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Chimeric Antigen Receptor (CAR) T Cell Immunotherapies for Leukemias and Lymphomas

Fatiha U. Iqbal

ABSTRACT

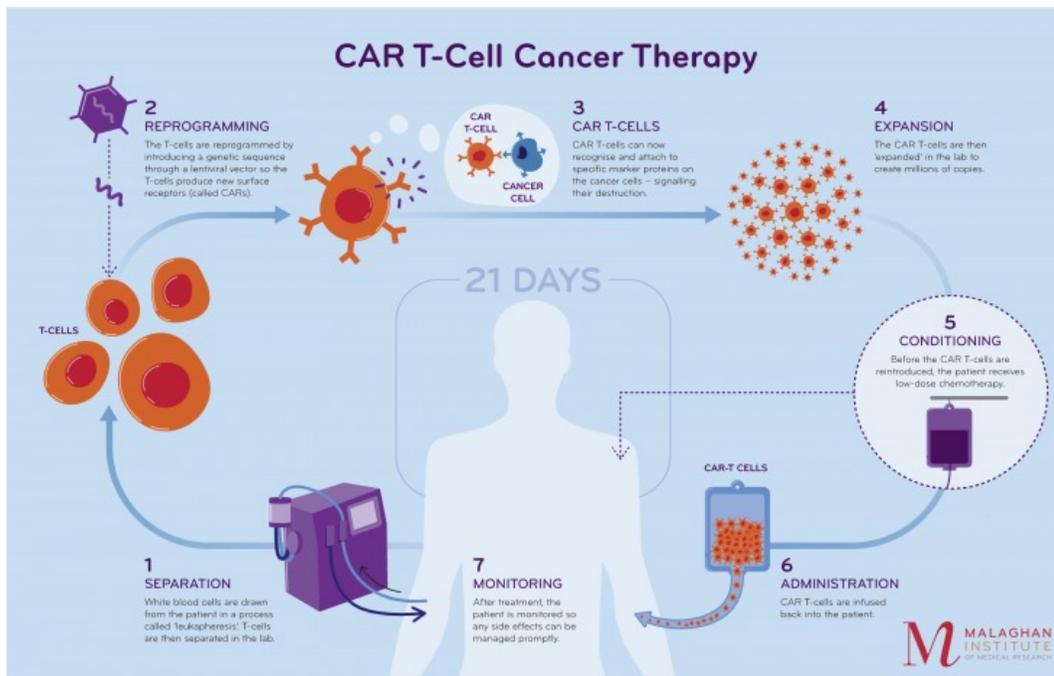
Adoptive cell transfer (ACT) is a rapidly emerging immunotherapeutic approach for cancers. This process includes using a patient's own immune cells and manipulating receptors to fight the cancer. The approach with the most significantly positive clinical results has been chimeric antigen receptor (CAR) T cell therapy. This includes the method of separating the T cells from patients' blood and using disabled virus to genetically engineer new receptors on the T cells. These artificial receptors allow T cells to bind to antigens on tumor cells to inhibit their growth and proliferation. The purpose of this review will be to discuss the typical therapeutic agents for cancers such as lymphomas and leukemias as well as introduce the newer approach of CAR-T cell immunotherapy. Current clinical outcomes and research in this field, including in canines, will be discussed.

INTRODUCTION

Acute lymphocytic leukemia (ALL) is the most commonly diagnosed cancer in children aged birth to 14 years. Acute myeloid leukemia (AML) and chronic lymphocytic leukemia (CLL) are the most

common types diagnosed in adults.

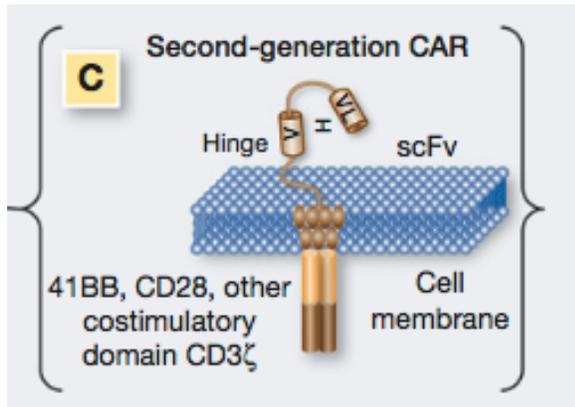
Lymphoma can be divided into two classes: Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL), with NHL being more common. While treatment option for these cancers have increased in the past three decades, survival rate is still not ideal as the



