 receptor blockade was identified, which is capable of providing a near-immediate reversal of CRS.[48] This lead to the front-line use of the tocilizumab for the treatment of sCRS after the administration of CAR – T cells.[47]

2. Neurological toxicities

There have been multiple neurological toxicities that have been reported post the administration of the CD19 specific CAR – T cells which include confusion, delirium, obtundation, seizure, expressive aphasia and obtundation.[49] Although the reason for the neurological adverse effects is unknown, similar side effects have been observed with the administration of blinatumomab.[50] It is assumed that the increase in the cytokine level is responsible for neurological toxicity. Though the direct toxicity on the CAR – T cell on the nervous system is not been proven clinically but could be possible. As of now, in a majority of cases, the neurological toxicity has been reversed and it is uncertain if the toxicity is limited only to CD19-specific CAR – T cells or may be extended to other targeted tumour associated antigens.[49]

3. On – target/off – target recognition

It is expected that the engineered cells would only target the tumour cells which is the ideal target antigen and allow the survival of the malignant clones. But, majority of the CAR – T cell targets usually share the expression on the normal tissue thus leading to on – target/off – target toxicity caused due to their action on the non-pathogenic tissues.[51] The range of this toxicity has been reported to be varying from manageable lineage depletion (B-cell aplasia) to severe (death). The effect of on – target/off – target toxicity can be observed in a range of organ systems including haematological, gastrointestinal and pulmonary systems.

Treating patients with colon cancer by targeting carcinoembryonic antigen while using CAR – T cells has resulted in the formation of cholestasis due to the expression of carboxyanhydrase-IX on the bile duct epithelium.[52] Now coming to a fatal example for the case of on – target/off – target recognition associated with the use of CAR – T cells specific for cancer-associated antigen HER2-neu resulted in a rapid respiratory failure, multiorgan dysfunction and finally death due to the reactivity against the pulmonary tissue expression of HER2/neu.[53] Nevertheless, the unpredicted toxicity was aggravated by the considerable amount of the infused CAR – T cells (1×10^{10} cells), though further studies that utilized a different HER2/neu-specific CAR – T cells without the preceding conditioning chemotherapy have been proven safer at a lower dose of CAR – T cells.[54]

4. Anaphylaxis

The immune system of the human body is adapted to identify and differentiate between self and foreign substances and fight against the foreign substance. As the majority of the genetically engineered T cell contains antigen recognition domains that are derived from the murine mABs, the incidence of both cellular and humoral rejection of CAR – T cells is expected and has been demonstrated by the foreign protein due to its immunogenicity.[51],[55] Studies and researches are being done to synthesise the components of the expressed proteins sourced from humans so as to improve persistence and potentially the efficacy.[56] These toxicities due to the host recognition due to infused foreign components have resulted in acute anaphylaxis. This has been observed in patients who received mesothelin-specific CAR – T cells.[57] In a study, it was reported that one of the four patients receiving multiple infusions of mesothelin-specific CAR – T cells developed cardiorespiratory failure at the end of the third infusion.[57] This study aimed to reduce the on – target/off – target toxicity by using multiple infusions of T cell which expressed a transient CAR (mRNA vector). Later in the investigation, it was understood that the cardiorespiratory failure occurred due to the presence of human anti-mouse antibodies and elevated trypsin levels in the patient's serum confirming it to be IgE-mediated anaphylactic event.[57] Thus it is important to have strict surveillance, quick recognition, and immediate treatment of a possible fatal adverse effect for the patients receiving the genetically engineered CAR – T cells.

5. Graft vs Host disease

Multiple clinical benefits have been demonstrated in cancer patients with the use of patient-derived tumour specific CAR – T cells in the Adoptive cell transfer. Even though there is a risk of having alloreactivity, the collected CD19 specific CAR – T cells post allo-HSCT, did not show any tendency to produce GVHD.[45] Regardless of the proven safety of the personalized T cells, the production of these on a patient to patient basis is quite unfeasible in both time and cost. Thus a requirement of an "off-the-shelf" has been suggested as a suitable solution by reducing both cost and time consumption. Currently, two methods are being developed.[58]

- Endogenous T cell receptor silencing
- CAR transduced viral-specific cells

6. Insertional oncogenesis

Various studies have established the risk of having insertional oncogenesis in human cells due to gene therapy of hematopoietic stem cells for cross-linked severe combined immunodeficiency and chronic granulomatous disease.[59] Most cases have shown the insertion of retroviral vector proximal to the LMO-2 oncogenes.[60] Though as of now, there has been no reported case of transformation post the infusion of the genetically engineered T cells. It is important to note that the LMO-2 oncogene is silent in T cells making this site highly unlikely site for retroviral insertion. In the clinical setting, the use of genetically engineered T cells has been proven to have a decade long safety profile, without the instance of a vector induced immortalization, clonal expansion or the enrichment for integration sites near genes implicated in growth control or transformation.[61] Though the chances of having an incidence of insertional oncogenesis are very low, the researchers must remain cautious and stick to the strict monitoring regime as planned by the clinical trial design.

