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Immune-Cell-Mediated Cancer Treatment: Advantages, Drawbacks And Future Direction

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ABSTRACT

Cancer ranks as the most lethal and prevalent non-communicable disease in clinical settings. Therapeutic options for cancer comprise chemotherapy, radiotherapy, surgery, and combined treatment. Cancer remission and relapse cases are widespread despite having various advanced medications and sophisticated dissection techniques. A new approach involving immune-cell-mediated cancer therapy has been adopted extensively for cancer treatments by utilizing immune cells. Immunotherapy has gained much attention to prevent and treat various types of cancer. Immunotherapy treatments operate in multiple contexts. Several immunotherapy therapeutic interventions assist the immune function in halting or reducing the advancement of cancer cells. Many also facilitate the immune cells in destroying cancerous cells or safeguarding against cancer from disseminating to certain other regions of the human body. Among other methods, genetic manipulation of immune cells offers hope for innovative anticancer treatment. T lymphocytes and natural killer cells have become the most extensively documented immune cells for immunotherapy. Chimeric antigen receptor T-cell therapy exhibits the most promising blood cancer treatment. However, adoptive NK cell transfer therapy displays potential anticancer treatment options, although more research is needed to be carried out. In addition, cytokine-induced immunomodulation is also plausible for cancer immunotherapy. This review will highlight the most comprehensive information, observations, and consequences associated with different cancer immunotherapy initiatives.

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1 Introduction

Cancer is one of the fastest-growing causes of fatalities across the globe, accounting for substantially 10 million mortalities in 2020 (WHO 2022). This horrifying disease presents an unacceptable health risk to all communities, regardless of economic or social status. Also, it is the fundamental cause of roughly 30 percent of all non-communicable (NCD) related deaths in people aged 30-69. Female breast carcinoma is the most widespread cancer after pulmonary, colorectal, and prostate tumours (Sung et al. 2021). Prevention strategies, such as monitoring, early recognition, multidisciplinary therapeutic interventions, longevity, and rehabilitation, are all part of the cancer management spectrum. Nowadays, surgical intervention, medical therapy (chemotherapy, immunotherapy, endocrine therapy), radiation therapy, or a combination of these treatments are used for cancer treatment (Figure 1). Cancer is a growing global public health concern and contributes to one out of every six deaths worldwide. The strain on individuals, societies, healthcare, and financial systems grows daily. The psychosocial impact of cancer ranges from profound physical devastating effects to financial crises within the family and suicidal attempts (WHO 2020).

After two famous research experts were awarded Nobel Prizes in Medicine or Physiology in 2018 for their pioneering work on cancer immunotherapy, interest in the treatment skyrocketed. These two researchers uncover the activation mechanism of the immune system's cells to fight against cancer, an advance in developing new cancer treatments. Their incredible discoveries have guided the development of several potential chemotherapeutic agents that mete effective cancer immunotherapy's routine use. Nowadays, immunotherapy stands apace with chemotherapy, radiotherapy, and surgery as a new way of cancer management. To date, immune-mediated cancer treatment is currently merely impactful in a few forms of cancer. The subsequent step for researchers is crucial to expand the percentage of patient populations who could benefit from this strategy (Simon and Uslu 2018).

For the past decade, the scientific community has gained a greater awareness of the connections between innate immune lymphocytes and tumour cells and the underlying biochemical pathways by which disease can avoid the immune system. The immunity is monitored by the active link of two distinct categories of cells, particularly lymphoid cell lines, i.e. T and B lymphocytes (natural killer "NK" cells), and myeloid cell lines (neutrophils, macrophages) and the by-products of their synthesis, for instance, cytokines and antibodies. In cancer, the immune system is dwindled primarily due to the effects of radiotherapy and chemotherapy, so research has been carried out in recent years to boost immune cell activity, and immunotherapy is explored to treat cancer (Grigore 2017). This new treatment seems to be a broader range of therapeutic approaches that induce immune-cell-mediated malignant cell breakdown (Mostafa and Morris 2014). As a result, this modern concept of cancer immunotherapy policies and procedures emerges that overrides the restrictions imposed by traditional treatment modalities (Gleichmann 2020). This article will provide information about the most comprehensive data, observations, and consequences related to various cancer immunotherapy initiatives.

