Review Article

Indian Journal of Cancer Education and Research
Volume 12 Number 2, July - December 2024
DOI: http://dx.doi.org/10.21088/ijcer.2321.9815.12224.3

CAR-T Immunotherapy Against Cancer

Nisarg Kumar Teli[1], Kinjal Upadhyay[2]

How to cite this article:

Nisarg Kumar Teli, Kinjal Upadhyay. CAR-T Immunotherapy Against Cancer. Ind J Canc Educ Res 2024;12(2):63-73.

Abstract

Treatments like chemotherapy for cancer have less efficiency and specificity than required; immunotherapy provides one of the best means to have specific and more efficient treatment. With the great advancement in molecular biology, immunology, and cancer biology specific treatments like CAR-T immunotherapy have been introduced. Out of the various cancer immunotherapies discussed here, CAR-T immunotherapy provides the most promising results against certain cancers. CAR-T therapy has been discussed in detail with fundamental discussions of the immune system's role in cancer and cancer immunotherapy. CAR-T immunotherapy a version of adoptive cell transfer therapy has given outstanding results that were beyond expectations. Some of them are discussed with minute details on CAR-T immunnnotherapy in this paper.

Keywords: Immunotherapy, Cancer, Immune system, Cytokine, Antibody, Immunoediting, Immunosurveillance, Cancer immunotherapy, Antigens, Receptors.

INTRODUCTION

Cancer, a destructive disease was the cause of about 10 million deaths (across the globe) in 2020 and almost a 19.3 million cancer cases were reported across 185 countries worldwide in the same year (Sung *et al.*, 2021). Not only in 2020 but each year the condition is nearly similar, so each year cancer takes the lives of many hence considering that in mind it becomes crucial to have proper treatment(s) for all the types of cancer. There are treatments available that are generally being employed against various cancers, but the problem with these treatments is that they are not specific as they affect the normal cells with cancerous cells also they are not very effective as they do not cure cancer completely. One such treatment is chemotherapy; it uses cytostatic drugs (these drugs block or cease the growth of the cancerous cells) and cytotoxic drugs (the drugs that directly kill the tumor cells). But the problem with chemotherapy is that it helps in curing cancers but with that, it has several side effects like vomiting (Schirrmacher, 2019). Treatments like radiotherapy have also been employed for certain cancers like breast cancer (Taylor & Kirby, 2015). Again with curing properties there are harsh side effects, for instance while having radiotherapy for breast cancer people will have exposure to radiation to their hearts hence one of the serious side effects of radiotherapy in breast cancer may result in a high risk of heart diseases, mainly ischemic heart diseases (Darby *et al.*, 2013; Taylor & Kirby, 2015). There are other iatrogenic effects of these treatments

Author's Affiliation: [1]B.Sc Student, [2]Assistant Professor, Department of Biochemistry and Biotechnology, St. Xaviers College (Autonomous), Ahmedabad 380009, Gujarat, India.

Corresponding Author: Kinjal Upadhyay, Assistant Professor, Department of Biochemistry and Biotechnology, St. Xaviers College (Autonomous), Ahmedabad 380009, Gujarat, India.

E-mail: kinjal.upadhyay@sxca.edu.in

Received on: 24-09-2024 **Accepted on:** 12-11-2024

like patients after having chemotherapy often have hair loss, nausea, brain damage, paralysis, spasm, and severe iatrogenic effects including infertility, and drug resistance (Hansen, 2007; Morrow & Dobkin, 1988; Schirrmacher, 2019)2019. Hence it is a necessity to have treatments with the least side effects. Recent advancements in molecular biology, immunology, and cancer biology have resulted in the discoveries of target-specific cancer treatments, these treatments may include checkpoint inhibitors which will inhibit the cell cycle, and may also include certain drugs that are specific to any of the signal transduction pathways (Schirrmacher, 2019).

Also, new advancements resulted in the discovery of immunotherapy which utilizes the immune system of the host, for instance, it may use the memory function of the immune system, and also the immunotherapy induces/enhances or simply activates specific immune responses against various cancers. Immunotherapy is a better option for the treatment of cancer as it eliminates various side effects that are caused by the treatments that are used generally i.e. - chemotherapy. Another advantage of immunotherapy against these treatments is that it is very specific for cancerous cells and in most cases does not affect the growth of the normal cells (Schirrmacher, 2019). Immunotherapy can make tumor cells more visible to the immune system (Eggermont *et al.*, 2014). There are various specific types of immunotherapies that have been employed for cancer treatments from which the CAR T cell (chimeric antigen receptor T cell) immunotherapy will be disused further in this paper with details. Immunotherapy can be one of the best possible treatments in the future because it activates the immune response of the host against cancerous cells and makes it more robust and specific, also in certain immunotherapies the immune cells of the hosts are used so it avoids the use of any foreign molecules (like foreign DNA, e.g. plasmid DNA) or cells, which would most certainly be used in certain types of gene therapies and cause other problems like unnecessary activation or over activation of the immune system of the host and also not all the hosts will accept the same cells or molecules that can be used in gene therapies for others, these molecules and cells are sometimes host-specific, hence it becomes tedious to perform such treatments. Hence therapies like CAR T immunotherapy provided new and better means of treatment with the least side effects possible.

Immune System and Cancer

There is a lot of information regarding how the immune system functions against various infections like a bacterial infection, viral infection, and parasite infection but there is very little information about how the immune system responds to cancers (Corthay, 2014). There are two types of immune responses; 1) innate immune response and 2) adaptive immune response. They work together coordinately to protect against these kinds of infections (Gasteiger & Rudensky, 2014). It is necessary to know more about how the immune system functions (mainly against cancer) in order to understand CAR T immunotherapy. The immune system can identify and eliminate the tumors without affecting the normal cells; this ability is termed cancer immunosurveillance (Vesely *et al.*, 2011). The immune system is considered a double-edged sword, this is because of its dual functions, and it has both promontory as well as inhibitory effects on tumors (Lakshmi Narendra *et al.*, 2013). This ability of the immune system to have both functions is termed immunoediting (Vesely *et al.*, 2011).

