2022

Chimeric Antigen Receptor T-Cell Therapy in Cancer

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Abstract:

The development of immune-checkpoint-inhibitors (ICIs) has led to promising advancements in the treatment of patients with cancers, leading with the use of cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) as a negative regulator of T cell activation in the mid-1990s. With the discovery of two ligands for program cell death protein-1 (PD-1) and promising checkpoint blockades in 2010, this sparked a cascade of hallmark immunotherapy drug patents, focusing on the mechanism of anti PD-1 and anti PD-L1 antibody inhibitors. Since then, chimeric antigen receptor (CAR)-engineered T (CAR-T) cells have emerged into the immuno-oncologic scene for treatment of hematological malignancies. These genetically modified T-cells focus on the destruction cancer cells without the need of chemotherapy.

One Sentence Summary: The history of the immune system, immunotherapy, then CAR-T cell therapy is discussed as an efficient method of combatting hematological (blood) cancers and supported by clinical case studies.

Abbreviations: APC=antigen presenting cell; CKI=checkpoint inhibition; FDA=Food and Drug Administration; ICIs=immune-checkpoint-inhibitors; CTLA-4=cytotoxic T lymphocyte-associated antigen 4; PD-1=Programmed cell death protein-1; PD-L1=Programmed cell death ligand-1; CAR=chimeric antigen receptor; PRRs=pattern recognition receptors; TLRs=Toll-like receptors; MHC=Major histocompatibility complex; TAAs=Tumor-associated antigens; T_h=Helper T; T_c=Cytotoxic T; TCR=T cell receptor; ALL=acute lymphoblastic leukemia; CRISPR=Cluster Regularly Interspaced Short Palindromic Repeats; IS=immunological synapse; CTLs=cytotoxic T lymphocytes; scFvs=single-chain variable fragments

Introduction

The Immune System

The immune system is a complex system made up of organs, tissues, cells, and molecules that protects the human body from infection and diseases. Body responses are broken down into two distinct categories: The innate immune response and the acquired immune response. The innate immune system is composed of chemical and physical barriers such as acidic pH levels within the stomach, the epithelial cells within the skin, and cilia lining the airways (*1*). If a pathogen succeeds at breaching a chemical or physical barrier, macrophages will respond by engulfing the unwanted substance. When pathogen concentration increases, pattern recognition receptors (PRRs) are activated, causing the release of cytokines, promoting inflammation and recruiting other myeloid cells. Toll-like receptors (TLRs) activate transcription factor, NF-κB, stimulating an inflammatory response and cytokine secretion, which causes vasodilation. Thus, the acquired immune response is activated.

The immune system can identify a threat, mount an attack, eliminate a pathogen, and remember the offender if it were to be encountered again. The acquired immune response consists of activated cells that differentiate between a diverse number of pathogens. Immune cells, such as T cells and B cells, will undergo monoclonal expansion and later memorize pathogenic substances to provoke a future, stronger response (*1*).

B cells express surface receptors that bind to specific antigens and can act as an antigen presenting cell (APC). Antigens are loaded onto the major histocompatibility complex (MHC) II Complex which then displays the antigen to a T cell. When a T cell is activated by the B cell/T cell conjugate, T cells will secrete cytokines that assist in B cell maturation into either a plasma cell or memory B cells, which secrete antibodies into the serum to tag pathogens for destruction

(*Figure 1*). T cells then mediate the destruction of the tagged pathogen via processes called apoptosis and granule exocytosis (*2*).

Cancer and Immunotherapy

Cancer

Cancer is considered to be a genetic disease caused by the mutations of genes that control the way cells grow and divide (*3*). This rapid duplication of cells may eventually lead to a tumor through a process called carcinogenesis (*1*). While cancer cells are recognized as foreign substances, the immune system fails to do so at times because some cancers may display similar features as normal cell. This is due to inadequate eradication or due to the cancer cells displaying similar features as normal cells. There are also some cases where the immune system may not respond well enough to destroy a particular cancer, causing further metastasis.

Tumors can be classified into two categories. Benign tumors are a collection of abnormal growing cells and are non-cancerous in nature. If these neoplastic cells were to break through the basement membrane of an epithelial cell and spread throughout the body, this would be defined as metastatic disease, requiring immediate attention (*3*). Metastatic disease can lead to many adverse effects depending on the location of the tumor and may necessitate treatments including chemotherapy, radiotherapy, or immunotherapy.

Staging

Staging of one's cancer is vital for clinicians to determine the location and severity of the disease for proper management options. Although cancer is unpredictable, knowing the stage may also help predict the course cancer will take based on the trend of previous data. Most

importantly, staging of a tumor assists physicians help describe its characteristics to their patients in order to make a decision that is right for them.

Although the TNM staging system is the most generally used cancer staging system, leukemia staging is slightly different as leukemia derives from the bone marrow. Staging is categorized based on one's blood cell count and accretion of leukemia cells within the organs. Not all leukemias are the same, as each subtype has its own individual differences in their staging. For example, acute myeloid leukemia uses a cytologic (cellular) system which characterizes the cancer based on the size and number of leukemia cells, change of appearance within the chromosomes of leukemia cells, the number of healthy blood cells, and other genetic abnormalities that have occurred in the patient (*Table 1*) (*5*).

Immunotherapy

Radiation and chemotherapy were the standard of care treatment options for many who were suffering from cancer. This changed in the early 2000s when a new approach to combatting this deadly disease was discovered. Programmed cell death protein 1 (PD-1) is a protein found on T cells that keep the body's immune system in check (*3*). Programmed cell death ligand-1 (PD-L1) is a protein which acts as an inhibitor to the immune response and is found on normal cells and in higher-than-normal amounts on certain cancer cells (*3*). When PD-L1 and PD-1 bind, PD-L1 acts as an antagonist towards the T-cell programmed cell death mechanism allowing cancer to flourish and spread. The discovery of this mechanism led to the creation of many anticancer drugs that inhibit the antagonist interactions between PD-1 and PD-L1 through a process known as checkpoint inhibition (CKI) (*Figure 2*). Diseases expressing high concentrations of PD-1 and PD-L1 receptors include colorectal, lung, gastric, bladder, pancreatic, prostate, and diffuse large B-cell lymphoma (*6*).

By 2011, the first checkpoint inhibition drug, ipilimumab (Yervoy), was approved for treatment for patients diagnosed with advanced melanoma (*7*). Within the next five years, inhibitor drugs including nivolumab (PD-1 inhibitor), and atezolizumab (PD-L1 inhibitor) had gained the approval by the Food and Drug Administration (FDA). CKI's are currently being used to treat various cancers such as non-small cell lung cancer, bladder carcinoma, melanoma, and renal cell carcinoma with associated high response rates (*7*).