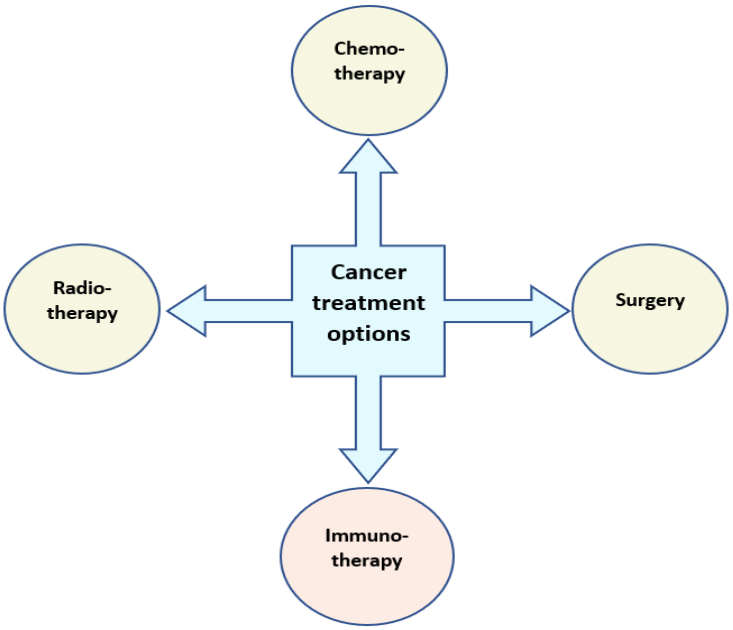

Figure 1 Types of cancer treatment.

2 Types of Immunotherapies

A few types of immunotherapies operate in an array of aspects. Several immunotherapeutic treatment methods assist the immune function in stopping or slowing cancer cell development. Some even aid the immune lymphocytes in destroying tumour cells or preventing cancer from extending toward other tissues throughout the body. Immunotherapy is effective in itself or conjunction with several other anticancer agents. Monoclonal antibodies, immune checkpoint blockers, oncolytic virus treatment, T-cell and NK-cell-mediated therapy, and cancer vaccines are presently offered as immunotherapeutic remedies (Chhabra and Kennedy 2021; Saxena et al. 2021).

Monoclonal antibodies are manufactured in sophisticated laboratories, and these proteins boost the body's natural immunities. Antibody production occurs when the immune cells identify potentially hazardous microbes or abnormally transformed cells during an early phase of the immune response. Then, these antibodies fight against invading organisms and cancerous cells by binding to specific antigens. Later, these proteins could restrict the function of unusual specific proteins in tumour tissue. Also, monoclonal antibodies boost the activity of the cells' immune system by modulating immune checkpoints (Zahavi and Weiner 2020).

Immune checkpoint blockers enhance the body's immune cell's ability to eradicate unnaturally dividing tumours. These blockers commonly target the programmed-cell-death-1 or its ligand (PD-1)/PD-L1 and cytotoxic-T-lymphocyte-associated antigen-4 (CTLA-4) processes. United States Food and Drug Administration (US-FDA) recently certified many immune checkpoint blockers to be used clinically for specific types of cancer. The most commonly used immune checkpoint blockers include ipilimumab, nivolumab, atezolizumab, avelumab, and durvalumab (Robert 2020).

Oncolytic virus treatment employs laboratory-modified viruses to suppress malignant cells. Firstly, the virus that has been genetically manipulated is administered into the tumour. Then, the virus invades tumour cells and replicates itself. As an outcome of this, the cancerous cells erupt and start dying. While the cells perish, viral proteins are released, which induce the immune function to identify any irregular cells throughout the body that contain similar protein molecules as the dying tumour cells. In 2015, the FDA granted the first oncolytic virus treatment to cure extensive melanoma that could not be managed surgically. The virus used in the therapy is designated as talimogene laherparepvec (T-VEC). However, various untoward effects of this treatment were reported, such as fatigue, flu-like illness, and discomfort at the injection site. Numerous viral vectors for various types of cancers are being studied in clinical studies (Lawler et al. 2017). Furthermore, the co-administration of T-VEC and immune checkpoint inhibitors appears to be a favourable melanoma therapeutic modality (Zhang et al. 2023).