Immunoediting consists of 3 phases known as 3 Es of immunoediting, 1) Elimination (immunosurveillance) 2) equilibrium (cancer persistence) and 3) escape (cancer progression) (Dunn *et al.*, 2004; Lakshmi Narendra *et al.*, 2013) supported by strong experimental data derived from murine tumor models and provocative correlative data obtained by studying human cancer, holds that the immune system not only protects the host against development of primary nonviral cancers but also sculpts tumor immunogenicity. Cancer immunoediting is a process consisting of three phases: elimination (i.e., cancer immunosurveillance. The components of both innate and adaptive immune systems take part in elimination (immunosurveillance) and if the immune system of the host is naturally strong then this would result in the complete eradication of tumor cells (Dunn *et al.*, 2004) supported by strong experimental data derived from murine tumor models and provocative correlative data obtained by studying human cancer, holds that the immune system not only protects the host against development of primary nonviral cancers but also sculpts tumor immunogenicity. Cancer immunoediting is a process consisting of three phases: elimination (i.e., cancer immunosurveillance. People with deficiencies in vital immune components/molecules or simply people with immunodeficiency have shown increased susceptibility towards cancer/tumor development, so this concludes that the immune system does have a role to play against cancer/ tumor development (Hadden, 2003). The immune

system can both eliminate and promote tumor growth. The main components that take part in antitumor action are T_C cells (cytotoxic T cells or CD8+ cells), T_H1 (helper T cells {Citation} type1 or CD4+ cells), NK cells, and certain cytokines like interferon-gamma (IFN-γ). Whereas the components taking part in tumor promoting action include TH2 cells myeloid derive suppressor cells with their cytokines take part in tumor promoting actions (Lakshmi Narendra *et al.*, 2013).

Let's see how the innate immune system helps! NK cells (natural killer cells) can detect the changes that happen on the surface of the transformed cells, for instance changes in the expression of MHC molecules. So NK cells identify the tumor cells in this way and then on activation they can kill/eliminate tumor cells by releasing perforin (granzyme), also NK cell-mediated cytotoxicity plays important role in the initial stages of tumor development (Lakshmi Narendra *et al.*, 2013; Langers *et al.*, 2012). Macrophages are attracted to the tumor progression site by certain chemokines and then these macrophages differentiate and release many other chemokines which in turn promote the recruitment of helper and cytotoxic T cells (Mantovani *et al.*, 2004). Certain cytokines (TNF-α, IL-1, IL-6, etc) secreted by M1 macrophages have shown cytotoxic activity against tumor cells, whereas certain macrophages also secrete kinds of cytokines that promote tumor progression (Fair weather & Cihakova, 2009; Hao *et al.*, 2012) such as parasitic worms, are strong inducers of alternatively activated or M2 macrophages. However, infections such as bacteria and viruses that require Th1-type responses may induce M2 as a strategy to evade the immune system. M2 are particularly efficient at scavenging self tissues following injury through receptors like the mannose receptor and scavenger receptor-A. Thus, M2 may increase autoimmune disease by presenting self tissue to T cells. M2 may also exacerbate immune complex (IC. Dendritic cells also play a very crucial role against tumor development in many ways, mainly they act as antigen-presenting cells, and these cells present the tumor antigen, and activate the immune system further (Lakshmi Narendra *et al.*, 2013).

As the paper focuses on CAR T immunotherapy, it becomes crucial how T cells, one of the components of the adaptive immune system plays a role against cancer/tumor development. Helper T cells and cytotoxic T cells recognize antigens that are presented by MHC 2 and MHC 1 respectively. The TCRs (T cell receptors) aid in doing so. The cytotoxic T lymphocytes (CD8+ cells) play a very crucial role against cancer, they identify the tumor antigens presented by dendritic cells via interaction through their TCRs, and then due to this signal and other co-stimulatory signals these naïve T cells are differentiated to memory and effector CD8+ T cells. The memory cells remember the antigen and the effector T cell will produce cytokines like IFN- γ which will be having a direct cytotoxic effect on the tumor cells (Lakshmi Narendra *et al.*, 2013). In the same way, the helper T cells are also activated via their interaction with APCs (antigen-presenting cells) through their TCRs and will further increase the amount of immune response by differentiating into subtypes like T_H1 and they also secrete certain cytokines that ramp up the process of antigen presentation and cytotoxic killing by T_C cells. Further, these T_H cells will activate the humoral immune response by activating the B cells (Lakshmi Narendra *et al.*, 2013; Wilson *et al.*, 2009). The role of B cells in cancer is much less understood in comparison to the role of T cells, certain studies suggest that B cells promote tumor growth (Gonzalez *et al.*, 2018). Studies have suggested that B cells producing IL-35 are responsible for the development of pancreatic tumor (Pylayeva-Gupta *et al.*, 2016) we report the prominent presence of B cells in human pancreatic intraepithelial neoplasia and PDAC lesions as well as in oncogenic Kras-driven pancreatic neoplasms in the mouse. The growth of orthotopic pancreatic neoplasms harboring oncogenic Kras was significantly compromised in B-cell-deficient mice (μMT. As one can see the crucial role played by TCRs in the immune response, hence by simply manipulating these TCRs one can have a better immune response against cancer, and how it can be done? That will be discussed further.

Cancer Immunotherapy

After discussing the immune system's role in cancer development, now it would be better to talk about different immunotherapies that have been employed against various cancer types, so cancer immunotherapies are the immunotherapies that can be used against cancer. The major focus of cancer immunotherapy is to enhance the antitumor responses of the host's immune system by manipulating the components of the immune system. Several immunotherapies have been used successfully to treat cancer; but cancer immunotherapy is not too advanced, it has been practiced for many years under different names, William Bradley Coley is known as the father of immunotherapy who first discovered and employed immune system based therapy to treat bone cancer (Dobosz & Dzieciątkowski, 2019; McCarthy, 2006).

Coley injected heat-killed streptococcal species of bacteria into the patients having tumors, the idea was to inject these bacteria at the tumor site and the side effects of this infection would eliminate the tumor cells, and he was successful in treating people and these bacterial products were known as Coley's toxins (Coley, 1991; McCarthy, 2006). This was the first immunotherapy and from this to the present time, many immunotherapies have been performed to treat cancers. Immunotherapies like antibody based therapies (monoclonal antibodies are used widely), cancer vaccine treatments, cytokine based therapies, immune checkpoint inhibitors based therapy, oncolytic virus-based therapies and adoptive cell therapy or adoptive cell transfer (ACT) therapy, etc have been performed successfully against cancer (Dobosz & Dzieciątkowski, 2019; Oiseth & Aziz, 2017; Waldman *et al.*, 2020).