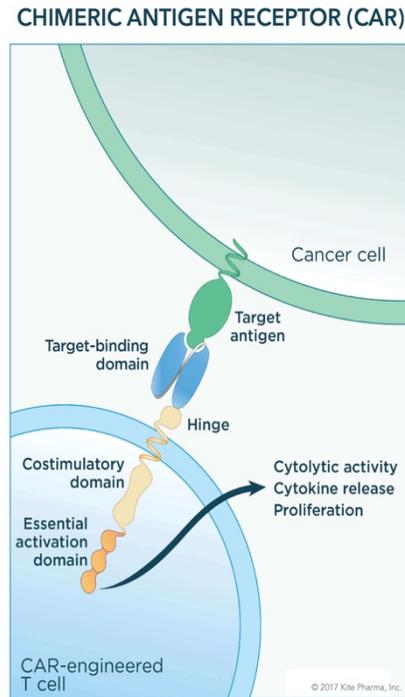
current 5-year survival rate for ALL is 46% for patients aged 20 to 39 years, 30% for those aged 40 to 64 years, and 15% for those aged 65 years and older (13). Patients who have disease that is resistant to chemotherapy and stem cell transplantation have extremely poor prognoses, and CAR-T cell immunotherapy can provide better response rates.

There are two general methods to introduce artificial antigen specificity onto T cells to create CARs. The first way is to clone T cell receptors specific to known antigens and enhance their affinity to overcome central tolerance. The second way is to utilize single chain variable fragments (scFvs) from antibodies that attach to known surface antigens. These fragments are cloned into an expression cassette that incorporates T-cell signaling domains and enables T cells to respond to human leukocyte antigen molecules on antigen presenting cells (7).

CARs are composed of an antigen binding domain, a hinge region, an intracellular signaling domain, and one or more co-stimulatory domains. The scFVs derived from tumor antigen-reactive antibodies are used as the antigen binding domains. All CARs contain the CD3-zeta chain domain as



the intracellular signaling domain. Second- or third-generation CARs improve proliferation, cytokine secretion, resistance to apoptosis, and *in vivo* persistence. Second generation CARs have been the most used in clinical trials. Most of the constructs in the second generation utilizes co-stimulatory transmembrane domains, like CD28 and CD8 to carry out their effects. The CD19-specific CAR enables CD28 co stimulation, which is a key receptor in many clinical trials and will be discussed later in this review (7).



It is not enough to simply genetically design a CAR; is also necessary to transfer specific genes into T cells. This can be done through gammaretroviral or lentiviral transduction. Nonviral gene transfer can also be used which is mostly done through

transposon/transposase systems techniques which already exist such as the Sleeping Beauty transposon system. This system results in sustained expression of CAR on T cells and anti-CD19 transposon CAR T cells in early-phase clinical trials. Finally, electroporation of T lymphocytes with in vitro transcribed RNA can also be done. This leads to high but temporary expression of the CAR, which is why this method is not as often used (3, 4, 7).

In 2017, the first two CAR T cell immunotherapies were approved by the Food and Drug Administration (FDA). The first, tisagenlecleucel (Kymriah™), was used in children and adults with advanced leukemia. The second, axicabtagene ciloleucel (Yescarta™), was utilized in patients with large B cell lymphomas (8).

In 2018, James Allison and Takuso Hogeno won the Nobel Prize in Physiology or Medicine for their work in discovering the cytotoxic T-lymphocyte antigen 4 (CTLA-4) receptor and the programmed death (PD-1) molecule on T cells respectively. Blocking these two mechanisms has shown to decrease tumor sizes by allowing T cells to launch better immune attacks against various cancers. PD-1 blocking drugs are also now approved for lung, kidney, and bladder cancers as well as melanomas (6). This approach utilizes the intrinsic properties of T cells and does not involve any genetic engineering like CAR T cell therapy.