MATERIALS AND METHODOLOGY

Search strategy

The studies were explored using MEDLINE, PubMed, Web of Science, Embase and Science direct for the published literature. Thes strategy of the study was designed to minimize the bias while maximizing the sensitivity and being broad and inclusive. This was achieved by utilizing a set of keywords, which helped in the comprehensive search. Each specific keyword had subset, enhancing the accuracy at the time of the search. The search continued by surfing on the web for some relevant works of literature. The keywords used the search were

1. Cancer Immunotherapy
2. Adoptive Immunotherapy
- Development
- Types
3. CAR – T cell Therapy
- Structure
- Generations
- Manufacturing
- Treatment method
- Available therapies
- Adverse effects

- Adverse effects management

The references cited in the identified literature were also searched to identify relevant information important for the study.

Clinical Trial data regarding the genetically engineered T – cell for the purpose of treatment of cancer mentioned in the systemic review was collected from ClinicalTrials.gov online database. The selection procedure of the clinical trials is given in the diagrammatic representation in Fig.8.

Selection criteria

The studies fulfilling the following criteria were included in the study.

Inclusion criteria

- Studies that discussed on Adoptive Immunotherapy
- Studies that included data on CAR – T cell therapy
- Studies that involved the use of CAR – T cell therapy for haematological cancers
- Press release of FDA

Exclusion criteria

- Studies that were not published in English
- Studies that focused on TILs and TCRs T cell-based adoptive immunotherapy
- Studies using duplicate data
- Conference proceedings or thesis.

The extensive search resulted in identifying of a total sum of 60 articles and 6 websites that were published between 1955 and 2020 which was then studied. The websites included the US-FDA, Dana-Farber Cancer Institue, CancerResearch and National Cancer Institute. The tables and graphs were made using MS Excel.

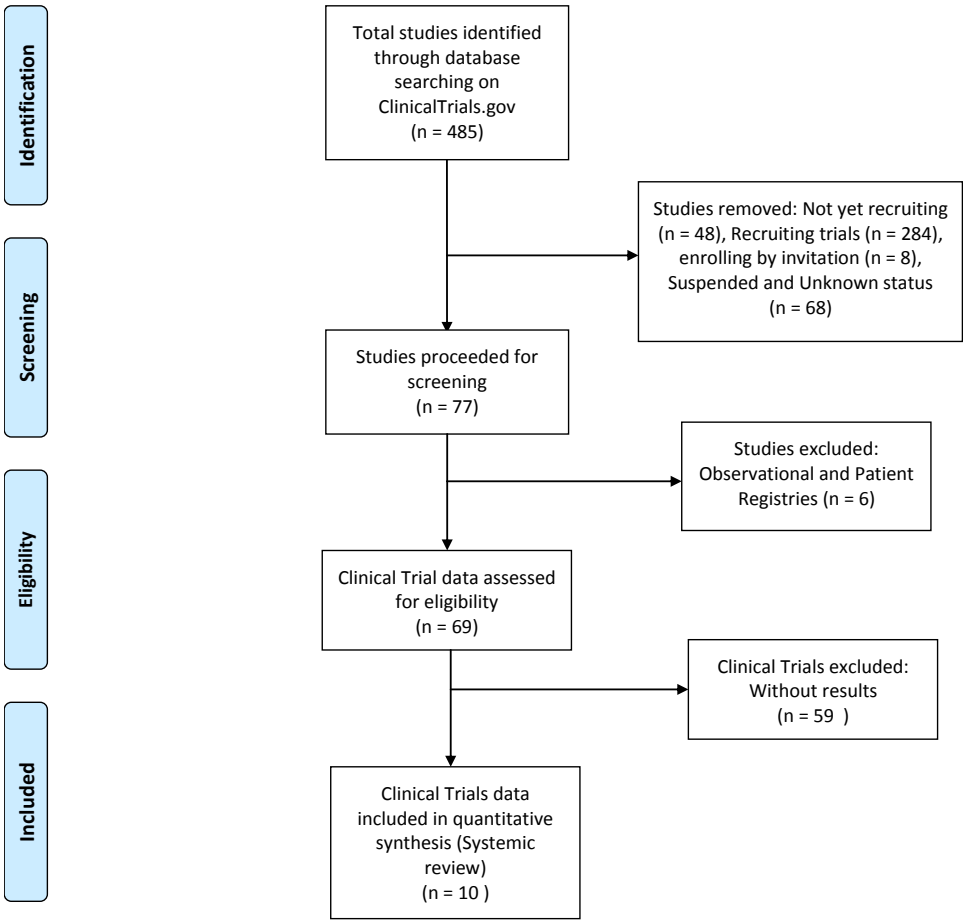

Fig. 8: Flowchart for selection of the clinical trials from ClinicalTrials.gov database by using keyword "CAR-T cell therapy"

RESULT AND DISCUSSION

A total of 66 different pieces of literature including published articles, websites and press releases were studied regarding Cancer immunotherapy, Adoptive cell therapy and Chimeric Antigen Receptor – T cell therapy. The initial study of the immunotherapy of cancer revealed that the concept of transferring of the immune cell-like lymphocytes obtained from a different animal for self-immuning the animal towards a particular tumour. This success led to further study for exploring various other methods to induce immune-based therapy for various cancers.