Antibody-based immunotherapies utilize the ability of antibodies to target specific tumor antigens present on tumor cells. There are certain mechanisms used by antibodies to eliminate the tumor cells, these include; antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), antibody-induced fixation of complement, etc (Borghaei et al., 2009; Weiner et al., 2012). By manipulating the structure of antibodies the efficiency and persistence of the therapy can be increased (Ruf & Lindhofer, 2001). Many cancer vaccines have been developed to protect against certain cancers. Prophylactic vaccines are used against cancers that develop due to the actions of oncolytic viruses like HPV. Whereas therapeutic vaccines are used against non-viral mediated cancers (Guo et al., 2013). Cytokine-based immunotherapies utilize cytokines that are released by immune cells in response to various infections and studies have suggested that many pro-inflammatory cytokines possess anti-tumor properties. Cytokines like interleukin-2 (IL-2) have been approved for cytokine-based immunotherapy against certain types of tumors (Rallis et al., 2021). Immune checkpoints are the molecules that provide self-tolerance, or simply these are the molecules that are responsible for the inactivation of the immune system, when T cells interact with receptor proteins present on other cells, this results in the signal that turns off the activity of T cells, hence they protect the host from autoimmune disorders (they inhibit the immune cells from attacking the host cells)(Haanen & Robert, 2015; Waldman et al., 2020). Many tumors activate these immune checkpoints to invade the immune system and protect themselves, hence blockade of certain immune checkpoint molecules like PD-1(programmed death-1) and CTLA-4 (cytotoxic-T lymphocyte associated antigem[4]) have shown promising results as cancer immunotherapy (Darvin et al., 2018; Pardoll, 2012). The molecules responsible for the blockade of these checkpoints are known as immune checkpoint inhibitors (Haanen & Robert, 2015). It's important to note that the Nobel Prize for physiology or medicine was awarded to James Allison and Tasuku Honjo for their discovery of new advances in the immune checkpoint blockade therapy (Smyth & Teng, 2018; Zang, 2018).

oncolytic viruses are the viruses that are naturally selected or are artificially engineered inside a laboratory (recombinant oncolytic viruses) to infect only the tumor cells (Bell *et al.*, 2003). These viruses lack virulence against normal cells, this is possible because when normal cells become tumor cells, they sacrifice a lot of anti-viral mechanisms that may protect them from a viral infection, and hence these oncolytic viruses have been approved for immunotherapy (Fukuhara *et al.*, 2016). Adoptive cell therapy is again a type of immunotherapy in which the immune cells are taken and modified for treating diseases like cancer, certain cells are used for this purpose but when T cells are used in this therapy then this therapy is termed adoptive T cell transfer therapy (Dobosz & Dzieciątkowski, 2019).

Adoptive cell immunotherapy or sometimes referred to as adoptive cell therapy (ACT) or adoptive cell transfer therapy is a type of cellular immunotherapy in which a patient's immune cells (autologous adoptive cell immunotherapy) or donor's immune cells (allogenic adoptive cell immunotherapy) are isolated and then they are modified and are infused back into the patient (Marcus & Eshhar, 2011; Oiseth & Aziz, 2017; Southam *et al.*, 1966). The first report on targeting malignancies with adoptive transfer of lymphocytes was done in 1955 with mouse models (June, 2007; Mitchison, 1955). Adoptive cell transfer immunotherapy includes isolating immune cells and modifying them to use against cancer, the cells include dendritic cells, NK (natural killer) cells, T cells, etc (Fan *et al.*, 2018; Hinrichs & Rosenberg, 2014; Rezvani, 2019; Waldman *et al.*, 2020). As in most of the cases of adoptive cell immunotherapy, T cells are utilized hence in most of the papers ACT is referred to as adoptive T cell transfer therapy. In several ways, the T cells were modified but the best results were obtained by CAR-T cells, hence the CAR-T immunotherapy was one of the most successful versions of adoptive cell therapy (Dobosz & Dzieciątkowski, 2019). Hence let us discuss about CAR-T immunotherapy.

CAR-T cell immunotherapy

Broadly there are three types or versions of adoptive cell transfer immunotherapy depending on the kind of cells employed and that include; TILs based immunotherapy, TCR gene-modified T cells based immunotherapy, and CAR-T cells based immunotherapy or simply CAR-T immunotherapy, Out of these CAR-T immunotherapy is considered as one of the most advanced versions or types of adoptive cell transfer therapy, and has been successfully employed against cancers like chronic lymphoid leukemia (Porter *et al.*, 2011), refractory large B cell lymphoma (Neelapu *et al.*, 2017) and metastatic melanoma (Soltantoyeh *et al.*, 2021). CAR-T immunotherapy has been successful in treating hematopoietic malignancies but the efficiency of CAR-T therapy for solid tumors is still questionable (Mohanty *et al.*, 2019). CAR-T immunotherapy includes the isolation of a patient's T cells and then these T cells are modified in the laboratory through genetic engineering to express CAR (chimeric antigen receptors) and then these T cells with highly specific CARs are infused back inside the patient's body (June *et al.*, 2018). As patient T cells are used CAR-T therapy is also personalized immunotherapy (Feins *et al.*, 2019). T cells are engineered to express chimeric antigen receptors that target specific tumor antigens and depending on the specific tumor-associated antigen targeted different types of T cells having chimeric antigen receptors with different specificities are synthesized (Ruella & Kalos, 2014). The specificity of CARs or any receptors is very important in immunotherapy as the effectiveness of immunotherapy depends on the specificity of the targeted antigens. This is a kind of reprogramming of T cells to make them more desperate for tumor cells and make these T cells able to survive in a certain tumor microenvironment(June *et al.*, 2014). What are CARs? Let's discuss.

What are CARs?

CARs are chimeric antigen receptors and this concept was first introduced by Eshhar in the mid-1980s with his colleagues the first CAR was also designed by them at the Weizmann Institute of Science in Israel (Gross et al., 1989). Chimeric antigen receptors are synthetic recombinant receptors (Feins et al., 2019; Sadelain et al., 2013). CARs are hybrids of T cell receptor and antibody (*fig. 1*)(Jin et al., 2021; Ramos & Dotti, 2011). This idea of CAR arose due to two major findings or observations the first is the cloning of the CD3 zeta (ζ) chain of the T cell receptor complex and the second finding indicated that the cytoplasmic tail of the zeta chain can activate the T cells independently of rest of the TCR complex (Irving & Weiss, 1991; Letourneur & Klausner, 1991). Initially, the studies on the efficiency of CARs were done with HIV patients and the results were promising as the CAR T cells persisted for more than 10 years after treatment (Mitsuyasu et al., 2000; Scholler et al., 2012). The first trials with cancer patients were carried out by using CAR-T cells expressing CARs specific for TAG-72 antigens, so the tumor cells expressing this particular antigen were eliminated (McGuinness et al., 1999).

CARs are composed of 4 well-defined regions that include; 1) an extracellular domain 2) A transmembrane (TM) domain 3) A hinge region and 4) an intracellular signaling domain (fig. 2)(Jin *et al.*, 2021). CARs generally have a single-chain variable fragment (scFv) of antibody (extracellular domain) combined with a transmembrane domain which in turn are combined with one or more intracellular signaling domains or co-stimulatory domains that are associated with T-cell signaling, this co-stimulatory domain helps to sustain antitumor activity (Ruella & Kalos, 2014) . ScFv, the antigen-binding site of CARs consists of variable light (V_L) and variable heavy (V_H) chains of the antibody that are joined by a 15 residues long peptide (fig. 1) (Mullaney & Pallavicini, 2001). In most CARs, the signaling domain is derived from the CD3ζ chain (Ruella & Kalos, 2014). The transmembrane domain provides stability and helps in the expression of receptors, and this TM domain has a hydrophobic-alpha helix structure which aids in the stability of the entire receptor (Ramos & Dotti, 2011). Depending on the structure of CARs, there are about 5 generations of CARs (Jin *et al.*, 2021). Out of these five three of them are discussed here.