T cells are the primary lymphocytes that fight against infection and abnormal cells. In T-cell-mediated cancer treatment, they are isolated from peripheral blood and then transformed by introducing particularly associated receptor proteins to the cells. These receptor proteins enable T cells to identify abnormally transformed cell populations. The modified T cells are then reinstated into the human organism. After entering one's body, they locate and eliminate tumour cells. This is widely recognized as chimeric antigen receptor (CAR) T-cell therapy. CAR T-cell treatment is successful in curing certain kinds of blood-related cancer. In exceptional cases, febrile reactions, disorientation, hypotension, and epileptic episodes are among the complications. Research teams nowadays are looking into avenues for enhancing this kind of treatment and certain other methods of changing T-cells to combat malignancy (Cerrano et al. 2020; Fischer and Bhattarai 2021; Martino et al. 2021).

Cancer vaccines can improve the body's ability to fight diseases. Vaccine uncovers the body's immune lymphocytes to an antigen, a foreign protein. This activates the immune system, causing it to detect and dismantle the antigen or associated compounds. There are two kinds of cancer vaccines presently offered: a vaccine for preventive measures and a vaccine for therapeutic options; nevertheless, their utilization remains relatively small (Saxena et al. 2021).

NK cells are lymphocytes with cytotoxic capabilities that are essential for natural immunity. NK cell adoptive transfer treatment is the latest area of interest in immunotherapy due to T cell therapy's severe unwanted side effects. NK cell immunotherapy is extensively developing, and many clinical trials have been going on and reaching phase II clinical trials. Several research investigations have taken place to assess the risks and benefits of adoptive NK therapeutic strategies to treat haematological malignancies (Veluchamy et al. 2017). But then again, as a result of inadequate NK cell relocation and incursion into the tumour, implementing the application of NK cells to cure solid tumours poses significant obstacles. Animal studies are now aimed at enhancing these NK cell processes for adoptive transfer. Contemporary NK cell treatment methods focus on autologous, allogeneic, or CAR-NK cells (Franks et al. 2020; van Vliet et al. 2021). Among all the available treatment options for cancer immunotherapy, CAR-T and adoptive NK cell transfer treatment are the most promising, although various side effects are still a significant problem. This article will address the positive and negative aspects of the two common types of immunotherapies. In addition, the possible ways for future developments in the area of immune-cell-mediated cancer treatment will also be highlighted.