Adoptive cell transfer emerged as one of the most promising methods of cellular immunotherapy for cancer as it showed promising results towards the treatment of melanoma. The three different types of ACTs have shown promising results in different types of cancers. Tumour infiltrating Lymphocytes (TILs) showed strong positive results towards

melanoma but was not much effective in other types of tumours and cancers. The T – cell Receptor T cells were engineered towards treating of various types of tumour, specifically solid tumours due to the broader range of antigen recognition. The next method of ACT came in the form of Chimeric Antigen Receptor (CAR) – T cell therapy which similar to the TCR – T cell where the T – lymphocytes collected from the patient were modified ex vivo before infusing them back into the patient to induce anti-tumour activities. The CAR – T cells proved effective in the treatment of haematological cancers especially Acute Lymphoblastic Leukaemia (ALL) and Diffused large B-cell Lymphoma (DLBCL). Though they were effective in the haematological cancers, their effectiveness in the solid tumours was not very successful. Various characteristics were identified of the CAR – T cell and The TCR – T cell, given in Table 1.

Table 1: Various properties compared between CAR and TCR engineered T cells.

Property	CAR – T cells	TCR T cells
Signalling	Signal amplification from synthetic biology: 200 targets can trigger CAR T cells	Sensitive signal amplification derived by the evolution of the TCR
Avidity	Controllable	Low, unless engineered
Target	Surface structures like protein and glycans	Intracellular proteome
MHC dependency	Independent recognition of tumour targets	MHC class 1 expression and HLA matching on the tumour
Lifespan	At least a decade	Lifelong
Toxicity	Cytokine release syndrome more severe than TCR T cell therapy	Off – tumour toxicity difficult to predict

The main focus of the review revolved around the development, manufacturing and treatment of cancer using the CAR – T cell therapy. During the study of the development of the therapy, it was identified that various generations of the CAR – T cells provided different outcomes in the treatment. The first generation of the CAR – T cells was formed of a single signalling intracellular domain, which despite producing an anti-tumour activity was not very successful due to the lack of amount of IL-2 produced for sustained anti-tumour activity. This resulted in the addition of a secondary/co-stimulatory molecule which was either CD28 or CD137 which made the second generation of CAR – T cells. The addition of either of these molecules enhanced the cytokine release which enhanced the anti-tumour activity. Further research led to the development of the third-generation CAR – T cells which involved the use of both CD28 and CD137 co-stimulatory molecule. Though it was expected that they would provide more sustained cytokine release for the ani-tumour activity, various studies revealed

that they were very similar in activity when compared with the previous generations. This was due to the fact that the additional co-stimulatory molecule was unable to activate the CD3ζ signalling molecule effectively. significant progress was made with the development of the fourth generation CAR – T cells which had an activated T cell nuclear factor transcriptional counterpart to the base of the second-generation CAR – T cells, that allowed significant production of IL2, that mediated the anti-tumour activity.

Multiple methods have been developed to manufacture these genetically engineered cells so that they can be produced at a commercial level while managing the quality of the product at an optimal level. This is achieved by stringent quality control standards both in-process and post-manufacturing. Toxicity management has been one of the most crucial steps toward the success of the therapy. Some of the identified methods for the management of the toxicities are mention in Table 2.

Table 2: Management method to overcome problems arising during CAR – T cell therapy.

Problem	Management method	Expected results
Cytokine Release Syndrome (CRS)	mABs: Tocilizumab, Siltuximab JAK kinase inhibitors Corticosteroids	IL6 effects blocked, fever suppressed, hypotension and hypoxia
Development of human anti-mouse antibodies	Use of scFv derived from humans	Longer survival rate of CAR – T cells
Minimized persistence of CAR – T cells	Understand mechanisms of signalling domains that impart increased longevity, use sorted memory or stem cells	Increased survival of CAR – T cells
Relapse due to loss of CD19 co-stimulatory molecule	Target CD22 and CD19	Combination of the molecules for targeting prevent escape

Two therapies for the treatment of ALL and DLBCL involving the use of CAR – T cell have been approved by the US-FDA in 2017 and since being used for treating multiple patients suffering from cancer. Multiple clinical trials have been identified using the ClinicalTrials.gov database. A total of 485 clinical trials were identified by using the keyword “CAR-T cell therapy” on ClinicalTrials.gov. A graph was plotted for the geographical distribution of the CAR – T cell therapy clinical trials from the data collected given in the Fig.9.