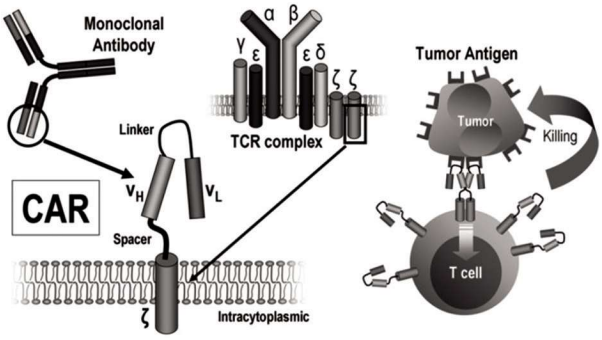

Fig. 1: one of the most common CARs has the parts of both monoclonal antibodies and the TCR complex. The interaction of CARs with tumor antigens initiates the signal transduction to activate the T- cells.

Adapted from (Ramos & Dotti, 2011)

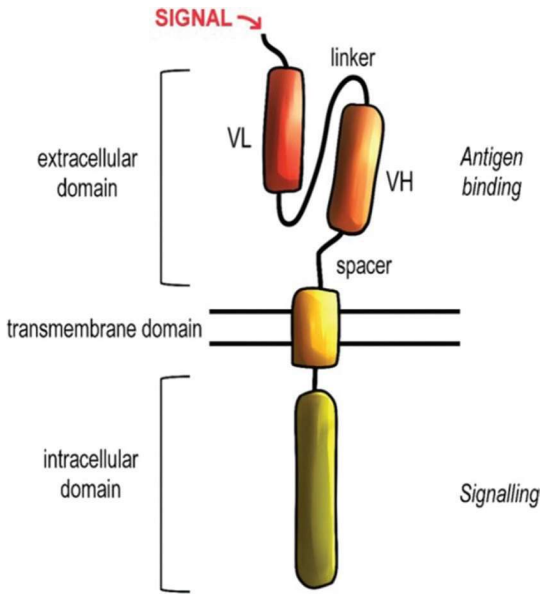

Fig. 2: General structure of Chimeric Antigen Receptor. Adapted from (Skorka *et al.*, 2020)

Generations of CARs

Depending on the structures there are different generations of CARs. The need to have different generations was a result of a lack of requirements in the first generation of CARs or the original CAR. The different CARs have different specificities, meaning the only generation cannot target all the types of cancers, because with the type of cancer the type of antigen expressed changes hence to target a wide range of antigens and so the wide range of cancers, the different generations of CARs are synthesized which are different in terms of their structure, specificity, target antigens as well as their efficiency (R. J. Brentjens & Curran, 2012) with the exception of chronic myelogenous leukemia and EBV-induced lymphoproliferative disease, allo-HSCT GVL lacks the potency to significantly affect disease progression or recurrence in most other hematologic malignancies. The inadequacy of a GVL effect using past approaches is particularly evident in patients with lymphoid malignancies. However, with the advent of improved gene transfer technology, genetically modified tumor specific immune effectors have extended cellular immunotherapy to lymphoid malignancies. One promising strategy entails the introduction of genes encoding artificial receptors called chimeric antigen receptors (CARs. Originally the CARs were termed as T- bodies and then subsequently the first generation of CARs (Eshhar *et al.*, 1996).

The first generation of CARs consists of mainly the CD3ζ chain as an intracellular signaling domain and it is scFv based generation, meaning it has scFv as an extracellular domain (Irving & Weiss, 1991). So the first generation of CARs only has CD3ζ as a co-stimulatory domain (Abate-Daga & Davila, 2016). The initial clinical trials against solid tumors were not satisfactory (June & Sadelain, 2018). But a few successful trials against cancers like neuroblastoma and ovarian cancer motivated the scientists to develop the next generations of CARs to overcome the shortcomings of the first generation of CARs (Louis *et al.*, 2011) but by 6 weeks, both subsets became low or undetectable. We now report the long-term clinical and immunologic consequences of infusions in 19 patients with high-risk neuroblastoma: 8 in remission at infusion and 11 with active disease. Three of 11 patients with active disease achieved complete remission, and persistence of either CAR-ATCs or CAR-CTLs beyond 6 weeks was associated with superior clinical outcome. We observed persistence for up to 192 weeks for CAR-ATCs and 96 weeks for CAR-CTLs, and duration of persistence was highly concordant with the percentage of CD4(+. One of the major shortcomings associated with the first generation CARs was their inability to produce efficient anti-tumor activity which was associated with their inability to produce the required amount of IL-2; hence with the first generation CARs, the need was to supply the extra amount of IL-2 (Abate-Daga & Davila, 2016). Hence the second generation of CARs was developed, and the second generation was derived from the first generation the major difference it has is the presence of another co-signaling domain. Like the first generation, this generation CARs also have a CD3ζ chain as one of the co-signaling domains but with that, they also have another co-signaling or co-stimulatory domain, for instance, the second generation anti-CD-19 CARs have CD-28 as another co-stimulatory domain and these anti-CD-19 CARs were used successfully against B-ALL (B-cell acute lymphoblastic leukemia)(R. Brentjens *et al.*, 2010; Mohanty *et al.*, 2019; J. H. Park & Brentjens, 2015) there is a need to develop a promising immunotherapy that targets tumors at both the cellular and genetic levels. Chimeric antigen receptor (CARs).

The results with second generation were great but to extend the anti-tumor activity the third generation of CARs were developed with two additional co-stimulatory domain such as CD3ζ-CD-28-4-1BB, so here this CAR has two additional co-stimulatory domain CD-28 and 4-1BB respectively, presence of more co-stimulatory domains resulted into more efficient anti-tumor response, better proliferation and increased cytokine production (Mohanty *et al.*, 2019). Structures of different generations are given

below in fig. 3.