Immunotherapy has now changed the way physicians' approach and treat numerous cancers as some studies favor the use of immunotherapy over certain chemotherapy regimens due to improved overall outcomes and significantly decreased adverse effects of cytotoxic therapies. The benefits over traditional treatments and rise in popularity have increased the importance of continued research to new interactions between the immune system and its rival, cancer cells.

Chimeric Antigen Receptor T Cells

Chimeric Antigen Receptors (CARs) are synthetic receptors that enable T cells to recognize tumor-associated antigens (TAAs) (*6*). Normally, naïve T cells require costimulation by an APC with an antigen loaded on its MHC class I or II molecules. In the case of a helper T (T_h) cell, its CD40L and CD28 ligands must interact with the APC's CD40 and B7 respectively. Cytotoxic T (T_c) cells also require costimulation for activation, however, only need the interaction of the CD40L and CD40. In the case of CAR T cells, these are synthesized to recognize TAAs without the interaction of the T cell receptor (TCR)-MHC complex. Instead, these CAR T cells will target the pan-B cell marker CD19 to activate its cell death mechanism (*Figure 3*). CAR T cells have shown great response rates in treating refractory B cell malignancies with drugs being implemented into clinical use (*8*).

CAR T cells emerged in 1993 by immunologist, Zelig Eshhar, when the first chimeric molecule was fused to part of a TCR and tumor-specific cytotoxicity was observed. Later tests proved these first-generation CAR T cells to be nonviable as they failed to elicit potent antitumor effects. This process was almost forgotten until 2002 when the first effective second-generation CAR T cell was created by the Memorial Sloan Kettering (MSK) Team (*9*). Second generation CAR T cells containing a costimulatory domain, targeted prostate cancer antigens. Using second generation cells were then modified to target the CD19 ligand which showed high efficacy in destroying leukemia cells in mice (*9*). By the year 2013, the first human CAR T cell clinical trial was published by the MSK group. Here, patients diagnosed with acute lymphoblastic leukemia (ALL) were treated with CD19 targeting CAR T cells showed significant response. This paved the way for many new CAR T cell therapies. Scientists continued to develop this generation of engineered cells and have since created "Armored CARs" that secrete active cytokines or express ligands based on the specific tumor environment that further armor CAR T cells to improve their efficacy and persistence. Cytokines included in the mechanism behind armored CAR T cells include IL-12, CD40L and 4-1BBL (*10*).

In recent years, the emergence of genome editing has played a vital role creating new CAR T cell with improved recognition of cancer. Dr. Sadelain and colleagues have since been able to use the genome-editing tool, Cluster Regularly Interspaced Short Palindromic Repeats (CRISPR), to place a CAR at designated sites of T cells for boosted function (*11*). Sadelain was quoted saying the CAR T cells within the mice "retained their ability to kill tumor cells for much longer than conventional CAR T cells, which burn out more quickly." Essentially, this mechanism involves the DNA slicing at a particular location with the insertion of a new gene (*11*). The improved efficacy combined with an extended life-span of these CAR T cells results in fewer cells required for therapy, the potential for decreased cytotoxicity, and improved tolerance

by the patient. The first CRISPR-CAR T cell clinical trial, *"A Phase I Study of CTX130 Immunotherapy in People with Persistent or Recurrent T-Cell or B-cell Cancers,"* is currently underway in New York and is scheduled to be completed in 2027.

Mechanism

Classical cytotoxic T cell activation and function rely on the interaction with APCs and the forces through the immunological synapse (IS). This includes the interactions of MHC I and TCR as well as CD40 and CD40L. Disturbances of this synapse occur in the presence of various cancers, decreasing the efficacy of CTLs. Engineered CAR T cells act independently of the IS and target tumor antigens independently of their presentations of antigens on MHC molecules (*12*).

CAR T cells resemble both an antibody and TCR in the sense that they are recombinant transmembrane receptors composed of an extracellular binding domain of a monoclonal antibody single-chain variable fragments (scFvs) for the respective TAA, a hinge domain, and an intracellular signaling domain of a TCR molecule (CD3ζ). The next generation of CARs are given a CD28 costimulatory domain proximal to the CD3ζ tail for supplementary signal strength (*13*). These newer, engineered T cells are specifically designed to recognize specific tumor antigens independent of an MHC expression complex.

In some cases, CKI drugs (anti PD-1, PD-L1, CTLA-4) may not work due to the cancer cells not properly expressing a sufficient amount of MHCs, causing T cells to remain inactive. CAR T cells avoid this because they act independently of MHC. Once the CAR T cell comes in contact with a cancer cell, signaling events induce and granule exocytosis, cytokine secretion, and T cell proliferation is conducted.

Generations of CAR T-cells

The second generation of CAR T cell frequently imbues a CD28 costimulatory domain nearing the CD3ζ tail to forte the activity of the CAR through phosphatidylinositol-3-kinase (PI3K) signaling (*Figure 4b*).

The third generation of CAR T cells focus on the additional costimulatory ligand 4-1BB, which joins CD28 at the intracellular domain. This connection prolongs the tenacity of the cytotoxic effects of a CAR T-cell through the extracellular signal-regulated kinase (ERK) signal (*Figure 4b*). The 4-1BB stimulus augments the CAR T-cell purpose and perseverance through the NF-κB signaling, encourages T-cell differentiation to central memory cells and protects against CAR T-cell exhaustion, allowing for a long-term purging of tumor cells (*13*).

Another third generation emphasizes the interaction between CD28-OX40. This costimulatory signal induces a significant increase in the production of IL-2 and IL-10 cytokines when compared with its older sibling of the second-generation CAR T-cell (*14*). The heightened expression of IL-2 and IL-10 is beneficial for the effector functions and persistence of CAR T-cells in vivo.

The fourth generation of CAR T-cell uses single costimulatory domains that elevate the expression of cytokines like IL-12, dispersing the intracellular signal from the costimulatory molecules like PD-1 (*14*). This results in the improvement of the efficiency of the CAR T-cell death mechanism (*Figure 4b*). A single activation of CAR instantaneously induces numerous signaling events including the release of perforin granzymes, cytokine secretion, and T-cell proliferation (*14*). Once the cancer cells are destroyed, some of the remaining T-cells become memory lymphocytes for future recurrences.

The peripheral molecules of the immunological synapse play significant regulatory functions on signaling the cytotoxic mechanism. This immunological synapse heavily relies on

adhesion molecules such as LFA-1 and talin. The engagements of these adhesion molecules play important roles in antigen recognition. Once these adhesion molecules are engaged with the antigen, this will result in a dampening of the TCR ligand density requirement for necessary signaling, increase the affinity of LFA-1 to the intercellular adhesion molecule 1 (ICAM-1), and LFA-1 ligation would boost actomyosin forces, further increasing the affinity of other glycoproteins on T-cells (*15, 16, 17*). The overall improved T-cell adhesion results in stabilization of the CAR TCR immunological synapse and extended signaling.