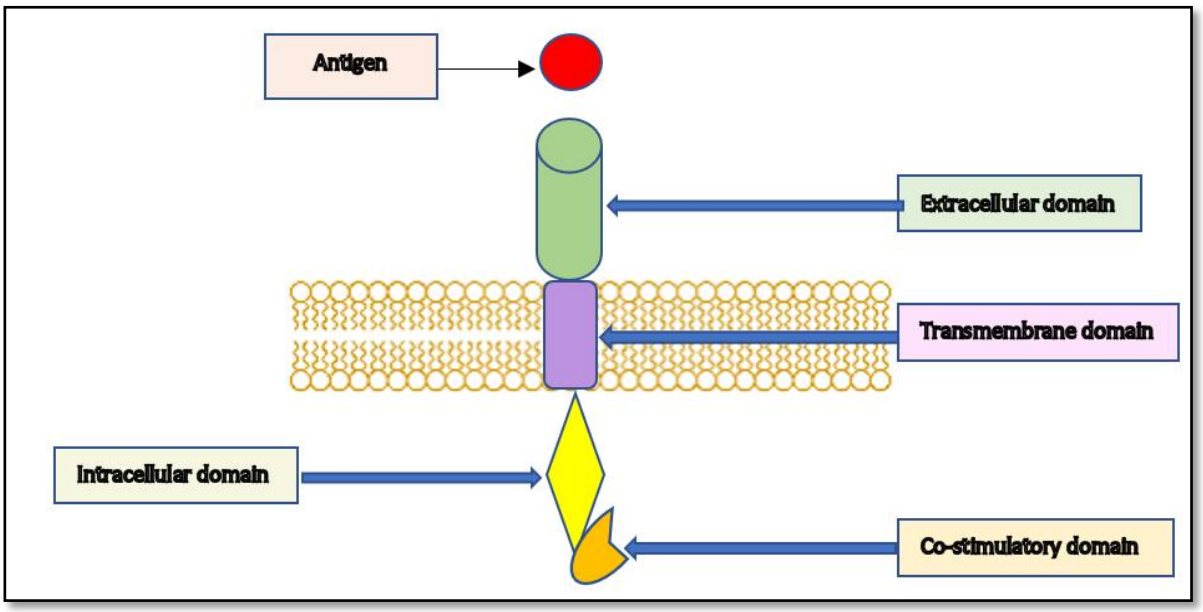

Figure 2 Schematic diagram of the domains of the chimeric antigen receptor.

2.1 Chimeric Antigen Receptor or CAR-T Therapy

The most popular adoptive cell transfer therapy nowadays is chimeric antigen receptor T cell or CAR-T cells that identify and destroy tumours independently of major histocompatibility complex (MHC) protein; thus, downregulation of MHC molecules overcomes immune escape of tumour cells (Zhang et al., 2020). CAR-T cells are genetically modified and manufactured to recognize tumour-associated antigen (TAA) and subsequent stimulation of T lymphocytes and lysis of cancer. As displayed in Figure 2, CARs are designed with several distinct regions: an extracellular domain, a transmembrane portion, and intracellular and costimulatory domains. Each domain possesses a specific operation, and the ideal biological configuration of the CAR can be attained through many modifications of the component amino acid sequences (Rafiq et al. 2020).

The external antigen binding region of CAR is in charge of antigen specificity. At first, antigen-binding regions were extracted from monoclonal antibodies and linked with a flexible linker to form a single-chain variable fragment (scFv). The scFv binds to the exterior protein of the cancer antigen, culminating in MHC-independent T-cell induction (Zhang et al. 2014). Essentially, the fundamental determinant of CAR function depends on the binding affinity of scFv to the target cell antigen. This binding must be precise enough to recognize cancer cells and subsequent induction of CAR signal transduction and T-cell stimulation; nevertheless, extremely high affinity could also result in activation-induced cell death (AICD) of the CAR-expressing cell and, plausibly, lead to toxic effects (Dholaria et al. 2019). The transmembrane domain links the antigen-binding region and the intracellular signal transduction domain. The transmembrane domain primarily stabilizes the CAR to the T-cell lipid bilayer (Guedan et al., 2018). It is extracted mainly from type-I protein molecules such as clusters of differentiation-3 (CD3, CD4, or CD28 (Alabanza et al. 2017). The intracellular signal transduction domain is the most critical component of CAR and primarily consists of an activation domain and one or more costimulatory sites. Most CARs invoke CAR-T cell lines via immunoreceptor tyrosine-based activation motifs sourced from CD3. Nevertheless, stimulation mediated through these motifs was ineffective and could lead to restricted *in vivo* T-cell longevity and interaction (Rafiq et al. 2020).

A costimulatory domain is crucial for effective T-cell activation, operation, proper metabolic activity, and consistency. The addition of the costimulatory domain not only improves cytokine production but also favours proliferation upon repeated antigen exposure (Tariq et al. 2018).

2.2 Eureka moment of CAR-T treatment

The breakthrough of CAR-T approval was determined after successful trials were done between 2015 and 2020. Firstly, Maude and co-researchers conducted the very first global CAR-T-cell therapy enrolment cohort study, which resulted in the FDA's recommendation of tisagenlecleucel (tisacel) for paediatric and teenage patients with relapsed/refractory B-cell-acute lymphoblastic leukaemia (R/R B-ALL). In that phase-II experiment, 75 patients were injected with tisacel, with a mean average follow-up of 13.1 months. The ideal overall response rate (ORR) was 81%, with 60% complete remission (CR), and all were measurable residual disease (MRD) negative (0.01% by flow

cytometry). Tisacel was found in the patient's blood for 20 months, even after injection. At twelve months, the event-free survival (EFS) and overall survival (OS) rates were 50% and 76%, respectively. However, 73% of the infused patients experienced severe adverse effects, and intensive care unit (ICU) treatment was needed in 47% of these patients for cytokine-related toxicity management. Neurotoxicity was also witnessed in 40% of the cases after eight weeks of such injection, with 13% severe scenarios, but none of the patients required ICU treatment (Maude et al., 2018).