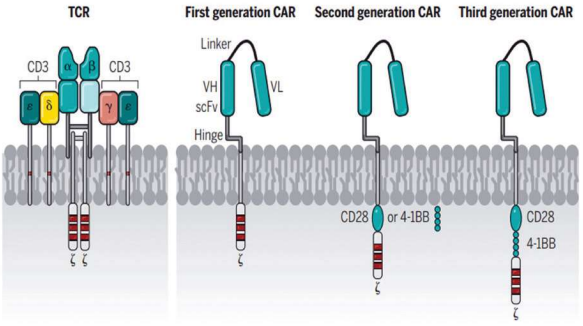

Fig. 3: Transgenic TCR and 3 generations of CARs. As per the figure it can be seen that the main difference between the generations of CARs is the number of co-stimulatory domains and with the increase in the number of co-stimulatory domains the efficiency and the range of CARs increase. Adapted from (June *et al.*, 2018)

HOW CAR-T CELLS WORK?

Many clinical trials with engineered T cells (especially with CARs) have shown the potential of CAR-T immunotherapy to target cancer (Grupp *et al.*, 2013; Kalos *et al.*, 2011). The efficiency of CAR-T immunotherapy depends on safe and stable gene transfer methods. CAR-T cell immunotherapy is one of the first instances of synthetic biology and personal cancer treatment to be available commercially (Feins *et al.*, 2019). In certain cases chemotherapy is given prior to the CAR-T immunotherapy, this helps in the elimination of immune cells that take part in immunosuppression, and this approach helps in the expansion of CAR-T cells. As the number of other immune cells is depleted, the CAR-T cells will have more space for expansion and also they will be more functional and active than any other immune cells (Graham *et al.*, 2018)000 allogeneic and autologous haematopoietic stem cell transplants (HSCTs. CAR-T cell immunotherapy does not only have advantages but it has also resulted in certain toxicities that have never been observed before(Graham *et al.*, 2018)000 allogeneic and autologous haematopoietic stem cell transplants (HSCTs. The general mechanism of CAR-T cell immunotherapy includes the infusion of CAR-T cells inside the patient's body then these CAR-T cells undergo proliferation and they increase in number after these CAR-T cells reach where the tumor is present and each of them will kill multiple tumor cells by recognizing the tumor antigen. They will also elicit the antitumor response through the process called cross-priming (Davenport *et al.*, 2015; June & Sadelain, 2018) there are important aspects of CAR-T-cell biology that have not been explored, particularly with respect to the kinetics of activation, immune synapse formation, and tumor cell killing. Moreover, the effects of signaling via the endogenous T-cell receptor (TCR. The general mechanism is illustrated in *fig. 4*.

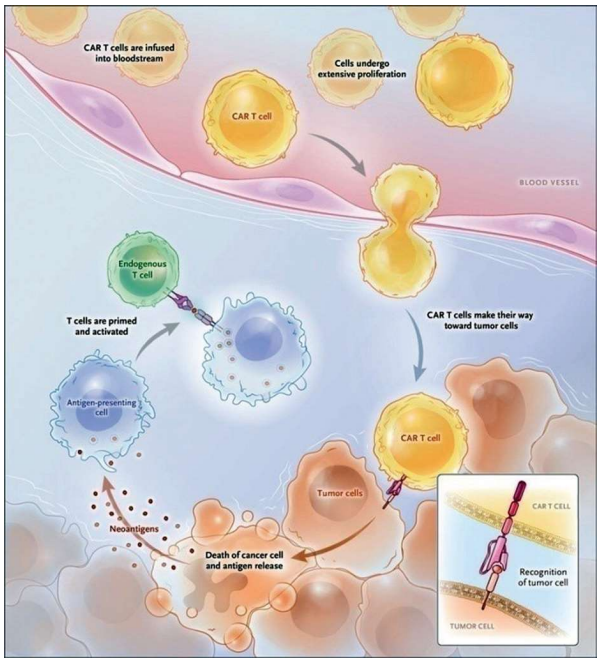

Fig. 4: CAR-T cells are injected into the bloodstream where these cells undergo extensive proliferation and then they travel to the location of the tumor. After reaching the tumor site, these cells recognize tumor antigens presented on tumor cells and will eliminate tumor cells. But the elimination of tumor cells will result in antigen release which will further activate immune response by antigen presentation. Adapted from (June & Sadelain, 2018)

Clinical trials

Many clinical trials were done against various cancers to check the efficiency and safety of CAR-T cell immunotherapy, in many cases, different generations of CARs were used against different types of cancers; the trials are listed in the table below.

Table 1: Different clinical trials for different cancers with different generations of CARs

Target antigen	Associated cancer	Generation of CAR	Clinical trial ID*
CD-19	Acute lymphocytic leukemia	3rd (CD28:TNFSF9)	NCT02186860
CD-174	Neuroblastoma	2nd (TNFSF9) and 3rd (CD28:TNFSF9)	NCT02311621
Her-2	Breast cancer	2nd (CD28)	NCT02547961

Target antigen	Associated cancer	Generation of CAR	Clinical trial ID*
CD133	Various malignancies	1st	NCT02541370
Her-2	Glioblastoma multiforme	2nd	NCT01109095

* The clinical trial ids will lead you to the clinical trials that were performed. Adapted from (Spear *et al.*, 2016)

After having these many successful trials scientists were more interested in exploring this therapy for as many cancer types as possible, many initial clinical trials were not that promising but by manipulating the CARs and changing the different target antigens, it was possible to get better results. Also, several clinical trials were performed on patients with cancers like refractory B-cell cancers (Kochenderfer *et al.*, 2010) and chronic lymphocytic leukemia (Porter *et al.*, 2011). All these clinical trials then resulted in having two FDA-approved CAR-T immunotherapy for the treatment of cancers like acute lymphoblastic leukemia, which are Tisagenlecleucel and Axicabtagene ciloleucel (Ahmad *et al.*, 2020). It is not that CAR-T immunotherapy does not have any problems/ challenges, there are many challenges and toxicities associated with it and few of them are discussed here.

Toxicities and challenges associated with CAR-T immunotherapy

With the promising results, there were many neo toxicities and challenges were observed that were never expected, hence intensive studies and research are going on to overcome these toxicities. A few of these toxicities and challenges are discussed here to give a brief account of them. Just like any other cancer therapy CAR-T immunotherapy also poses many adverse side effects. One of the most commonly occurring and one of the most adverse side effects is Cytokine Release Syndrome (CRS)(Giavridis *et al.*, 2018) especially acute lymphoblastic leukemia (ALL Severe CRS is associated with specific target CAR-T cell therapies like CAR-T immunotherapy that are specific for CD19 (June *et al.*, 2018). Although the CRS was quite expected as a side effect, this much of the severity was not expected, and in many severe cases of CRS, the symptoms were similar to syndromes like macrophage activation syndrome (Barrett *et al.*, 2014). In many patients, CAR-T immunotherapy also resulted in clinical symptoms like fever, hypoxia, and many neurological changes (Davila *et al.*, 2014; Lee *et al.*, 2014) which allowed us to transition most of these patients to a standard-of-care allogeneic hematopoietic stem cell transplant (allo-SCT. The big challenge of the CAR-T immunotherapy is to have a team of professionals that can fulfill all the requirements to perform CAR-T immunotherapy, for instance, the hospitals giving CAR-T treatments should have trained professionals to perform CAR-T, and then they should also have a team to observe the patients for the possible side effects (Graham *et al.*, 2018)000 allogeneic and autologous haematopoietic stem cell transplants (HSCTs).