Synergies

There are additional factors that affect CAR T-cell signaling induction. The first is the action of actomyosin. When the CAR TCR ligates with its respective antigen, actomyosin synergistically polymerize retrograde flow in the immunological synapse. Retrograde flow involves the actin filaments located in the leading edge of the migrating T-cell and flowing back into the body of the cell, creating an efficient binding of the TCR–pMHC and CAR–antigen complex. T-cells will create F-actin-rich overhangs, organizing the secretion of perforins (*18*). Actomyosin polymerization encourages the dispersion of T-cells across its cell surface for optimal antigen–receptor binding.

Microtubules are also vital mechanisms of the CAR TCR immunological synapse (*19*). The microtubules aid as the supporting framework to guide the reorientation of centrosome in the direction of the antigen-receptor complex (*20*). This transformation process is important for the release of lytic granules in central immunological synapse. Microtubules also regulate the killing efficiency of CAR T-cell. They act as anchors for the cytotoxic granules and transport the granules toward the center of the immunological synapse, inducing the rapid secretion of lytic

granules for the imminent cytolysis of cancer cells (*21*). The secretion of granules is much faster in the CAR T-cell mechanism when compared to the typical CTL death process.

Metabolism

CAR T-cell activation initiates remodeling and influences numerous chromatins and organelles, which are mirrored in the change of metabolic status of the T-cell. The metabolism of T-cell governs the progression of the immune responses. A naïve T-cell typically has low-rate fatty acid metabolism, which require remarkable upregulation upon activation (*22*). Given this, naïve and memory T-cell development rely heavily on oxidative phosphorylation and mitochondrial metabolism. Phenotypic transformation is conducted with the activation of naïve CD8+ T-cells. Glycolysis is enhanced to meet the energetic demands for the rapid proliferation, force generation, signaling transduction, and production of cytolytic granules in the activated T-cell (*22*). CD28 costimulation has been reported to enhance the signaling by the CAR TCR through the PI3K signaling and effectively elevates glycolysis and mitochondrial oxidative metabolism of the activated CTLs through the upregulated expression of glucose transporter 3 (GLUT3) to fulfill effector functions (*23*). Increased metabolism results in the synthesis of metabolic enzymes including pyruvate kinase and oxidized nicotinamide adenine dinucleotide (NAD+), both of which partake the histone acetylation and chromatin remodeling that regulate the cytokine secretion of T lymphocytes (*24*).

Cytokine Release Syndrome

Cytokine Release Syndrome (CRS) is defined as a systemic inflammatory response that can be activated by infection and therapeutic drugs (*25*). Given the growing success of CAR T cell therapy, CRS has been at the forefront of debate regarding the genetically-engineered cell's

safety when administered to patients. Research has shown CD19-targeted T cells revealed that CRS is the most significant adverse event, with cytokine storms being reported upwards of 100% of the patients within the clinical trials *(Table 1)* (*26, 27*).

CRS usually begins with flu-like symptoms including headache, fatigue, and arthralgia. If untreated or monitored, symptoms can worsen over time and become life-threatening in severity. If the CAR T cells are unable to stop signaling the inflammatory response of the immune system, lab abnormalities will begin to arise, such as increased creatinine, liver enzymes, and cytopenias (*25*). If treatment is not adjusted, this may result in intravascular coagulation and possible organ failure.

Recent studies have shown progression in controlling CAR T cell induced CRS. Currently, inhibiting IL-6R has been approved for treatment of CRS. Additionally, in 2019, Sterner and colleagues investigated in targeting monocytes and macrophages by neutralizing granulocyte macrophage colony-stimulating factor (GM-CSF) (*28*). This study involving GM-CSF and CD19 targeted CAR T cells have since entered Phase II studies.

Clinical Trials

Many clinical trials have been published in support the use of CAR T-cell therapy. Wang and colleagues published their findings in April 2020 with the use of their anti-CD19 drug, KTE-X19 in relapsed or refractory mantle-cell lymphoma. In this phase-II clinical trial, 60 patients who had up to five previous therapies, including the use of BTK inhibitor therapy underwent leukapheresis, optional bridging therapy, followed by conditioning chemotherapy. Patients' T-cells were genetically altered and given back in a single-infusion setting (*29*).

Results of the clinical trial were promising. Objective response expression was 93%, 67% being a complete response (*29*). At the median follow-up of 12.3 months, 57% remained in

remission (*29*). One-year Progression-free survival and overall survival were noted to be 61% and 83% respectively (*29*). Common adverse events of grade 3 or higher were cytopenias (in 94% of the patients) and infections (in 32%) (*29*). Grade 3 or higher cytokine release syndrome and neurologic events occurred in 15% and 31% of patients, respectively; none were fatal (*29*). Two grade 5 infectious adverse events occurred (*Figure 5*) (*29*).

A small phase I study was conducted in 2017 on patients diagnosed with refractory diffuse large B cell lymphoma (DLBCL). Seven patients were treated with KTE-C19 CAR T cells following three days' worth of conditioning chemotherapy cyclophosphamide and fludarabine. Five patients (71%) experienced an overall response with four (57%) experiencing a complete response (*30*). Three patients were noted to have ongoing complete responses at the 1-year mark (*30*). Toxicities were noted within the study (one patient with grade 4 cytokine release syndrome, one with grade 3 neurotoxicity), however, all resolved within one month (*30*). Given the safety and effectiveness of the trial, phase II was strongly recommended for patients with refractory DLBCL.

Finally, a study investigating the efficiency in relapsed or refractory multiple myeloma in 2019 also showed promising results where CAR T cells targeted B-cell maturation antigens (BCMAs). In this phase I study, 33 patients diagnosed with relapsed/refractory multiple myeloma were given bb2121 CAR T cell infusions following at least three lines of previous therapy (*31*). The objective response rate 6 months following their last infusion was 85% including fifteen patients (45%) visualizing complete responses (*31*). Unfortunately, six of the fifteen patients experienced a relapse (*31*). Median progression-free survival was 11.8 months with the CAR T cells persisting in the bodies of the patients up to 1 year after the first set of infusions (*31*). Raje concluded antitumor activity was distinguished with BCMA-targeted CAR T cell immunotherapy.

CAR T-cell combined with chemotherapy

Many clinical trials have shown that CAR T-cell monotherapy had insufficient efficacy to treat solid tumors including trials with patients diagnosed with renal carcinoma and neuroblastoma, though the mechanistic reasons for failure are unknown (*32, 33, 34*). Combination of CAR T-cell therapy and chemotherapy have been suggested to show possible synergetic capabilities in the future (*35, 36*). Chemotherapy agents such as cyclophosphamide, fluorouracil, doxorubicin, oxaliplatin, and gemcitabine, are capable of reducing tumor burden (*37*). Chemotherapy is able to sensitize tumor cells by upregulating mannose-6-phosphate receptors on tumor cell surfaces. This results in the release of granzymes by CTLs to pervade tumor cells, sensitizing tumor cells to immunotherapy (*38*).