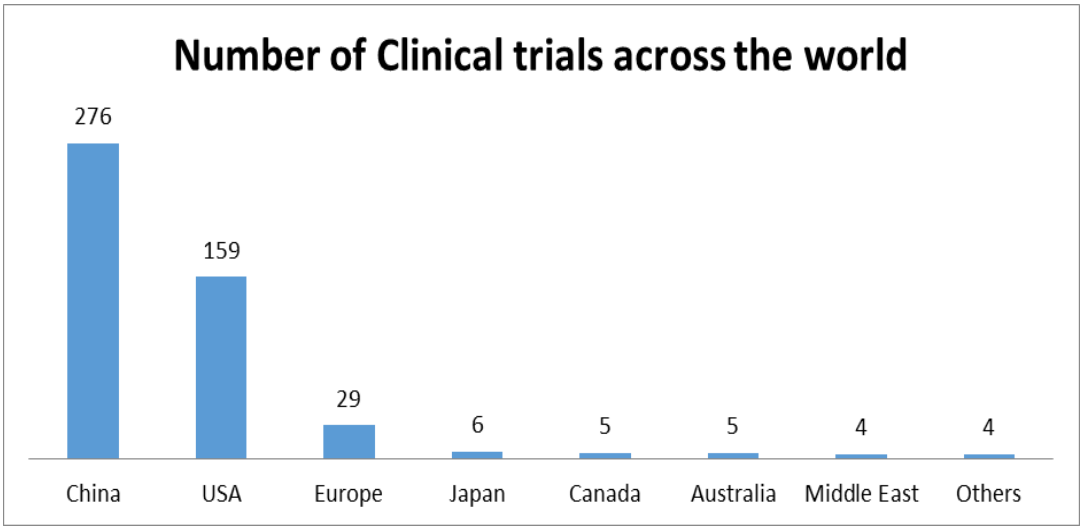

Fig. 9: Geographical distribution of the clinical trials across the world.

The list of clinical trials was then further scrutinized by the methodology mentioned in the Fig.8 and thus identifying 10 Clinical trials of which the results were available. Information including the Study title, NCT number, number of participants, study conditions, phase and status was utilized for Table 3. Two graphs were plotted with the given data to estimate the proportion of phases and status of the trials, given in Fig.10 and Fig.11.

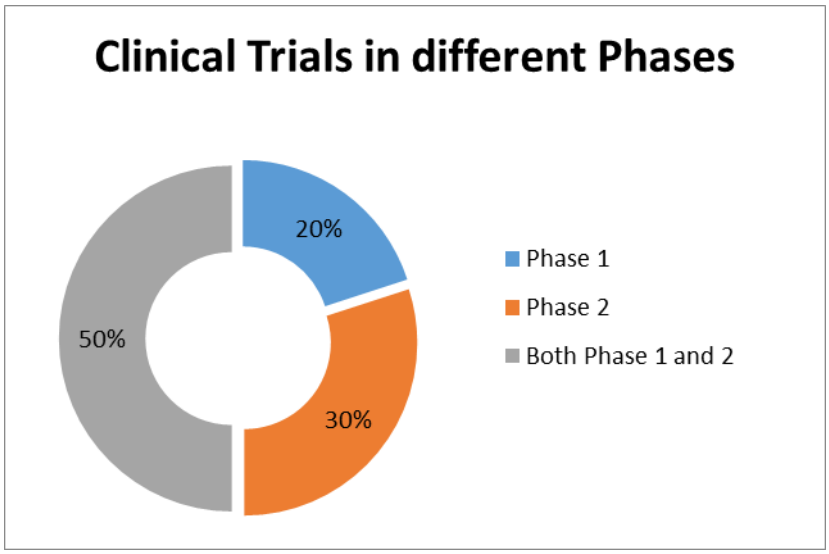

Fig. 10: Percentage of clinical trials in different phases.

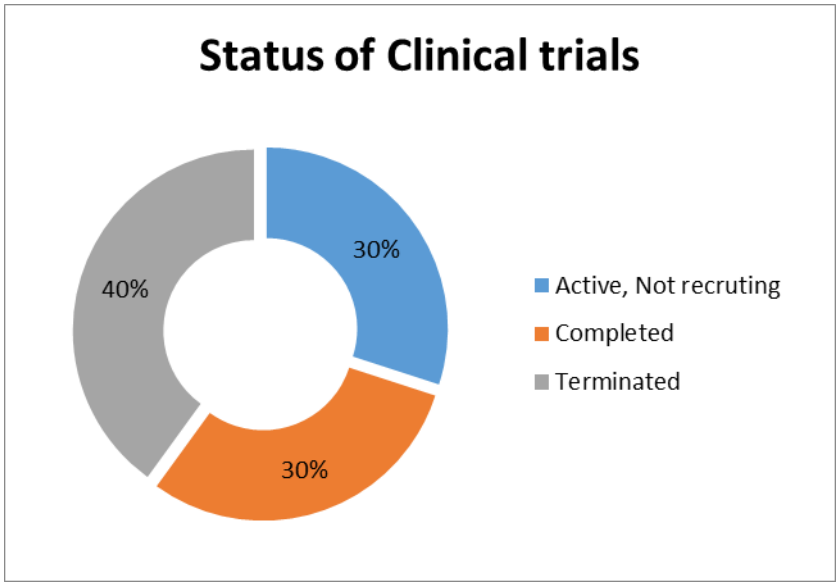

Fig. 11: Percentage of the status of different clinical trials.

Table 3: Clinical trials data of studies with results available.