Future perspectives

After getting this much idea about CAR-T immunotherapy, it would be better to discuss what will be the future or what is the future of this treatment? Or how CAR-T immunotherapy will help create a better future! As discussed in the paper there are many toxicities and challenges associated with CAR-T immunotherapy and not all of them have been overcome! Hence in the future, it becomes the utmost necessity that all the issues should be solved before the commercialization of CAR-T immunotherapy. One of the biggest challenges is to make the treatment available to each person in need. This would require that the cost of CAR-T treatment is affordable to everyone; this would not only face biotechnological problems but will also encounter problems like political issues, etc. hence biotechnology-based companies should come together and find the best solution for all the problems associated with CAR-T immunotherapy and help in make the CAR-T immunotherapy the ultimate weapon to fight cancer. Another big challenge that needs to be solved is CAR-T immunotherapy's poor response to the solid tumors, hence intensive research is required to fulfil all the criteria that can make CAR-T immunotherapy as common as chemotherapy for cancer treatment

CONCLUSIONS

- There is a need for a specific and robust treatment for cancer.
- Due to immune tolerance gained by tumors, treatments like adoptive cell therapy become a necessity.
- The clinical trials employing different generations of CARs were promising but neo toxicities were also observed.

Still, there is a lot to explore, but depending on the results and observations obtained in clinical trials it would not be wrong to say that **CAR-T**

immunotherapy could arise as a boon to cancer patients.

Conflicts of interest: No Conflicts of Intrest

ACKNOWLEDGEMENTS

We are grateful to Dr. Sudeshna Menon (Head of the Department of Biochemistry and Biotechnology, St. Xavier's College Autonomous, Ahmedabad) for providing this opportunity to accomplish by providing us with all kinds of support and resources required for our work.

REFERENCES

1. Abate-Daga, D., & Davila, M. L. (2016). CAR models: next-generation CAR modifications forenhanced T-cellfunction. *Molecular Therapy-Oncolytics*, *3*, 16014.
2. Ahmad, A., Uddin, S., & Steinhoff,M.(2020). Car-tcelltherapies: An overview of clinical studies supporting their approved use against acutely mphoblasticleukemia and largeb-celllymphomas. *InternationalJournal of Molecular Sciences*, *21*(11),3906.
3. Barrett, D.M., Teachey, D.T., & Grupp, S.A. (2014). Toxicitymanagementforpatientsreceiving novelT-cellengagingtherapies.*Current opinion in pediatrics*, *26*(1),43.
4. Bell, J. C., Lichty, B., & Stojdl, D. (2003). Getting oncolyticvirus therapies off theground. *Cancercell*, *4*(1), 7-11.
5. Berraondo, P., Sanmamed, M. F., Ochoa, M. C., Etxeberria, I., Aznar, M. A., Pérez-Gracia, J. L., ... & Melero, I. (2019). Cytokinesinclinicalcancerimmunotherapy.*British journalofcancer*,*120*(1),6-15.
6. Borghaei, H., Smith, M. R., & Campbell, K. S. (2009). Immunotherapy of cancer. *European journal ofpharmacology*, *625*(1-3),41-54.
7. Brentjens, R. J., & Curran, K. J. (2012). Novel cellular therapies for leukemia: CAR-modified Tcells targeted to the CD19 antigen. *Hematology 2010, the American Society of Hematology EducationProgramBook*,*2012*(1),143-151.
8. Brentjens,R.,Yeh,R.,Bernal,Y.,Riviere,I.,&Sadelain, M. (2010). Treatment of chroniclymphocytic leukemia with genetically targeted autologous T cells: case report of an unforeseen adverseevent ina phaseIclinicaltrial.*MolecularTherapy*,*18*(4),666-668.
9. Corthay, A. (2014). Does the immune system naturally protect against cancer?. *Frontiers in immunology*, *5*,197.
10. Darby, S. C., Ewertz, M., McGale, P., Bennet, A. M., Blom-Goldman, U., Brønnum, D., ... & Hall, P. (2013). Risk of ischemic heart disease in women after radiotherapy for breast cancer. *New England Journal of Medicine*, *368*(11), 987-998.
11. Darvin, P., Toor, S. M., Sasidharan Nair, V., & Elkord, E. (2018). Immune checkpoint inhibitors: recent progress and potential biomarkers. *Experimental & molecular medicine*, *50*(12),1-11.
12. Davenport, A. J., Jenkins, M. R., Cross, R. S., Yong, C. S., Prince, H. M., Ritchie, D. S., ... &Neeson,P.J.(2015). CAR-Tcellsinflictsequentialkillingofmultipletumor targetcells. *Cancerimmunologyresearch*,*3*(5),483-494.
13. Davila, M. L., Riviere, I., Wang, X., Bartido, S., Park, J., Curran, K., ... & Brentjens, R. (2014).Efficacyandtoxicitymanagementof19-28zCARTcelltherapyinBcellacutelymphoblasticleukemia. *Science translationalmedicine*, *6*(224),224ra25-224ra25.
14. Dobosz, P., & Dzieciątkowski, T. (2019). The intriguing history of cancer immunotherapy. *Frontiers inimmunology*,2965.
15. Dunn, G. P., Old, L. J., & Schreiber, R. D. (2004). The immunobiology of cancer immunosurveillance andimmunoediting. *Immunity*,*21*(2),137-148.
16. Eggermont, L. J., Paulis, L. E., Tel, J., & Figdor, C. G. (2014). Towards efficient cancer immunotherapy: advances in developing artificial antigen-presenting cells. *Trends in biotechnology*, *32*(9), 456-465
17. Eshhar, Z., Bach, N., Fitzer-Attas, C. J., Grosse, G., Lustgarten, J., Waks, T., & Schindler, D. G.(1996,June).TheT-bodyapproach:potentialforcancerimmunotherapy.In *Springerseminarsinimmunopathology*(Vol.18,No.2,pp.199-209). Springer-Verlag.
18. Fairweather,D.,&Cihakova,D.(2009).Alternativelyactivatedmacrophagesininfectionandautoimmunity. *Journalofautoimmunity*,*33*(3-4),222-230.
19. Fan, J., Shang, D., Han, B., Song, J., Chen, H., & Yang, J. M. (2018). Adoptive cell transfer: is it promisingimmunotherapyforcolorectalcancer?. *Theranostics*, *8*(20),5784
20. Feins, S., Kong, W., Williams, E. F., Milone, M. C., & Fraietta, J. A. (2019). An introduction tochimericantigenreceptor(CAR)T□cellimmunotherapyforhumancancer. *Americanjournalofhematology*, *94*(S1),S3-S9.
21. Fukuhara,H.,Ino,Y.,&Todo,T.(2016).Oncolyticvirus therapy:A new era of cancertreatmentatdawn. *Cancerscience*,*107*(10),1373-1379.
22. G.(1999).Anti-tumoractivityofhumanTcellsexpressingtheCC49-zetachimericimmunereceptor. *Humangenetherapy*,*10*(2),165-173.
23. Gasteiger, G., & Rudensky, A. Y. (2014). Interactions between innate and adaptive lymphocytes. *NatureReviewsImmunology*,*14*(9),631-639.
24. Giavridis, T., van derStegen, S. J.,Eyquem, J., Hamieh, M., Piersigilli,A.,& Sadelain,M.(2018). CAR T cell–induced cytokine release syndrome is mediated by macrophages and abated by IL-1blockade. *Nature medicine*,*24*(6),731-738.