Mechanistically, some chemotherapy agents may induce autophagy resulting in the release of ATP and increasing the recruitment of dendritic cells (DCs) and CTLs. Dying cancer cells secrete damage-associated molecular patterns (DAMPs) which are recognized by TLR4. By promoting DC activation, T-cell response is also increased. Chemotherapy is noted to also create type I interferons (interferon alpha and interferon-beta) which also promote DC activation in the innate immune response system (*39*). This trickle-down effect leads to interferon-gamma secretion by DCs and activation of the adaptive immune response.

Radiotherapy may also create synergetic effects as studies have shown sensitizing tumor cells have reportedly demonstrated improved MHC I molecules for CD8+ CTLs to bind to the tumor-specific sites. Radiographic areas also promote the release of Interferon-gamma and DAMPs, resulting in the activation of T lymphocytes. Given the excitation of DAMPs, it can be assumed tumor antigen presentation is also improved due to canonical mechanism of DC

maturation via type I interferon environment, serving as a connecting bridge of the innate to adaptive immune system (*40*).

CAR T cells efficiency and battle against solid tumors

CAR T cells encounter a number of challenges to combat the complex state of a solid tumor. For promising results, CAR T cells must meet a sequential order of tasks to be effective. Currently, these cells lack the unique TAAs, are inefficient in targeting the specific tumor site, and remain weak to the immunosuppressive environment of solid tumors. TAAs are diverse as they contain novel peptide sequences, are expressed in a specific sequence, can be expressed during fetal development or at immunocompromised states, and expressed higher when tumor cells are prevalent (*41*). If a TAA sequence, expression, and prevalence is compromised, CAR T cell effectiveness diminishes. 'On target/off tumor,' or a direct attack on normal tissues that have the shared expression of a target antigen, side effects may occur due to the overexpression of those antigens (*41*).

The interest in modifying CAR T cells to effectively neutralize a solid-state tumor launched many continued trials. A study by Adachi and colleagues in 2018 confirmed CAR T cell survival and infiltration into solid tumors relies heavily on IL-7 and CCL19 expression (*42*). Chemokine ligand 19 (CCL19), a protein that is involved in immunoregulatory and inflammatory processes. It is suggested that the cytokine encoded by this gene may play a role in normal lymphocyte recirculation, homing, and T cell trafficking. Knowing this, Adachi was able to implement both IL-7 and CCL19 with results showing abrogated the migration of responder cells, suggesting that the modified CAR T cells mediated their chemotactic activity. The investigation was continued in vivo using mouse models and a specified tumor. The mice were treated with conventional or IL-7/CCL19 modified CAR T cells.

Results favored the IL-7/CCL19 as this induced a complete regression of the tumor, leading to long-term survival of the mice without tumor recurrence (*42*). It was also noted that dosing of the IL-7/CCL19 was increased which correlated to increased mice survival, suggesting conventional CAR T cells have tumor-lytic capabilities. Immune checkpoint molecules were also investigated in vivo. It was found that PD-1, lymphocyte-activation gene 3 (LAG3), and T cell immunoreceptor with immunoglobulin and ITIM domains (TIGIT) on the IL-7/CCL19 CAR T cells were lower when compared to the conventional CAR T cells (*42*). This proposes and confirms the idea of IL-7 signaling can prevent and/or restore T cell exhaustion.

Adachi noted the IL-7/CCL19 CAR T cells were considered to be a third-generation line as it consisted of the CD3ζ, CD28, and 4-1BB domains (*42*). Second-generation CAR T cells are considered to exercise a more potent anti-cancer effect when compared to the third generation (Zhong 2010 – CAR combining 4-1BB and CD28 signaling domains augment). Finally, the IL-7/CCL19 cells were tested in mice expressing Lewis lung carcinoma showing prolonged survival, although debated whether a new target should be selected (*42*).

Alizadeh and colleagues also investigated the efficiency of a CAR T cell and concluded cytokine IL-15 promotes T cell antiapoptotic factors and inhibits T cell exhaustion. It was also determined that IL15 enhances the antioxidant capacity of T cells, resulting in amplified T cell perseverance (*43*). Correlating with the inhibition of T cell exhaustion was the lowered expression of PD-1, LAG3, and 2B4 checkpoint inhibitor molecules (*43*). Finally, IL-15 cultured CAR T cells exhibited less mammalian target of rapamycin (mTOR) activity and reduced expression of glycolytic enzymes, suggesting the CAR T cells were in fact displaying antitumor activity (*43*).

Conclusion

The use of CAR T cell therapy in patients expressing refractory B cell lymphomas have presented positive outlooks on metastatic control and patient survival. These genetically-engineered cells have been proven effective as patients receiving KTE-X19 concluded with promising objective response, complete response, and progression-free survival rates. Although cytopenia's were experienced during the clinical case trial, "KTE-X19 CAR T-Cell Therapy in Relapsed or Refractory Mantle-Cell Lymphoma," Wang and collegues were able to implement a safe, efficient approach to patients who previously exhausted first-line therapies including BTK inhibitor treatment. Future studies on CAR T cell therapy may include combination therapeutics alongside current chemotherapies and radiation for solid-state tumors.

The future of oncology and the administration of immunotherapy will continue to be a challenge for practicing physicians and scientists worldwide. Understand how the human body interacts with cancer cells is vital to the future of standard of care of patients diagnosed with cancer. This will be accomplished by discovering new ligands and receptors on associated t-cells, APC's and cancer cells, providing new opportunities to combat this deadly disease.