In 2018 the Food and Drug Administration also published a guide on cellular and gene

therapies in order to propose more guidance regarding manufacturing of these drugs and improved clinical development. As of October 2018, the FDA also had over 700 active investigational drug applications related to gene therapy which shows this is one of the fastest growing areas for many genetic diseases as well as cancer (3).

TRADITIONAL THERAPEUTIC APPROACHES

Ibrutinib is a drug which is traditionally used in B cell cancers, such as chronic lymphocytic leukemia. This drug binds permanently to the protein Bruton's tyrosine kinase (BTK). This kinase normally plays a critical role in B cell maturation and mast cell activation through IgE receptors. However, primary (inherent) and secondary (acquired) resistances have been seen while using this drug. This may be due to mutations which impair the affinity of Ibrutinib for BTK or alterations in other pathways downstream of BTK. The survival of patient's receiving Ibrutinib is also very low with a median three-month survival rate after use. For the patient's that do experience success with Ibrutinib, many patients relapse within three years of treatment (5).

Residual or relapsed lymphomas are often treated with oral compounds such as cyclophosphamide, which are immune suppressors. However, these types of compounds can be toxic, which is why it can only be used in patients with severe or relapsed disease. They also cannot be given at very high concentrations and many side

effects exist including anemia, emesis, thrombocytopenia, allergic reactions and pulmonary fibrosis. These types of compounds are also not effective in leading to complete response. In a clinical trial done by Ricconi et. al, 18 patients with residual/relapsed lymphoma received 100 mg oral cyclophosphamide every day for 15 days every month for six months. Three of these patients had no response, ten had partial remission and only five had complete response. The overall survival in patients with aggressive history of cancer was only 20 months after receiving this treatment (13).

Rituximab is another compound which is used with non-Hodgkins lymphoma and leukemia. Rituximab is an anti CD20 monoclonal antibody and can play a key role in the apoptosis of cancerous B cells, especially when used in conjunction with compounds such as cyclophosphamide. However, not all patients respond equally well to rituximab, and in vitro studies has shown resistance to rituximab involving the anti-complement inhibitors CD55 and CD59. This drug also has many unwanted side effects including rash, hypotension, shortness of breath, leukoencephalopathy, and epidermal necrolysis. In a clinical trial done by Maury et. al, 209 patients were split into two groups and treated with rituximab or a control substance. Two-year event free survival rates were 65% for the treatment group and 52% for the control group, but the severe adverse events did not differ significantly between the two groups. The survival difference is only 7%, but because both groups had severe adverse effects, the

adverse effects do not outweigh the small change in survival rate (9).

Finally, stem cell transplantation (SCT) including allogenic and autologous can be used for treating leukemias and lymphomas. Many times, SCT allows a patient to receive higher doses of chemotherapeutic drugs as severely depleting and damaging the bone marrow is less of a concern. In allogenic SCT, donors who have a similar human leukocytic antigen (HLA) to the patient are chosen. These donors are typically family members who have close tissue matches to the patient. A downside to this method is the ability to develop graft-versus-host-disease (GVHD) in which the donor's immune system will attack the patient's own body tissues. A major part of the development of GVHD is donor T cells recognizing recipient minor histocompatibility antigens which facilitates an adverse immune response. In addition, it can be difficult to find a good match if family members are not good candidates and utilizing the matched unrelated donor method has been linked to more complications. In autologous SCT, a patient's own stem cells are removed and stored while the patient is receiving chemotherapy, and then reinfused after completion of treatment. While GVHD is not a problem with this method, it can be difficult to separate normal stem cells from leukemia cells in the bone marrow of the patient. A laboratory technique called purging is used to remove the leukemia cells, but there is still a risk of returning some leukemia cells with the SCT (15).

A study done by Bleakley et. al was done to see if the risk of GVHD could be reduced in patients with high risk leukemia.

Researchers conducted in vitro studies in mice to observe the effects of naïve versus memory T cells in donor stem cells. It was found that the frequency of human CD8⁺ T cells specific for minor H antigens was at least 5 to 20-fold higher in naïve T cells compared to memory T cells. The naïve T cells were then depleted from peripheral blood stem cells and implanted into 35 patients after they had received chemotherapy consisting of total body irradiation, thiotepa and fludarabine. The incidence of acute GVHD did not decrease, but there was less amount of chronic GVHD (9% of patients). This is compared to 50% of chronic GVHD in patients with naïve T cell grafts. In addition, including memory T cells within the SCT resulted in rapid T cell recovery and transfer of protective virus specific immunity. The overall survival rate of the 35 patients was 78% after two years (1).