25. Gonzalez, H., Hagerling, C., & Werb, Z. (2018). Roles of the immune system in cancer: from tumorinitiationtometastaticprogression. *Genes&development*,*32*(19-20), 1267-1284.
26. Graham, C., Hewitson, R., Pagliuca, A., & Benjamin, R. (2018). Cancer immunotherapy withCAR-Tcells–beholdthe future.*ClinicalMedicine*,*18*(4),324.
27. Gross, G., Waks, T., & Eshhar, Z. (1989). Expression of immunoglobulin-T-cell receptor chimericmolecules as functional receptors with antibody-type specificity. *Proceedings of the National Academy ofSciences*, *86*(24),10024-10028.
28. Grupp, S. A., Kalos, M., Barrett, D., Aplenc, R., Porter, D. L., Rheingold, S. R., ... & June, C. H.(2013). Chimeric antigen receptor–modified T cells for acute lymphoid leukemia. *New England Journal ofMedicine*, *368*(16),1509-1518.
29. Guo,C.,Manjili,M.H.,Subjeck, J.R.,Sarkar,D.,Fisher,P.B.,&Wang,X. Y.(2013).Therapeuticcancervaccines:past,present, andfuture.*Advancesincancerresearch*,*119*,421-475.
30. H.(2012).Decade-longsafetyandfunctionofretroviral-modifiedchimeric antigen receptor Tcells.*Science translationalmedicine*, *4*(132),132ra53-132ra53.
31. Haanen, J. B., & Robert, C. (2015). Immunecheckpointinhibitors. *Immuno-Oncology*,*42*,55-66.
32. Hadden,J.W.(2003).Immunodeficiencyandcancer:prospectsforcorrection. *International immunopharmacology*,*3*(8),1061-1071.
33. Hansen, H. P. (2007). Hair loss induced by chemotherapy: An anthropological study of women, cancer and rehabilitation. *Anthropology & Medicine*, *14*(1), 15-26.
34. Hao, N. B., Lü, M. H., Fan, Y. H., Cao, Y. L., Zhang, Z. R., & Yang, S. M. (2012). Macrophages in tumor micro environments and the progress ion of tumors. *Clinical and Developmental Immunology*, *2012*.
35. Hinrichs, C. S., & Rosenberg, S. A. (2014). Exploiting the curative potential of adoptive T-cell therapy forcancer. *Immunological reviews*, *257*(1),56-71.
36. Irving, B. A., & Weiss, A. (1991). The cytoplasmic domain of the T cell receptor ζ chain is sufficient to couple to receptor-associatedsignaltransduction pathways. *Cell*, *64*(5), 891-901.
37. Jin, K. T., Chen, B., Liu, Y. Y., Lan, H., & Yan, J. P. (2021). Monoclonal antibodies and chimericantigenreceptor (CAR)Tcells in the treatment of colorectal cancer. *Cancercell international*, *21*(1),1-15
38. June, C.H. (2007). Principles of adoptive T cell cancer therapy. *The Journal of clinical investigation*, *117*(5), 1204-1212.
39. June, C. H., & Sadelain, M. (2018). Chimeric antigen receptor therapy. *New England Journal of Medicine*, *379*(1),64-73.
40. June, C. H., Maus, M. V., Plesa, G., Johnson, L. A., Zhao, Y., Levine, B. L., ... & Porter, D. L. (2014). Engineered Tcellsfor cancer therapy. *Cancer Immunology, Immunotherapy*, *63*(9), 969-975.
41. June, C. H., O'Connor, R. S., Kawalekar, O. U., Ghassemi, S., & Milone, M. C. (2018). CAR T cellimmunotherapyforhumancancer. *Science*, *359*(6382),1361-1365.
42. Kalos, M., Levine, B. L., Porter, D. L., Katz, S., Grupp, S. A., Bagg, A., & June, C. H. (2011). Tcells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. *Science translational medicine*, *3*(95), 95ra73-95ra73
43. Kochenderfer,J.N.,Wilson,W.H.,Janik,J.E.,Dudley, M.E.,Stetler-Stevenson,M.,Feldman,
44. Lakshmi Narendra, B., Eshvendar Reddy, K., Shantikumar, S., & Ramakrishna, S. (2013). Immune system:adouble-edgedswordincancer.*Inflammation Research*,*62*(9), 823-834.
45. Langers, I., Renoux, V. M., Thiry, M., Delvenne, P., & Jacobs, N. (2012). Natural killer cells: role in local tumor growth and metastasis. *Biologics: targets & therapy*, *6*,73.
46. Lee, D. W., Gardner, R., Porter, D. L., Louis, C. U., Ahmed, N., Jensen, M., ... & Mackall, C. L.(2014). Current concepts in the diagnosis andmanagement of cytokine release syndrome. *Blood, The Journal of the American Society of Hematology*, *124*(2), 188-195.
47. Louis, C.U., Savoldo, B., Dotti, G., Pule, M., Yvon, E., Myers, G.D., ... & Brenner, M.K. (2011). Antitumor activity and long-term fate of chimeric antigen receptor–positive T cells in patients with neuroblastoma. *Blood, The Journal of the American Society of Hematology*, *118*(23), 6050-6056.
48. Mantovani, A., Sica, A., Sozzani, S., Allavena, P., Vecchi, A., & Locati, M. (2004). The chemokine systemindiverseforms of macrophageactivationand polarization.*Trendsin immunology*,*25*(12),677-686.
49. Marcus, A., & Eshhar, Z. (2011). Allogeneic adoptive cell transfer therapy as a potent universal treatmentfor cancer.*Oncotarget*,*2*(7),525.
50. McCarthy, E.F. (2006). Thetoxins of WilliamB.Coley and the treatment of boneandsoft-tissuesarcomas. *The Iowaorthopaedic journal*, *26*,154.
51. Mcguinness, R.P., Ge,Y., Patel, S.D., Kashmiri, S.V., Lee, H.S., Hand, P.H., ... & Mcarthur, J.
52. Mitchison, N.A.(1955). Studies on the immunological response toforeign tumor transplants in the mouse:
53. Mohanty, R., Chowdhury, C. R., Arega, S., Sen, P., Ganguly, P., & Ganguly, N. (2019). CAR Tcelltherapy:A new era for cancer treatment. *Oncologyreports*, *42* (6), 2183-2195.
54. Morrow, G. R., & Dobkin, P. L. (1988). Anticipatory nausea and vomiting in cancer patients undergoing chemotherapy treatment: Prevalence, etiology, and

behavioral interventions. *Clinical Psychology Review*, *8*(5), 517-556.