References

1. Mak, T. W., Saunders, M. E., & Jett, B. D. (2014). *Primer to the immune response*. AP Cell Press, an imprint of Elsevier.
2. Jaime-Sanchez, P., Uranga-Murillo, I., Aguilo, N., Khouili, S. C., Arias, M. A., Sancho, D., & Pardo, J. (2020). Cell death induced by cytotoxic CD8+T cells is immunogenic and primes caspase-3–dependent spread immunity against endogenous tumor antigens. *Journal for ImmunoTherapy of Cancer*, *8*(1). https://doi.org/10.1136/jitc-2020-000528
3. Weinberg, R. A. (2014). *The Biology of Cancer*. Garland science.
4. BJ;, R. P. S. S. (n.d.). *Cancer and cure: A critical analysis*. Indian journal of cancer. Retrieved March 27, 2022, from https://pubmed.ncbi.nlm.nih.gov/28244479/
5. Rai, K. R., & Gupta, N. (2004). Staging of chronic lymphocytic leukemia. *Chronic Lymphocytic Leukemia*, 193–199. https://doi.org/10.1007/978-1-59259-412-2_10
6. Highly activated PD-1/PD-L1 pathway in gastric cancer with PD-L1 expression. (2018). *Anticancer Research*, *38*(1). https://doi.org/10.21873/anticanres.12197
7. Alsaab, H. O., Sau, S., Alzhrani, R., Tatiparti, K., Bhise, K., Kashaw, S. K., & Iyer, A. K. (2017). PD-1 and PD-L1 checkpoint signaling inhibition for cancer immunotherapy: Mechanism, combinations, and clinical outcome. *Frontiers in Pharmacology*, *8*. https://doi.org/10.3389/fphar.2017.00561
8. Neelapu, N. R. (2017). Role and regulation of transcriptional factors in gastric cancer. *Role of Transcription Factors in Gastrointestinal Malignancies*, 107–130. https://doi.org/10.1007/978-981-10-6728-0_9
9. Maher, J., Brentjens, R. J., Gunset, G., Rivière, I., & Sadelain, M. (2002). Human T-lymphocyte cytotoxicity and proliferation directed by a single chimeric TCRZ /CD28 receptor. *Nature Biotechnology*, *20*(1), 70–75. https://doi.org/10.1038/nbt0102-70
10. Yeku, O. O., & Brentjens, R. J. (2016). Armored car T-cells: Utilizing cytokines and pro-inflammatory ligands to enhance car T-cell anti-tumour efficacy. *Biochemical Society Transactions*, *44*(2), 412–418. https://doi.org/10.1042/bst20150291
11. Tontonoz, M. (2017). Crispr Genome-Editing Tool Takes Cancer Immunotherapy to the Next Level. *Memorial Sloan Kettering Cancer Center*.
12. Brentjens, R. J., Davila, M. L., Riviere, I., Park, J., Wang, X., Cowell, L. G., Bartido, S., Stefanski, J., Taylor, C., Olszewska, M., Borquez-Ojeda, O., Qu, J., Wasielewska, T., He, Q., Bernal, Y., Rijo, I. V., Hedvat, C., Kobos, R., Curran, K., … Sadelain, M. (2013). CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. *Science Translational Medicine*, *5*(177). https://doi.org/10.1126/scitranslmed.3005930
13. Li, R., Ma, C., Cai, H., & Chen, W. (2020). The car T-Cell Mechanoimmunology at a glance. *Advanced Science*, *7*(24), 2002628. https://doi.org/10.1002/advs.202002628
14. Park, J. H., & Brentjens, R. J. (2015). Are all chimeric antigen receptors created equal? *Journal of Clinical Oncology*, *33*(6), 651–653. https://doi.org/10.1200/jco.2014.57.5472
15. M. L. Dustin, J. M. Miller, S. Ranganath, D. A. A. Vignali, N. J. Viner, C. A. Nelson, E. R. Unanue, J. Immunol. 1996, 157, 2014.
16. Y. Wang, D. Li, R. Nurieva, J. Yang, M. Sen, R. Carreno, S. Lu, B. W. McIntyre, J. J. Molldrem, G. B. Legge, J. Biol. Chem. 2009, 284, 12645.
17. B. Graf, T. Bushnell, J. Miller, J. Immunol. 2007, 179, 1616; b) E. Tabdanov, S. Gondarenko, S. Kumari, A. Liapis, M. L. Dustin, M. P. Sheetz, L. C. Kam, T. Iskratsch, Integr. Biol. 2015, 7, 1272.

18. F. Tamzalit, M. S. Wang, W. Jin, M. Tello-Lafoz, V. Boyko, J. M. Heddleston, C. T. Black, L. C. Kam, M. Huse, Sci. Immunol. 2019, 4, eaav5445.
19. K. L. Hui, A. Upadhyaya, Proc. Natl. Acad. Sci. USA 2017, 114, E4175.
20. J. C. Stinchcombe, E. Majorovits, G. Bossi, S. Fuller, G. M. Griffiths, Nature 2006, 443, 462; b) A. Hashimoto-Tane, T. Yokosuka, K. Sakata-Sogawa, M. Sakuma, C. Ishihara, M. Tokunaga, T. Saito, Immunity 2011, 34, 919.
21. F. Bertrand, S. Müller, K.-H. Roh, C. Laurent, L. Dupré, S. Valitutti, Proc. Natl. Acad. Sci. USA 2013, 110, 6073.
22. F. Nicoli, L. Papagno, J. J. Frere, M. P. Cabral-Piccin, E. Clave, E. Gostick, A. Toubert, D. A. Price, A. Caputo, V. Appay, Front. Immunol. 2018, 9, 2736.
23. K. E. Beckermann, R. Hongo, X. Ye, K. Young, K. Carbonell, D. C. C. Healey, P. J. Siska, S. Barone, C. E. Roe, C. C. Smith, JCI Insight 2020, 5, e138729.
24. M. Saitakis, S. Dogniaux, C. Goudot, N. Bufi, S. Asnacios, M. Maurin, C. Randriamampita, A. Asnacios, C. Hivroz, Elife 2017, 6, e23190.
25. Shimabukuro-Vornhagen, A., Gödel, P., Subklewe, M., Stemmler, H. J., Schlößer, H. A., Schlaak, M., Kochanek, M., Böll, B., & von Bergwelt-Baildon, M. S. (2018). Cytokine release syndrome. *Journal for ImmunoTherapy of Cancer*, *6*(1). https://doi.org/10.1186/s40425-018-0343-9
26. Morgan RA, Yang JC, Kitano M, Dudley ME, Laurencot CM, Rosenberg SA. Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. Mol Ther. 2010;18:843–51. 24.
27. Brudno JN, Kochenderfer JN. Toxicities of chimeric antigen receptor T cells: recognition and management. Blood. 2016;127(26):3321-30.
28. Sterner 2019 – GM-CSF inhibition reduces cytokine release syndrome and neuroinflammation but enhances CAR T cell function in xenografts
29. Simoneaux, R. (2020). KTE-X19 Car T cells in relapsed/refractory mantle cell lymphoma. *Oncology Times*, *42*(S10). https://doi.org/10.1097/01.cot.0000668140.38010.d4
30. Locke, F. L., Neelapu, S. S., Bartlett, N. L., Siddiqi, T., Chavez, J. C., Hosing, C. M., Ghobadi, A., Budde, L. E., Bot, A., Rossi, J. M., Jiang, Y., Xue, A. X., Elias, M., Aycock, J., Wiezorek, J., & Go, W. Y. (2017). Phase 1 results of Zuma-1: A multicenter study of KTE-C19 anti-cd19 car T cell therapy in refractory aggressive lymphoma. *Molecular Therapy*, *25*(1), 285–295. https://doi.org/10.1016/j.ymthe.2016.10.020
31. Berdeja, J. G., Lin, Y., Raje, N., Siegel, D., Munshi, N., Turka, A., Lam, L. P., Quigley, M. T., & Kochenderfer, J. N. (2016). Clinical remissions and limited toxicity in a first-in-human multicenter study of BB2121, a novel anti-BCMA car T cell therapy for relapsed/refractory multiple myeloma. *European Journal of Cancer*, *69*. https://doi.org/10.1016/s0959-8049(16)32614-4
32. Lamers CH, Sleijfer S, van Steenbergen S, van Elzakker P, van Krimpen B, Groot C, Vulto A, den Bakker M, Oosterwijk E, Debets R and Gratama JW: Treatment of metastatic renal cell carcinoma with CAIX CAR-engineered T cells: Clinical evaluation and management of on-target toxicity. Mol Ther 21: 904-912, 2013.
33. Lamers CH, Klaver Y, Gratama JW, Sleijfer S and Debets R: Treatment of metastatic renal cell carcinoma (mRCC) with CAIX CAR-engineered T-cells-a completed study overview. Biochem Soc Trans 44: 951-959, 2016.
34. Park JR, Digiusto DL, Slovak M, Wright C, Naranjo A, Wagner J, Meechoovet HB, Bautista C, Chang WC, Ostberg JR and Jensen MC: Adoptive transfer of chimeric