S. No.	NCT Number	Study Title	Conditions	Phase
1	NCT02030847	T Cells Engineered to Contain Anti-CD19 Attached to TCR and 4-1BB Signaling Domains in Patients With Chemotherapy Resistant or Refractory Acute Lymphoblastic Leukemia	•Patients With B Cell ALL, Relapsed or Refractory, With no Available Curative Treatment Options	Phase 2
2	NCT01218867	CAR T Cell Receptor Immunotherapy Targeting VEGFR2 for Patients With Metastatic Cancer	•Metastatic Cancer •Metastatic Melanoma •Renal Cancer	Phase 1 Phase 2
3	NCT02535364	Study to Evaluate the Safety and Efficacy of KITE-585 in Participants With Relapsed/Refractory Multiple Myeloma	•Acute Lymphoblastic Leukemia	Phase 2
4	NCT03318861	Study to Evaluate the Safety and Efficacy of KITE-585 in Participants With Relapsed/Refractory Multiple Myeloma	•Relapsed/Refractory Multiple Myeloma	Phase 1
5	NCT02650999	Pembrolizumab in Patients Failing to Respond to or Relapsing After CAR T Cell Therapy for Relapsed or Refractory Lymphomas	•CD19+ Diffuse Large B-cell Lymphomas •Follicular Lymphomas •Mantle Cell Lymphomas	Phase 1 Phase 2

Conditions	Phase	Numbers Enrolled	Study Start	Study Completion	Status
•Malignant Glioma •Glioblastoma •Brain Cancer •Gliosarcoma	Phase 1 Phase 2	18	May-12	Jan-19	Completed
•Acute Lymphoblastic Leukemia	Phase 2	1	Oct-16	Dec-18	Terminated
•Cervical Cancer •Pancreatic Cancer •Ovarian Cancer	Phase 1 Phase 2	15	May-12	Dec-18	Terminated
•Primary Mediastinal B-cell Lymphoma •Diffuse, Large B-cell Lymphoma •Diffuse Large B-Cell Lymphoma Transformed From Follicular Lymphoma •Mantle Cell	Phase 1 Phase 2	43	Feb-09	Dec-20	Terminated
•Myeloma, Plasma-Cell •Myeloma-Multiple	Phase 1	30	Aug-14	Aug-19	Completed

Numbers Enrolled	Study Start	Study Completion	Status
42	Feb-14	Apr-18	Completed
24	Nov-10	Dec-15	Terminated
82	Aug-15	Sep-17	Active, not recruiting
17	Oct-17	Nov-33	Active, not recruiting
12	Jan-16	Dec-21	Active, not recruiting

S. No	NCT Number	Study Title
6	NCT01454596	CAR T Cell Receptor Immunotherapy Targeting EGFRvIII for Patients With Malignant Gliomas Expressing EGFRvIII
7	NCT01454596	CART19 in Adult Patients With Minimal Residual Disease During Upfront Treatment for ALL
8	NCT01583686	CAR T Cell Receptor Immunotherapy Targeting Mesothelin for Patients With Metastatic Cancer
9	NCT00924326	CAR T Cell Receptor Immunotherapy for Patients With B-cell Lymphoma
10	NCT02215967	Study of T Cells Targeting B-Cell Maturation Antigen for Previously Treated Multiple Myeloma

CONCLUSION

The cancer immunotherapy in the past few decades has developed into much-sophisticated treatment method of cancer. Currently, multiple types of cancer are being treated with various types of cancer immunotherapy. Of those, adoptive immunotherapy has been one of the most promising techniques by showing remarkable success in the treatment of metastatic melanoma. The broad spectrum of adoptive cell transfer therapy can be divided into three categories TILs, TCRs and CAR – T cells.

CAR – T cell therapy has drawn the attention of many clinicians as one of the most promising treatments for cancers specifically leukaemia. Since the approval of the first CAR – T cell therapy for ALL and DLBCL in late 2017 by the US – FDA, it has started a new era of synthetic biology and medicine. The ability of these engineered cells to overcome the tolerance, multiply in vivo after the infusion, work along with the natural immune system of the body and finally surviving in the body for long-duration post-therapy has allowed producing permanent anti-tumour effects in the patients. These types of engineered T cells have multiple benefits over the anti-body treatment regimens both at therapeutic and economic levels. This has been due to the fact that other targeted inhibitors as they require multiple administrations and are not usually curative. Along with that, the administration of the genetically modified CAR – T cells can potentially eliminate the requirement of allogeneic stem cell salvage procedure that is required in some types of haematopoetic cancers.

Though these CAR – T cells have shown promising outcomes in the treatment of haematological cancers, they have not been successful in the treatment of solid tumours. The use of this therapy for the treatment of malignancy will depend upon both the ease of manufacturing them at a large scale at a lower price point and improving the safety profile of the therapy. The toxicity management should be an important aspect for its growth in future. More studies in the future will enable the use of CAR – T cells other than the CD19 – specific CAR – T cells in the treatment of various other diseases and not being limited to the treatment of CD19-positive B cell malignancies. With the development of newer techniques for synthesising genetically engineered cells and combination therapy strategies, soon CAR – T cells will become an off-the-shelf, inexpensive and become possibly a curative treatment for multiple human cancers.

ACKNOWLEDGMENTS

This project becomes a reality due to the kind support of various individuals to whom I would like to express my sincere and heartily gratitude. Without the help of these individuals it would have been next to impossible to complete this project.

First and foremost, I would like to offer this endeavour to our **GOD Almighty** for the wisdom he bestowed upon me, the peace of mind, good health and the strength in order to finish this research work. I am highly indebted to **DELHI PHARMACEUTICAL SCIENCES AND RESEARCH UNIVERSITY, NEW DELHI** for giving me this opportunity to accomplish my research project during the final semester of my undergraduate program.