CD-19 SPECIFIC CAR

CD19 is a type 1 transmembrane protein within the immunoglobulin superfamily. Functioning of CD19 is dependent on three cytoplasmic tyrosine residues – Y391, Y482 and Y513. Prior experiments have shown substituting tyrosine for another amino acid leads to inhibition of phosphorylation and downstream signaling. CD19 is a biomarker used for normal and neoplastic B cells and it reduces the threshold required for receptor-dependent signaling in B cells. Its concentration is also three times higher in

mature B cells compared to immature ones, and it is critical in enabling the body to mount an immune response. CD19's expression is highly conserved on B cell tumors and it is also believed these tumors may maintain surface CD19 expression in order to amplify transmembrane signals and promote their expansion and survival. It is also believed that CD19's autoimmunity works via MHC class II receptors in vivo (2, 16, 17).

Poe et al., showed how c-myc and CD19 expression worked synergistically to influence B cell lymphoma severity. In this study Eμ-Myc transgenic (c-Myc^{Tg}) mice which normally develop aggressive and lethal B cell lymphomas were made CD19 deficient (c-Myc^{Tg}CD19^{-/-}). The CD19 deficient mice had significantly prolonged lifespan (81-83%). This was done through reduction in c-Myc phosphorylation at the S62, which is a regulatory site that contributes to the stability of the c-Myc protein. Reduction in c-Myc phosphorylation was only seen in CD19^{-/-} mice showing that there is a synergistic effect between c-Myc and CD19 (12).

The level of CD19 needs to be tightly regulated within animal models. It has been shown CD19 deficient mice have weak T-cell dependent humoral responses. In addition, total number and frequency of peripheral and splenic B cells decreases with deficient amounts of CD19. On the other hand, over expression of CD19 also manifests problems as it is linked with greater than 80% reduction in peripheral B cells due to impaired generation and

development of precursors in the bone marrow. Over expression can also dramatically alter the levels of immunoglobulins in the body. IgG2b levels increased by 168%, IgG3 levels decreased by 77%, and IgA levels remained unchanged. CD19 is also expressed at normal to high levels in acute lymphoblastic leukemias, chronic lymphocytic leukemias (CLL) and B cell lymphomas (12).

CAR-T cell therapy was also found to be effective in patients who had already received cyclophosphamide (Cy)-based lymphodepletion chemotherapy with or without fludarabine (Flu). In a study done by Turtle et.al, CAR-T cells were manufactured from defined T cell subsets and given in a one to one ratio of CD4+/CD8+ CAR-T cells to patients with relapsed and/or refractory B cell non-Hodgkin lymphoma. Results showed that there was more expansion and persistence of CAR-T cells in patients who had received both Cy and Flu in the past compared to patients only receiving Cy. Complete response was seen in 62% of the patients who had received both Cy and Flu in the past. It is unclear if these results could be seen with other types of chemotherapeutic agents (17, 13).

CANINE RESEARCH

CAR-T cell immunotherapy can also be applied to veterinary medicine in dogs. B cell lymphomas are the most common hematopoietic cancer in dogs and the blood systems are conserved in both humans and dogs. With the fact that the epidemiology of canine cancer is not well known and with

little data available for how dogs with cancer are treated, CAR-T cell immunotherapy could be a key therapeutic approach to use in veterinary medicine (10).

There are only a handful of FDA approved veterinary oncology therapeutics available and only one drug available for B cell lymphomas (abacfosadine). Typically, dogs with high grade lymphomas are treated with single agents such as prednisone or doxorubicin or combination chemotherapy. It has been agreed among veterinary oncologists that doxorubicin-based combination chemotherapy gives provides the longest period of disease control and overall survival. Unfortunately, response to chemotherapy can most often be suboptimal and animals can have recurrent disease. Bone marrow transplant is also difficult to do in the veterinary setting. Rituximab was also used in canines but was not found to be effective in binding to or depleting B cell lymphocytes (10).