antigen receptor re-directed cytolytic T lymphocyte clones in patients with neuroblastoma. Mol Ther 15: 825-833, 2007.
35. Bracci L, Schiavoni G, Sistigu A and Belardelli F: Immune-based mechanisms of cytotoxic chemotherapy: Implications for the design of novel and rationale-based combined treatments against cancer. Cell Death Differ 21: 15-25, 2014.
36. Vierboom MP, Bos GM, Ooms M, Offringa R and Melief CJ: Cyclophosphamide enhances anti-tumor effect of wild-type p53-specific CTL. Int J Cancer 87: 253-260, 2000.
37. Alizadeh D, Trad M, Hanke NT, Larmonier CB, Janikashvili N, Bonnotte B, Katsanis E and Larmonier N: Doxorubicin eliminates myeloid-derived suppressor cells and enhances the efficacy of adoptive T-cell transfer in breast cancer. Cancer Res 74: 104-118, 2014.
38. Trapani JA, Sutton VR, Thia KY, Li YQ, Froelich CJ, Jans DA, Sandrin MS and Browne KA: A clathrin/dynamin- and mannose-6-phosphate receptor-independent pathway for granzyme B-induced cell death. J Cell Biol 160: 223-233, 2003.
39. Sistigu A, Yamazaki T, Vacchelli E, Chaba K, Enot DP, Adam J, Vitale I, Goubar A, Baracco EE, Remédios C, et al: Cancer cell-autonomous contribution of type I interferon signaling to the efficacy of chemotherapy. Nat Med 20: 1301-1309, 2014.
40. Hong, M., Clubb, J. D., & Chen, Y. Y. (2020). Engineering car-T cells for next-generation cancer therapy. *Cancer Cell*, *38*(4), 473–488. https://doi.org/10.1016/j.ccell.2020.07.005
41. Kakarla, S., & Gottschalk, S. (2014). Car T cells for solid tumors. *The Cancer Journal*, *20*(2), 151–155. https://doi.org/10.1097/ppo.0000000000000032
42. Adachi, K., Kano, Y., Nagai, T., Okuyama, N., Sakoda, Y., & Tamada, K. (2018). IL-7 and CCL19 expression in car-T cells improves immune cell infiltration and car-T cell survival in the tumor. *Nature Biotechnology*, *36*(4), 346–351. https://doi.org/10.1038/nbt.4086
43. Alizadeh, D., Wong, R. A., Yang, X., Wang, D., Pecoraro, J. R., Kuo, C.-F., Aguilar, B., Qi, Y., Ann, D. K., Starr, R., Urak, R., Wang, X., Forman, S. J., & Brown, C. E. (2019). IL15 enhances car-T cell antitumor activity by reducing mtorc1 activity and preserving their stem cell memory phenotype. *Cancer Immunology Research*, *7*(5), 759–772. https://doi.org/10.1158/2326-6066.cir-18-0466
44. Cavanagh, O. author- M. (n.d.). *T-cell activation*. British Society for Immunology. Retrieved March 27, 2022, from https://www.immunology.org/public-information/bitesized-immunology/systems-and-processes/t-cell-activation
45. Saglam, O., & Conejo-Garcia, J. (2018). PD-1/PD-L1 immune checkpoint inhibitors in advanced cervical cancer. *Integrative Cancer Science and Therapeutics*, *5*(2). https://doi.org/10.15761/icst.1000272
46. Hughes-Parry, H. E., Cross, R. S., & Jenkins, M. R. (2019). The evolving protein engineering in the design of chimeric antigen receptor T cells. *International Journal of Molecular Sciences*, *21*(1), 204. https://doi.org/10.3390/ijms21010204

Figures and Tables

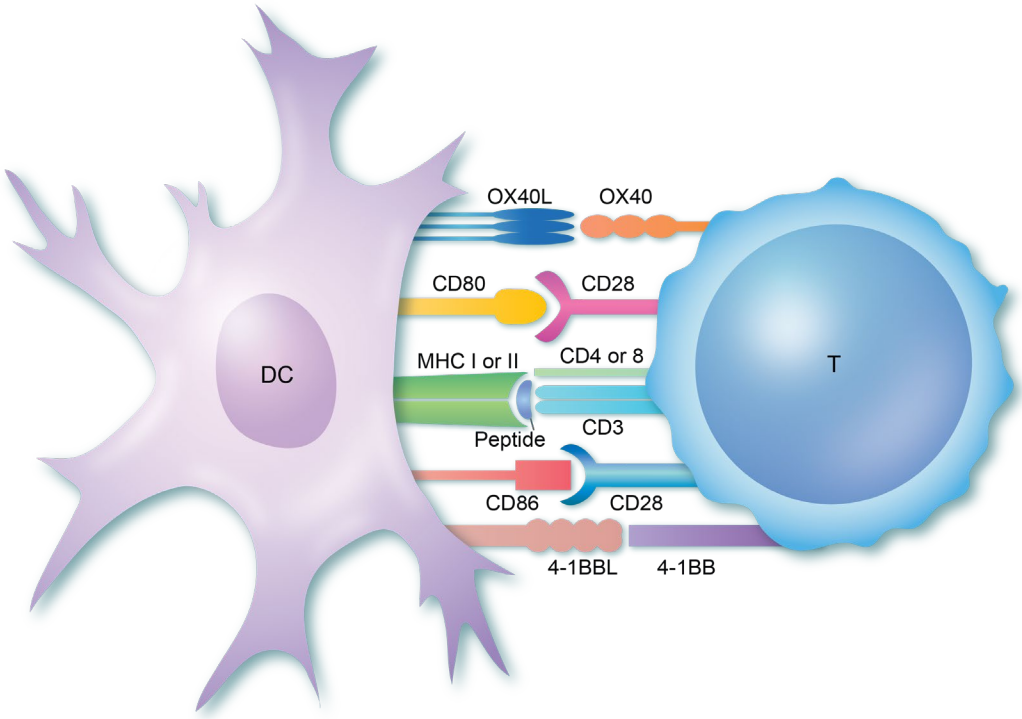

Fig. 1. Schematic Diagram of T cell activation. A dendritic cell (DC) carrying a peptide and MHC molecule will come into contact with a T cell. The binding occurs through the MHC complex. Co-stimulation will occur following CD86, CD80, OX40L and 4-1BBL binding. The end result is full activation in the T cell (*44*).