Similarly, the product's registration resulted in another multicenter phase-II clinical experiment using axicabtagene ciloleucel (axicel) in advanced B-cell-non-Hodgkin lymphoma (B-NHL). In this phase II trial, 108 cancer patients were administered axicel, and 101 were evaluated for clinical response. The enrolled in-patients began receiving CAR-T cells at a 2×10^6 cells/kg target dose following lymphodepletion chemotherapy. According to Locke and colleagues' updated experiment evaluation, the best ORR and CR rates were 83% and 58%, respectively. However, 93% of patients reported cytokine-released syndrome (CRS), with 13% severe symptoms. Neurotoxicity was also observed in 64% of patients, 28% severe and very severe (Neelapu et al. 2017; Bouchkouj et al. 2019; Locke et al. 2019).

Following the second multicenter clinical phase II trial, the FDA approved Tisacel for adult patients with R/R diffuse large B-cell lymphoma (DLBCL). Overall, 111 enrolled patients were infused with tisacel, and clinical response was assessed in 93 patients. The highest ORR was 52%, with a CR of 40% and a partial response (PR) of 12%. Severe and very severe CRS rate was observed in 22% and 24% of the patients, respectively, and they were referred to the ICU for CRS treatment. Neurotoxicity occurred in 21% of patients, with severe complications experienced in 12% of cases, with no fatalities(Schuster et al. 2019).

The US FDA and the European Medicine Agency (EMA) have both granted two distinct CAR-T cell products, namely tisacel and axicel, for the treatment of R/R B-ALL in patients under the age of 25 and adult patients with different types of lymphoma in late 2017. However, more improvements are required to improve CAR-T efficacy to widen the spectrum of target cancer cells and lessen unwanted complications. Furthermore, more research is needed to translate innovations in early-stage clinical research into the clinical setting (Cerrano et al. 2020).

2.3 CAR-T cell toxic effects and potential solutions

Despite being a ground-breaking cancer therapy weapon, high numbers of systemic toxicity and fatal accidents have prevented CAR-T immunotherapy from becoming a mainstream cancer therapeutic option. The severity of complications during CAR-T cell treatment becomes a significant disturbance in developing effective CAR-T. Developing a more significant breakthrough will be challenging if these hindrances are not solved. There are some serious adverse side effects reported on CAR-T therapy, and Cytokine Release Syndrome (CRS) is the most frequent negative impact, followed by neuronal toxicities, off-tumour toxicity, and additional side effects (Zhang et al., 2020).

2.3.1 Cytokine Release Syndrome (CRS)

Cytokine Release Syndrome is a severe and, in some cases, deadly consequence. In many clinical trials, the elevation of systemic cytokine production was endorsed in patients administered with CAR-T cells. In substantial clinical studies, CRS is prominently featured in 50-90% of the clients and most commonly occurs within the following day after the infusion of CAR-T cells (Schuster et al. 2019; Neelapu et al. 2017). CRS can vary from self-contained influenza-like symptoms to potentially fatal multi-organ impairment requiring urgent attention and rigorous life-sustaining therapeutic interventions. Hypotensive shock, decreased blood supply to the kidneys, and pulmonary congestion can result from CRS-associated capillary leak syndrome (Giavridis et al. 2018; Norelli et al. 2018). For severe CRS, supplemental Tocilizumab or corticosteroids alone would be required for intensive care. Conversely, there remain incidents where clinical manifestations could not improve or worsen following aggressive therapy (Brudno and Kochenderfer 2016).