I would like to express my special gratitude and thanks to my guide and mentor for this research project **DR MUKESH NANDAVE** (Associate Professor, HOD Pharmacology) for imparting his knowledge and expertise in the project. His visionary ideology helped me explore the concept and gain a vast sum of knowledge. Without his constant support it would have been utterly difficult to progress in the research.

Next, I would share the credit to my **PARENTS,** who stood beside me to provide all kind of help during this journey of this research project. Also I would like to extend my thanks to my beloved friends, **MOHAMMAD SAFDAR** and **SANDEEP CHAND** who provided me their valuable suggestions and encouragement until the end of the project and support for the last four years of college.

Special thanks to **MR AJAY KUMAR PAL** (Research Scholar, DPSRU) for this insightful knowledge and support to help me finish the project on time.

REFERENCES

1. S. A. Rosenberg, N. P. Restifo, J. C. Yang, R. A. Morgan, and M. E. Dudley, "Adoptive cell transfer: A clinical path to effective cancer immunotherapy," *Nat. Rev. Cancer*, 2008; 8(4): 299–308. doi: 10.1038/nrc2355.
2. M. E. Dudley *et al.*, "Adoptive cell therapy for patients with metastatic melanoma: Evaluation of intensive myeloablative chemoradiation preparative regimens," *J. Clin. Oncol.*, 2008, doi: 10.1200/JCO.2008.16.5449.
3. M. T. Lotze, A. E. Chang, C. A. Seipp, C. Simpson, J. T. Vetto, and S. A. Rosenberg, "High-Dose Recombinant Interleukin 2 in the Treatment of Patients With Disseminated Cancer: Responses, Treatment-Related Morbidity, and Histologic Findings," *JAMA J. Am. Med. Assoc.*, 1986, doi: 10.1001/jama.1986.03380220083027.
4. U. S. Kammula, D. E. White, and S. A. Rosenberg, "Trends in the safety of high dose bolus interleukin-2 administration in patients with metastatic cancer," *Cancer*, 1998, doi: 10.1002/(SICI)1097-0142(19980815)83:4<797::AID-CNCR25>3.0.CO;2-M.
5. G. Q. Phan *et al.*, "Cancer regression and autoimmunity induced by cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma," *Proc. Natl. Acad. Sci. U. S. A.*, 2003, doi: 10.1073/pnas.1533209100.
6. P. Attia *et al.*, "Autoimmunity correlates with tumor regression in patients with metastatic melanoma treated with anti-cytotoxic T-lymphocyte antigen-4," *J. Clin. Oncol.*, 2005, doi: 10.1200/JCO.2005.06.205.
7. S. A. Rosenberg, J. C. Yang, and N. P. Restifo, "Cancer immunotherapy: Moving beyond current vaccines," *Nature Medicine*. 2004, doi: 10.1038/nm1100.
8. S. A. Rosenberg *et al.*, " Tumor Progression Can Occur despite the Induction of Very High Levels of Self/Tumor Antigen-Specific CD8 + T Cells in Patients with Melanoma ," *J. Immunol.*, 2005, doi: 10.4049/jimmunol.175.9.6169.
9. K. Gowrishankar, L. Birtwistle, and K. Micklethwaite, "Manipulating the tumor microenvironment by adoptive cell transfer of CAR T-cells," *Mammalian Genome*, 2018, doi: 10.1007/s00335-018-9756-5.
10. D. M. Barrett, S. A. Grupp, and C. H. June, "Chimeric Antigen Receptor– and TCR-Modified T Cells Enter Main Street and Wall Street," *J. Immunol.*, 2015, doi: 10.4049/jimmunol.1500751.

11. National Institutes of Health, "NCI Dictionary of Cancer Terms," *Natl. Cancer Inst.*, p. 2020, 2019, [Online]. Available: https://www.cancer.gov/publications/dictionaries/cancer-terms/def/hematopoietic-stem-cell.
12. Y. Kuwana *et al.*, "Expression of chimeric receptor composed of immunoglobulin-derived V resions and T-cell receptor-derived C regions," *Biochem. Biophys. Res. Commun.*, 1987, doi: 10.1016/0006-291X(87)90502-X.
13. G. Gross, T. Waks, and Z. Eshhar, "Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity," *Proc. Natl. Acad. Sci. U. S. A.*, 1989, doi: 10.1073/pnas.86.24.10024.
14. J. Liu, J. F. Zhong, X. Zhang, and C. Zhang, "Allogeneic CD19-CAR-T cell infusion after allogeneic hematopoietic stem cell transplantation in B cell malignancies," *Journal of Hematology and Oncology*. 2017, doi: 10.1186/s13045-017-0405-3.
15. C. A. Ramos and G. Dotti, "Chimeric antigen receptor (CAR)-engineered lymphocytes for cancer therapy," *Expert Opin. Biol. Ther.*, 2011; 11(7): 855–873. doi: 10.1517/14712598.2011.573476.
16. Q. Zhang *et al.*, "CAR-T Cell Therapy in Cancer: Tribulations and Road Ahead," *Journal of Immunology Research*, 2020, doi: 10.1155/2020/1924379.
17. T. Brocker and K. Karjalainen, "Signals through T cell receptor-ζ chain alone are insufficient to prime resting T lymphocytes," *J. Exp. Med.*, 1995, doi: 10.1084/jem.181.5.1653.
18. G. Dotti, B. Savoldo, and M. Brenner, "Fifteen years of gene therapy based on chimeric antigen receptors: Are we nearly there yet?," *Human Gene Therapy*. 2009, doi: 10.1089/hum.2009.142.
19. Y. Hu *et al.*, "Predominant cerebral cytokine release syndrome in CD19-directed chimeric antigen receptor-modified T cell therapy," *J. Hematol. Oncol.*, 2016, doi: 10.1186/s13045-016-0299-5.
20. S. Wilkie *et al.*, "Retargeting of Human T Cells to Tumor-Associated MUC1: The Evolution of a Chimeric Antigen Receptor," *J. Immunol.*, 2008, doi: 10.4049/jimmunol.180.7.4901.
21. M. C. Ramello *et al.*, "An immunoproteomic approach to characterize the CAR interactome and signalosome," *Sci. Signal.*, 2019, doi: 10.1126/scisignal.aap9777.
22. C. Zhang, J. Liu, J. F. Zhong, and X. Zhang, "Engineering CAR-T cells," *Biomarker Research*, 2017, doi: 10.1186/s40364-017-0102-y.