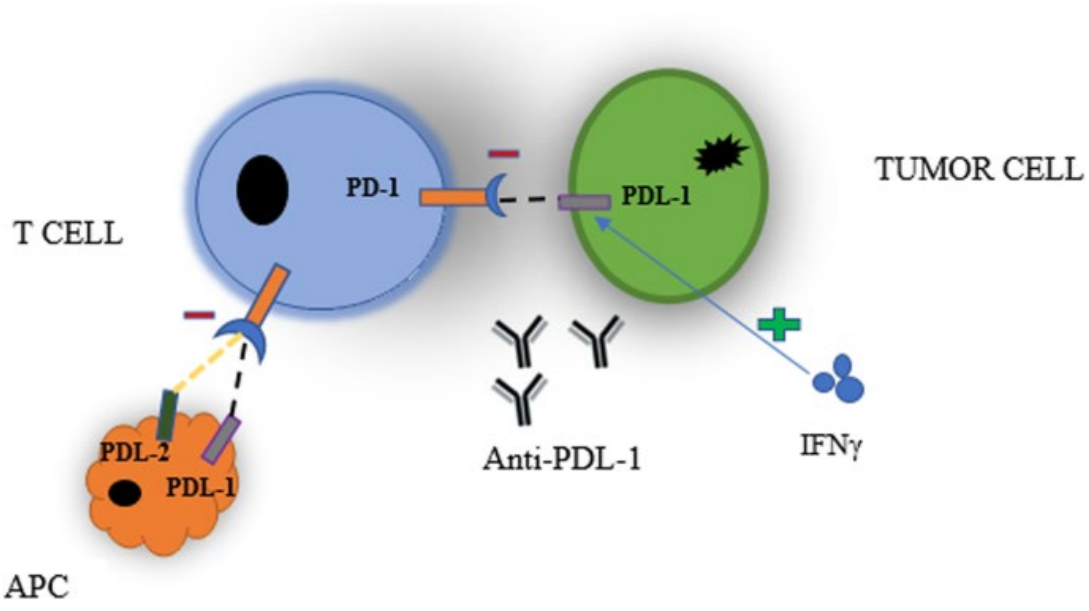

Fig. 2. PD-1 (T cell) and PD-L1 (tumor cell) interaction. IFNγ will induce and maintain expression of PD-L1. CKI's will act as PD-1 and PD-L1 inhibitors to induce the cell death mechanism of a T cell (*45*).

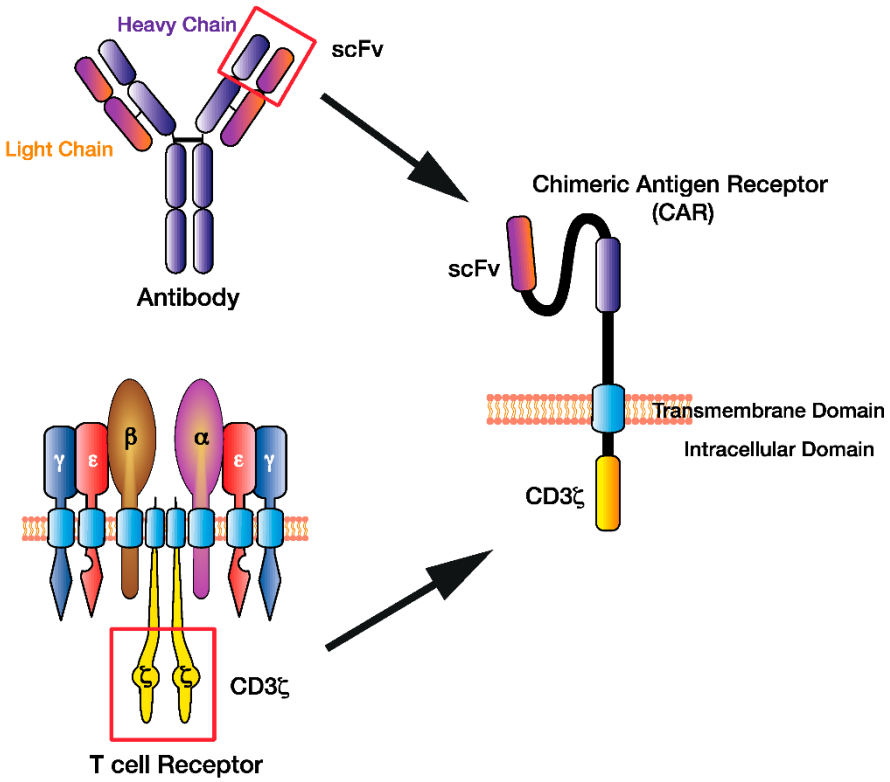

Fig. 3. Anatomy of a CAR T Cell highlighting major components (*46*).

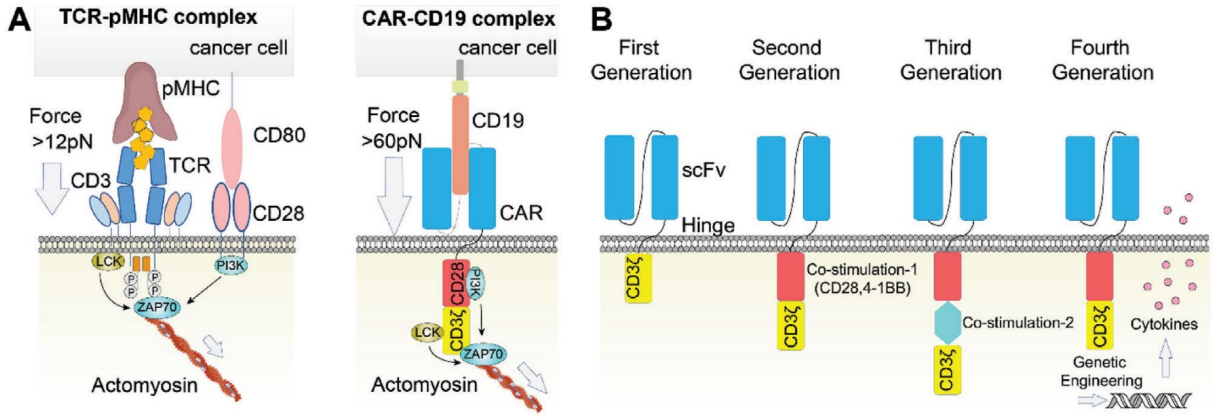

Fig. 4. Schematic Diagram of CAR T cells. A) Comparison of a classical TCR-MHC and CAR-CD19. B) The generations of CAR T cell designs (*13*).