Pankwaj et. all were the first to use mRNA electroporation to expand canine T cells ex vivo. Artificial antigen-presenting cells genetically modified to express human CD32 and canine CD86 were used. These artificial antigen-presenting cells were loaded with a canine CD3 monoclonal antibody and used in combination with human IL-2 and IL-21 to preferentially expand CD8+ T cells in canines. The expanded T cells were then injected into a dog with relapsed B cell lymphoma. The dog tolerated the treatment well, but the anti-tumor activity was short lasting (10).

Future research is underway to observe the clinical efficacy of more stable CAR-T cells in order to make anti-tumor activity longer lasting (10).

CLINICAL TRIALS IN HUMANS

As mentioned in the introduction, tisagenlecleucel was the first anti-CD19 CAR-T cell therapeutic drug made. This drug is still under investigation in pediatric and young adult patients with relapsed or refractory B cell acute lymphoblastic leukemia (ALL). The Children's Hospital of Philadelphia conducted a single-phase study of Tisagenlecleucel with 60 children and young adults with relapsed or refractory ALL. The results from this study showed a remission rate of 93% (8).

On the basis of these results, a phase two clinical trial was done by Maude et al. on five study sites in eleven countries across North America, Europe, Asia, and Australia. Research participants were 75 patients between 3 and 21 years in age and had not previously received anti-CD19 therapies. Tisagenlecleucel was produced ex-vivo with CD137 domain used to provide a costimulatory signal along with the CD3-zeta domain and was infused into patients (8).

The overall remission rate was 81% within three months of the infusion. These patients were also tested for residual minimal disease through flow cytometry and were not found to have residual disease. Event-free survival was 73% at six months and 50% at twelve months. Thirty days after tisagenlecleucel

infusion, 17 of the 75 patients died due to relapse ALL or progressed disease (8).

In a study done by Neepalu et al., researchers utilized axicabtagene ciloleucel (axi-cel) which is an autologous anti CD-19 CAR. Axicabtagene ciloleucel was the second CAR-T cell immunotherapeutic agent approved by the FDA in 2017. This drug has a single-chain variable fragment extracellular domain targeting CD19 proteins with CD3-zeta and CD28 co-stimulatory domains. Prior to this study, single institution studies, such as at the National Cancer Institute, have found that many responses to axi-cel and other anti-CD19 CARs have been ongoing beyond 4 years, which suggests that this therapy may be potentially curative for lymphomas. This study was done in patients who did not see any progress with conventional treatments. 101 patients with diffuse large B cell lymphoma, primary mediastinal B cell lymphoma, and transformed follicular lymphoma were enrolled in a phase two clinical trial (11).

Patients received a target dose of 2×10^6 anti-CD19 CAR T cells per kilogram of body weight. 85% of the patients receiving this treatment had stage III or VI cancer and 69% had received at least three prior therapies which were not effective. 82% of the patients who were treated had an objective response, and 54% had a complete response. Researchers followed up with the patients 15 months later and saw responses ongoing in 42% of the patients. 40% of the patients still had complete response 15 months after treatment. 52% of the patients

had also survived 18 months post treatment. Three of the patients died during the treatment. The median duration of response was 11.1 months and the median duration of progression-free survival was 5.8 months (11).

SIDE EFFECTS

Like the traditional chemotherapeutic agents used for leukemias and lymphomas, severe side effects can also occur with the use of CAR-T cell therapy. In the tisagenlecleucel study, 73% patients experienced grade three or four adverse events which were directly related to the use of the T cell therapy. The most common event being severe cytokine release syndromes which included increases interleukin-6, interferon gamma, and ferritin levels. Higher grade cytokine release syndromes were also associated with more

neurological side effects. Other common side effects were hypotension, and decreased lymphocyte count. All patients who responded to the treatment also developed B cell aplasia and had to be given immunoglobulin replacement. Six months after infusion, B cell aplasia continued to persist in 83% of patients (8).