23. M. Chmielewski and H. Abken, "TRUCKs: The fourth generation of CARs," *Expert Opinion on Biological Therapy*, 2015, doi: 10.1517/14712598.2015.1046430.
24. D. Sommermeyer *et al.*, *Chimeric antigen receptor-modified T cells derived from defined CD8+ and CD4+ subsets confer superior antitumor reactivity in vivo*, vol. 30, no. 2. Nature Publishing Group, 2016.
25. J. V. Kim, J. B. Latouche, I. Rivière, and M. Sadelain, "The ABCs of artificial antigen presentation," *Nature Biotechnology*, 2004, doi: 10.1038/nbt955.
26. D. Hollyman *et al.*, "Manufacturing validation of biologically functional T cells targeted to CD19 antigen for autologous adoptive cell therapy," *J. Immunother.*, 2009, doi: 10.1097/CJI.0b013e318194a6e8.
27. W. X. *et al.*, "Comparison of CTSb Dynabeadsc CD3/CD28, Miltenyi TransAct CD3/28 and ExpAct Beads for large-scale CAR T cell manufacturing," *Hum. Gene Ther.*, 2015, doi: http://dx.doi.org/10.1089/hum.2015.29008.abstracts.
28. C. Freimüller *et al.*, "Selection of adenovirus-specific and Epstein-Barr virus-specific T cells with major histocompatibility class I streptamers under Good Manufacturing Practice (GMP)-compliant conditions," *Cytotherapy*, 2015, doi: 10.1016/j.jcyt.2015.03.613.
29. R. J. Brentjens *et al.*, "Eradication of systemic B-cell tumors by genetically targeted human T lymphocytes co-stimulated by CD80 and interleukin-15," *Nat. Med.*, 2003, doi: 10.1038/nm827.
30. A. Deichmann and M. Schmidt, "Biosafety Considerations Using Gamma-Retroviral Vectors in Gene Therapy," *Curr. Gene Ther.*, 2014, doi: 10.2174/1566523213136660004.
31. A. D. Miller, J. V Garcia, N. von Suhr, C. M. Lynch, C. Wilson, and M. V Eiden, "Construction and properties of retrovirus packaging cells based on gibbon ape leukemia virus.," *J. Virol.*, 1991, doi: 10.1128/jvi.65.5.2220-2224.1991.
32. L. Naldini *et al.*, "In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector," *Science (80-.).*, 1996, doi: 10.1126/science.272.5259.263.
33. Y. Ni *et al.*, "Generation of a packaging cell line for prolonged large-scale production of high-titer HIV-1-based lentiviral vector," *J. Gene Med.*, 2005, doi: 10.1002/jgm.726.
34. P. Bajgain *et al.*, "Optimizing the production of suspension cells using the G-Rex M series," *Mol. Ther. - Methods Clin. Dev.*, 2014, doi: 10.1038/mtm.2014.15.
35. P. Kumaresan *et al.*, "Automated cell enrichment of cytomegalovirus-specific T cells for clinical applications using the cytokine-capture system," *J. Vis. Exp.*, 2015, doi:

10.3791/52808.