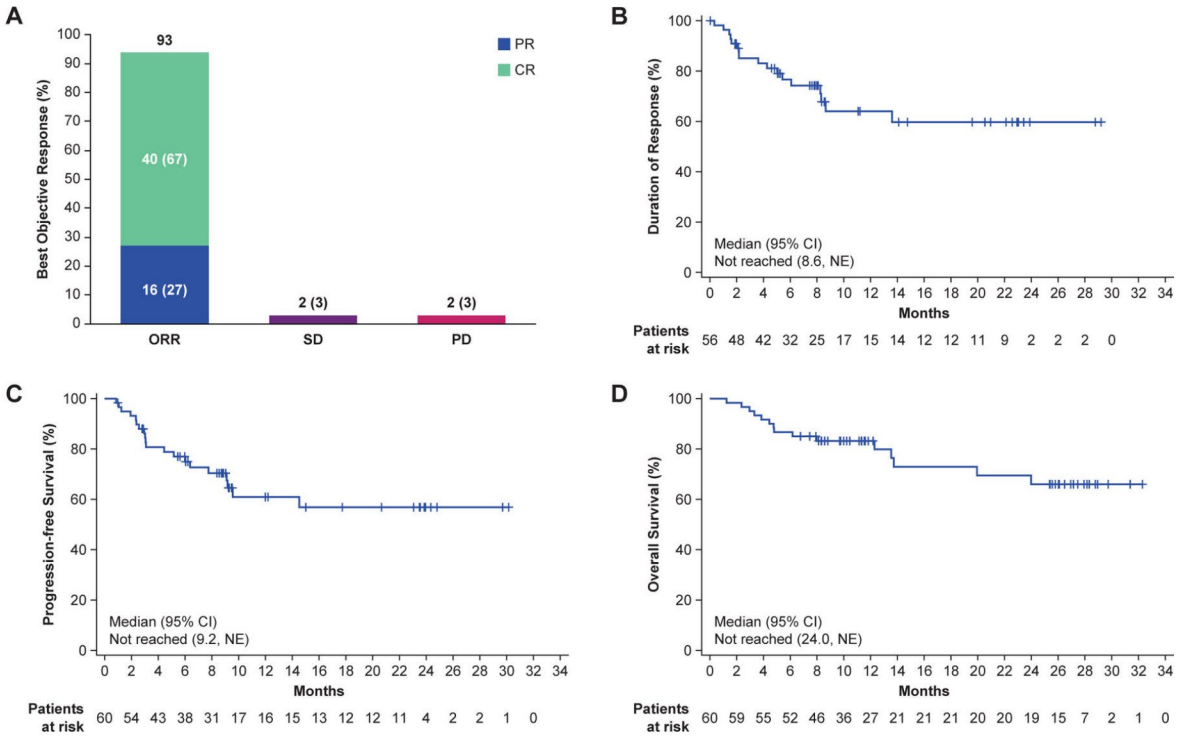

Fig. 5. Objective Response, Duration of Response, Progression-free Survival, and Overall Survival in XTE-X19 Phase 2 clinical trial (29). A) Number and percentages of patients with an objective response (complete or partial). B) Duration of response. C and D) Progression free survival and overall rates amongst the 60 patients, respectively.

Author/Year	Maude et al, 2018 [87]00	Park et al, 2018 [88] 00	Neelapu et al., 2017 [44] 00	Schuster et al, 2017 [89] 00	Turle et al, 2017	Gardner et al., 2017 [80] 8]	Ali et al, 2016 [79] 7]	Garfall et al, 2015 [90] 3]	Lee et al, 2015 [39] 5]	Maude et al, 2015 [32] 8]	Davila et al., 2014 [33] 9]	Kantarjian et al, 2017 [27] 4]	Stackelberg et al, 2016 [91] 5]	Topp et al, 2015 [68] 7]	Topp et al, 2014 [92] 6]
Institution	25 centers	MSKCC	22 centers	UPenn	FHCRC	SCHRI	NCI	UPenn	NCI	UPenn/ UPhil	MSKCC	101 centers	26 centers	37 centers	9 centers
Applied therapy	CD19 CAR (4-1BB)	CD19 CAR (CD28)	CD19 CAR (CD28)	CD19 CAR (4-1BB)	CD19 CAR (4-1BB)	CD19 CAR (4-1BB)	BCMA CAR (CD28)	CD19 CAR (4-1BB)	CD19 CAR (CD28)	CD19 CAR (4-1BB)	CD19 CAR (CD28)	blinatumomab	blinatumomab	blinatumomab	blinatumomab
Disease	B-ALL	B-ALL	DLBCL/ TFL/ PMBCL	DLBCL/ TFL	CLL	B-ALL	MM	MM	B-ALL	B-ALL	B-ALL	B-ALL	B-ALL	B-ALL	B-ALL
Number of patients	75	53	101	28	24	45	12	11	20	30	16	267	70	189	36
Incidences															
% CRS	77	85	93	57	83	93	50[1]	18	66	100	NR	14,2	11	NR	NR
% sCRS (>=II)	46	26	13	18	8	23	17[1]	9	32	27	44	4,9	5	3	0,8
% sNeurotox (>=II)	13	42	28	11	25	21	0	NR	1	3	13	9,4	4	11	14
treatment related deaths	1[2]	1[3]	3[4]	1[5]	1[6]	0	0	0	0	0	0	3[7]	6[8]	1[9]	3[10]
sCRS correlates															
tumor burden	NR	y*	NR	NR	y	n	NR	NR	y	y	y	NR	NR	NR	y
CRP/ferritin	n/y	NR	NR	NR	y*/y*	NR	NR	NR	y*/y*	y*[11]/y*	y*/NR	NR	NR	NR	NR
IL-6/IFNg	y/y	NR	y/n	NR	y*/y*	y/NR	NR	NR	y*/y*	y*/y*	n*/n*	NR	NR	NR	NR
Therapy															
tocilizumab response	NR	NR	NR	1/1	5/6	NR	100	NR	50%[12]	100%	y	NR	NR	NR	NR
steroid response	NR	NR	NR	0/0	5/6	NR	NR	NR	y	y	y	NR	NR	NR	NR
Prognosis															
CRS related to ORR?	NR	NR	NR	NR	NR	NR	NR	NR	y	NR	NR	NR	NR	2/3 CR	y
tocilizumab related to reduced ORR?	NR	NR	n	n	NR	n	n	NR	n	possible[13]	n	NR	NR	NR	NR
steroids related to reduced ORR?	NR	NR	n	NR	NR	n	NR	NR	n	possible[13]	y	NR	NR	NR	NR

Table 1: CRS reported in clinical trials involving CD19-targeted CAR T cell therapeutic agents.