55. Neelapu, S. S., Locke, F. L., Bartlett, N. L., Lekakis, L. J., Miklos, D. B., Jacobson, C. A., ... & Go, W. Y.(2017). Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. *New EnglandJournalofMedicine*,*377*(26),2531-2544.
56. Oiseth, S. J., & Aziz, M. S. (2017). Cancer immunotherapy: a brief review of the history, possibilities, andchallengesahead.*JournalofCancerMetastasisandTreatment*,*3*, 250-261.
57. Pardoll, D. M. (2012). The blockade of immune checkpoints in cancer immunotherapy. *Nature ReviewsCancer*, *12*(4),252-264.
58. Park, J. H., & Brentjens, R. J. (2015). Are all chimeric antigen receptors created equal?. *Journal ofClinicalOncology:OfficialJournaloftheAmericanSocietyof Clinical Oncology*,*33*(6),651-653.
59. Porter, D. L., Levine, B. L., Kalos, M., Bagg, A., & June, C. H. (2011). Chimeric antigen receptor–modified Tcellsinchronic lymphoid leukemia. *NengljMed*,*365*, 725-733.
60. Porter, D.L.,Levine, B.L.,Kalos,M.,Bagg,A.,& June,C.H.(2011). Chimericantigenreceptor–modified Tcellsinchroniclymphoid leukemia. *NengljMed*, *365*,725-733.
61. Pylayeva-Gupta, Y., Das, S., Handler, J. S., Hajdu, C. H., Coffre, M., Koralov, S. B., & Bar-Sagi, D.(2016). IL35-producing B cells promote the development of pancreatic neoplasia. *Cancer discovery*, *6*(3),247-255.
62. Ramos, C. A., & Dotti, G. (2011). Chimeric antigen receptor (CAR)-engineered lymphocytes forcancertherapy.*Expert opinionbiological therapy*,*11*(7),855-873.
63. Rezvani,K.(2019).Adoptivecelltherapyusingengineerednaturalkillercells. *Bonemarrowtransplantation*,*54*(2),785-788.
64. Ruella, M., & Kalos, M. (2014). Adoptive immunotherapy for cancer. *Immunological reviews*, *257*(1), 14-38.
65. Ruf,P.,&Lindhofer,H.(2001).Inductionofalong-lastingantitumorimmunitybyatrifunctionalbispecificantibody. *Blood,TheJournal oftheAmerican SocietyofHematology*,*98*(8),2526-2534.
66. S. A., ... & Rosenberg, S. A. (2010). Eradication of B-lineage cells and regression of lymphoma in a patienttreatedwith autologousTcells genetically engineered to recognize CD19. *Blood, The Journal of theAmerican Society of Hematology*,*116*(20),4099-4102.
67. Sadelain, M., Brentjens, R., & Rivière, I. (2013). The basic principles of chimeric antigen receptordesign. *Cancerdiscovery*,*3*(4),388-398.
68. Schirrmacher, V. (2019). From chemotherapy to biological therapy: A review of novel concepts to reduce the side effects of systemic cancer treatment. *International journal of oncology*, *54*(2), 407-419.
69. Scholler,J.,Brady,T.L.,Binder-Scholl,G.,Hwang,W.T.,Plesa,G.,Hege,K.M.,...&June,C.
70. Skorka, K., Ostapinska, K., Malesa, A., & Giannopoulos, K. (2020). The application of CAR-Tcellsinhaematological malignancies.*ArchivumImmunologiaeetTherapiaeExperimentalis*,*68*(6),1-19.
71. Smyth, M. J., & Teng, M. W. (2018). 2018 Nobel Prize in physiology or medicine. *Clinical & translationalimmunology*,*7*(10).
72. Soltantoyeh, T., Akbari, B., Karimi, A., Mahmoodi Chalbatani, G., Ghahri-Saremi, N., Hadjati, J., ... &Mirzaei, H.R. (2021). Chimericantigenreceptor (CAR) Tcell therapy formetastaticmelanoma: challenges and road ahead. *Cells*, *10*(6), 1450.
73. Southam, C.M., Brunschwig, A., Levin, A.G., & Dizon, Q.S. (1966).
74. ectofleukocytesontransplantabilityofhumancancer. *Cancer*, *19*(11),1743-1753.
75. Spear, T. T., Nagato, K., & Nishimura, M. I. (2016). Strategies to genetically engineer T cells for cancerimmunotherapy. *CancerImmunology,Immunotherapy*,*65*(6),631-649.
76. Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a cancer journal for clinicians*, *71*(3), 209-249.
77. Taylor, C. W., & Kirby, A. M. (2015). Cardiac side-effects from breast cancer radiotherapy. *Clinical Oncology*, *27*(11), 621-629.
78. The role of lymph node cells in conferring immunity by adoptive transfer. *The Journal of experimentalmedicine*, *102*(2),157-177.
79. Vesely, M. D., Kershaw, M. H., Schreiber, R. D., & Smyth, M. J. (2011). Natural innate and adaptiveimmunitytocancer.*Annualreview ofimmunology*,*29*, 235-271.
80. Waldman,A.D.,Fritz,J.M.,&Lenardo,M.J.(2020).
81. idetocancerimmunotherapy: from Tcell basic science to clinical practice. *Nature Reviews Immunology*, *20*(11), 651-668.
82. Weiner,L.M.,Murray,J.C.,&Shuptrine,C.W.(2012). Antibody-basedimmunotherapyofcancer. *Cell*,*148*(6),1081-1084.
83. Wilson,C.B.,Rowell,E.,&Sekimata,M.(2009). EpigeneticcontrolofT-helper-celldifferentiation. *Nature reviewsimmunology*,*9*(2),91-105.
84. Zang, X. (2018). 2018 Nobel Prize in medicine awarded to cancer immunotherapy: Immune checkpointblockade–Apersonal account. *Genes&diseases*, *5*(4),302.