36. Dana-Farber Cancer Institute, "How CAR T-Cell Therapy Works," *Dana-Farber Cancer Institute*, 2019. https://www.dana-farber.org/cellular-therapies-program/car-t-cell-therapy/.
37. Cleveland Clinic, "CAR T-Cell Therapy: Procedure Details," 2018. https://my.clevelandclinic.org/health/treatments/17726-car-t-cell-therapy/procedure-details.
38. D. (FDA) Grady, "FDA Approval Brings First Gene Therapy to the United States," *Mol. Cell. Pharmacol.*, 2017.
39. V. Prasad, "Immunotherapy: Tisagenlecleucel — the first approved CAR-T-cell therapy: implications for payers and policy makers," *Nat. Rev. Clin. Oncol.*, Oct. 2017; 15. doi: 10.1038/nrclinonc.2017.156.
40. Leukemia & Lymphoma Society, "CHIMERIC ANTIGEN RECEPTOR (CAR) T-CELL THERAPY." https://www.lls.org/treatment/types-of-treatment/immunotherapy/chimeric-antigen-receptor-car-t-cell-therapy.
41. F. News Release, "FDA Approval Brings First Gene Therapy to the United States," *Mol. Cell. Pharmacol.*, 2017; 9(3): 17–18.
42. U. Winkler, M. Jensen, O. Manzke, H. Schulz, V. Diehl, and A. Engert, "Cytokine-release syndrome in patients with B-cell chronic lymphocytic leukemia and high lymphocyte counts after treatment with an anti-CD20 monoclonal antibody (rituximab, IDEC-C2B8)," *Blood*, 1999, doi: 10.1182/blood.v94.7.2217.419k02_2217_2224.
43. R. J. Brentjens *et al.*, "CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia," *Sci. Transl. Med.*, 2013, doi: 10.1126/scitranslmed.3005930.
44. D. W. Lee *et al.*, "Current concepts in the diagnosis and management of cytokine release syndrome," *Blood*, 2014, doi: 10.1182/blood-2014-05-552729.
45. M. L. Davila *et al.*, "Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia," *Sci. Transl. Med.*, 2014, doi: 10.1126/scitranslmed.3008226.
46. S. L. Maude *et al.*, "Chimeric antigen receptor T cells for sustained remissions in leukemia," *N. Engl. J. Med.*, 2014, doi: 10.1056/NEJMoa1407222.
47. S. L. Maude, D. Barrett, D. T. Teachey, and S. A. Grupp, "Managing cytokine release syndrome associated with novel T cell-engaging therapies," *Cancer Journal (United States)*. 2014, doi: 10.1097/PPO.0000000000000035.

48. S. A. Grupp *et al.*, "Chimeric antigen receptor-modified T cells for acute lymphoid leukemia," *N. Engl. J. Med.*, 2013, doi: 10.1056/NEJMoa1215134.
49. D. W. Lee *et al.*, "T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: A phase 1 dose-escalation trial," *Lancet*, 2015, doi: 10.1016/S0140-6736(14)61403-3.
50. M. S. Topp *et al.*, "Safety and activity of blinatumomab for adult patients with relapsed or refractory B-precursor acute lymphoblastic leukaemia: A multicentre, single-arm, phase 2 study," *Lancet Oncol.*, 2015, doi: 10.1016/S1470-2045(14)71170-2.
51. K. J. Curran, H. J. Pegram, and R. J. Brentjens, "Chimeric antigen receptors for T cell immunotherapy: Current understanding and future directions," *Journal of Gene Medicine*. 2012, doi: 10.1002/jgm.2604.
52. M. R. Parkhurst *et al.*, "T cells targeting carcinoembryonic antigen can mediate regression of metastatic colorectal cancer but induce severe transient colitis," *Mol. Ther.*, 2011, doi: 10.1038/mt.2010.272.
53. R. A. Morgan, J. C. Yang, M. Kitano, M. E. Dudley, C. M. Laurencot, and S. A. Rosenberg, "Case report of a serious adverse event following the administration of t cells transduced with a chimeric antigen receptor recognizing ERBB2," *Mol. Ther.*, 2010, doi: 10.1038/mt.2010.24.
54. N. Ahmed *et al.*, "Human epidermal growth factor receptor 2 (HER2) - Specific chimeric antigen receptor - Modified T cells for the immunotherapy of HER2-positive sarcoma," *J. Clin. Oncol.*, 2015; 33(15): 1688–1696. doi: 10.1200/JCO.2014.58.0225.
55. M. C. Jensen *et al.*, "Antitransgene rejection responses contribute to attenuated persistence of adoptively transferred CD20/CD19-specific chimeric antigen receptor redirected T cells in humans," *Biol. Blood Marrow Transplant.*, 2010; 16(9): 1245–1256. doi: 10.1016/j.bbmt.2010.03.014.
56. S. L. Maude *et al.*, "Efficacy and Safety of Humanized Chimeric Antigen Receptor (CAR)-Modified T Cells Targeting CD19 in Children with Relapsed/Refractory ALL," *Blood*, Dec. 2015; 126(23): 683. doi: 10.1182/blood.V126.23.683.683.
57. M. V. Maus *et al.*, "T cells expressing chimeric antigen receptors can cause anaphylaxis in humans," *Cancer Immunol. Res.*, 2013, doi: 10.1158/2326-6066.CIR-13-0006.
58. L. P. B. Philip *et al.*, "Multiplex genome-edited T-cell manufacturing platform for 'off-the-shelf' adoptive T-cell immunotherapies," *Cancer Res.*, 2015, doi: 10.1158/0008-5472.CAN-14-3321.
59. D. B. Kohn, M. Sadelain, and J. C. Glorioso, "Occurrence of leukaemia following gene

therapy of X-linked SCID," *Nature Reviews Cancer*. 2003, doi: 10.1038/nrc1122.

60. S. Hacein-Bey-Abina *et al.*, "Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1," *J. Clin. Invest.*, 2008, doi: 10.1172/JCI35700.
61. J. Scholler *et al.*, "Decade-long safety and function of retroviral-modified chimeric antigen receptor T cells," *Sci. Transl. Med.*, 2012, doi: 10.1126/scitranslmed.3003761.