To tackle this problem, the American Society for Transplantation and Cellular Therapy strongly advocated a principle scoring system for CRS and neurotoxic effects linked to the immune impacts of cell therapies (Lee et al. 2019). In addition to intense therapeutic interventions and ICU monitoring, severe CRS management requires cytokine inhibitors. Tocilizumab, an IL-6 receptor blocker, has been granted FDA approval as a first-line intervention for CRS and is also being studied as a preventive initiative. Systemic corticosteroids are used as a second-line drug for clients with a dissatisfying effect. In this context, current stats demonstrated that earlier stage therapeutic approach during the initial signs and symptoms of CRS, which includes administering glucocorticoids, can preclude severe conditions without affecting outcomes, dispelling concerns that these drugs could impair the efficacy of CAR-T therapy (Gardner et al., 2019; Liu et al., 2020).

CRS is a widespread inflammatory process triggered by T cells' immediate activation and propagation following interaction with target tissues, releasing unnecessarily high cytokines. Furthermore, activated monocytes are mainly accountable for interleukin-6 (IL-6) and IL-1 secretion, a significant occasion in the advancement of CRS. Although the detailed pathophysiology has yet to be understood, the latest evidence has shed light on the potential molecular pathways of CAR-T cell-induced CRS (Bouchkouj et al.

2019). CAR-T cells proliferate after the scFv attaches to the target cell antigens, become activated, and secrete many proinflammatory cytokines as an acute response (Hay et al. 2017; Neelapu et al. 2017). The secreted cytokines stimulate lymphocytes, particularly T cells and non-immune cells like epithelial cells, to emit more cytokines of different forms and amounts (Shimabukuro-Vornhagen et al. 2018). According to study results, interferon-gamma (IFN-γ) activates macrophages and provokes the discharge of tumour necrosis factor (TNF), IL-6, IL-15, IL-1, and IL-12, thereby sustaining or improving following immune responses, and IL-6 is one of the crucial cytokines amongst these. The amount of IL-6 released by immune cells coincides with the amount of CD40L displayed on the outer layer of CAR-T cells**.** Two other research investigations have revealed that extreme CRS is coupled with vascular endothelial stimulation or impairment (Giavridis et al. 2018; Obstfeld et al. 2017). In clinical trials, Tocilizumab, siltuximab, kinase inhibitors, and corticosteroids that block IL-6 can quickly reverse fever, hypotension, and hypoxia (Zhang et al. 2020).

2.3.2 Neurotoxicity

Neurotoxicity, also known as immune effector cell-associated neurotoxicity syndrome (ICANS), is CAR-T therapy's next-most prevalent detrimental impact. ICANS is characterized by an interruption of the blood-brain-barrier (BBB) and elevated cerebrospinal fluid (CSF) inflammatory cytokines, which may manifest as dysarthria, altered psychological condition, involuntary movements, convulsions, headache, and life-threatening brain oedema, frequently concomitantly or continuing to follow CRS (Santomasso et al. 2018; Rafiq et al. 2020). As a result, central nervous system perivascular stress and over-expression of endothelium-activating proinflammatory cytokine increased BBB permeability in an endless cycle (Gust et al. 2017). Furthermore, in an animal study, CAR and non-CAR-T cells were found to accumulate in the CSF and nervous system tissue, implying a central role in neurotoxicity development (Taraseviciute et al. 2018). The most current neurotoxicity scoring method incorporates a 10-point grading scale based on the Immune Effector Cell-Associated Encephalopathy (ICE) evaluation system and considers five significant neurological aspects (Lee et al. 2019).