In the axi-cel study, the most common adverse events 3 were pyrexia (85%), neutropenia (78%), anemia (43%), and thrombocytopenia (38%). Grade 3 or higher cytokine release syndrome occurred in 13%. Compared to the Tisagenlecleucel study, there were less patients with cytokine release syndrome. Patient's with cytokine release syndrome did respond well to vasopressors. There were more neurological symptoms with axi-cel than Tisagenlecleucel. 64% of patients

Table 2. Grade 3 or 4 Adverse Events Suspected to Be Related to Tisagenlecleucel That Occurred in at Least 5% of Patients.

Event	≤8 Wk after Infusion (N=75)		>8 Wk to 1 Yr after Infusion (N=70)	
	Grade 3	Grade 4	Grade 3	Grade 4
	<i>number of patients (percent)</i>			
Any grade 3 or 4 adverse event	19 (25)	33 (44)	8 (11)	4 (6)
Cytokine release syndrome	16 (21)	19 (25)	—	—
Hypotension	7 (9)	6 (8)	—	—
Decrease in lymphocyte count	5 (7)	4 (5)	1 (1)	—
Hypoxia	5 (7)	3 (4)	—	—
Increase in blood bilirubin	8 (11)	—	—	—
Increase in aspartate aminotransferase	5 (7)	2 (3)	—	—
Pyrexia	5 (7)	2 (3)	—	—
Decrease in neutrophil count	1 (1)	6 (8)	1 (1)	1 (1)
Decrease in white-cell count	—	7 (9)	—	—
Decrease in platelet count	3 (4)	4 (5)	—	—
Decrease in appetite	6 (8)	1 (1)	—	—
Acute kidney injury	3 (4)	3 (4)	—	—
Hypophosphatemia	5 (7)	1 (1)	—	—
Hypokalemia	6 (8)	—	—	—
Pulmonary edema	4 (5)	1 (1)	—	—
Thrombocytopenia	1 (1)	4 (5)	—	1 (1)
Encephalopathy	4 (5)	—	—	—
Increase in alanine aminotransferase	4 (5)	—	—	—
Fluid overload	4 (5)	—	—	—

experienced neurological symptoms with 25% percent of patients experiencing grade three or higher neurologic symptoms. The most common grade three or higher symptoms included encephalopathy, confusion and aphasia. However, the median onset of these neurological symptoms was on day 5, with median resolution on day 17. The prevalence of cytokine release syndromes and neurological symptoms also decreased during the course of the study. Three of the patients died during treatment (11).

However, the difference with this type of therapy is that additional therapy was not needed. Patient's only received one single infusion of tisagenlecleucel or axi-cel without the need for SCT or other oral compounds. Many of the adverse side effects were mitigated through supportive measures such as utilizing tocilizumab, a monoclonal antibody against IL-6, for the cytokine release syndromes and vasopressors for hypotension.

CONCLUSION

To conclude, CAR T-Cell therapy has been effective in many patients with leukemia and lymphoma in inducing remission and improved symptoms. This type of therapy is most popular in patients in which typical therapies have not been effective. CAR T-Cell drugs have also been approved by the FDA, but unwanted side effect profiles continue to be a problem with this type of therapeutic agent. These treatments are also very cost prohibitive and the criteria to qualify to obtain the treatment is very

stringent. However, research in this field continues to become more popular and is advancing rapidly (8, 11).

Future directives in this field have already began. A newer approach is the development of CAR T cell therapies which use immune cells collected from healthy donors instead of cancer patients. The goal of this is to create CAR T cell therapies which can be readily available for use instead of manufacturing therapies individually for each patient, which can be both expensive and time consuming. The French company Cellectis, has launched a phase I trial of this type of CD19-targeted CAR T-cell product in the United States for patients with advanced acute myeloid leukemia. Cellectis uses a gene-editing technology known as TALEN which has already been tested in Europe, including in two infants with ALL who had already gone through all of the available therapeutic options. In both instances, the treatment was effective. It will be important to continue to use CAR-T cells from donor patients in a larger sample size to look at efficacy.

Numerous other researchers are also looking to develop CAR T cells in vivo with the ability to turn the cells "off or on" with the hope of relieving unwanted side effects. In addition, utilization of CRISPR/Cas 9 systems are also being considered to more precisely engineer T cells. So far, this type of therapy has not been effective against solid tumors, but researchers are looking to develop more advanced T cells which would be able to penetrate solid tumors.

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