Individuals with serious ICANS must always be performed assertively, and a comprehensive strategy, including neurologic collaboration, is frequently required. An electroencephalography, brain magnetic resonance imaging, and CSF examination are all necessary in the practice of a presumed ICANS to sort out other causal factors of neuronal damage (Dholaria et al. 2018). Levetiracetam, an anti-epileptic, could be a preventive remedy for avoiding convulsions, starting immediately following CAR-T cell administration or at the first sign of Neurotoxicity. The first-line intervention for neuronal damage has now become dexamethasone or high-dose methylprednisolone (Neelapu 2019). Recently conducted studies have found that granulocyte-macrophage colony-stimulating factor (GM-CSF) played an essential player in the aetiopathogenesis of Neurotoxicity. In preclinical studies, lenzilumab, an anti-GM-CSF monoclonal antibody, successfully blocked CD19-CAR-mediated Neurotoxicity and CRS (Sterner et al. 2019). Although life-threatening complications were encountered, ICANS, like CRS, is manageable in some instances, including a few fatal reports, and most sick people even have self-limited courses (Gust et al. 2017; Cerrano et al. 2020). There is no specific treatment guideline to prevent the above toxicities. Therefore, optimization of CAR genetic manipulation and employing other approaches to reducing CAR-induced toxicity are essential (Sterner and Sterner 2021).

2.3.3 On-target-off-tumour

On-target-off-tumour is an additional common complication of CAR-T treatment. T-cell antigen receptors, upon genetically engineered, recognize tumour cells by identifying specific antigenic proteins on the tumour cells' membrane (Leyfman 2018). Meanwhile, these antigens could also be depicted in healthy cells. Thus, administering the CAR-T cell line could attack healthy cells,

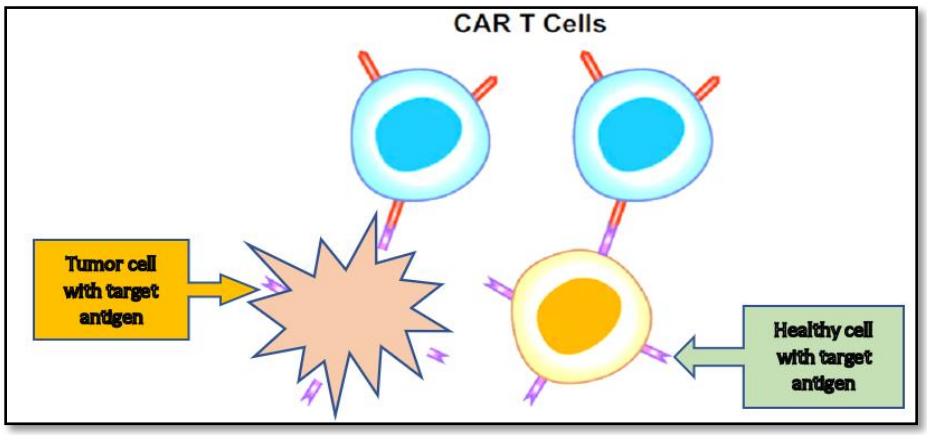

Figure 3 Schematic presentation of On-target Off-tumour side effects.

leading to tissue and organ damage, the on-target/- off-tumour effect (Morgan et al. 2010). The TAA identified by CAR-T treatment is not only specific to these abnormal cells, and when CAR-T cells attach non-tumour target proteins, it generates an off-target effect, as illustrated in Figure 3. CAR-T cells that observe two antigens have evolved to avert off-target adverse effects. Initially, CAR-T cells recognize malignant cell antigenic protein and invoke the transcription of CAR embedding codons within the cell. The single-chain immunoglobulin identifies antigen B after CAR translation, restricting CAR-T cells from attempting to kill healthy tissue (Zhang et al. 2020).

However, the problem remains a challenge in recognizing antigens expressed by solid tumours. The solid tumour antigens are frequently expressed at different levels of complexity in healthy tissue. Hence, antigen selection is essential in the CAR construct to secure curative potency and reduce the "on-target off-tumour" toxic effect. Designed to target tumour-restricted post-translational alteration, it could help overcome the attacking of antigens on solid tumours likewise found in normally functioning tissues. To broaden the medical usage of available CAR-T cell therapies in haematological malignancies and solid tumours, more ground-breaking methods to minimize antigen escape and then choose antigens that can generate adequate antitumor activity whilst also lessening toxicity issues will be required (Du et al. 2019; Sterner and Sterner 2021).

2.3.4 Additional Negative Impacts

Additional negative impacts of CAR-T treatment include tumour lysis syndrome, prolonged B-cell aplasia, hypogammaglobulinemia, and severe pancytopenia, which is prevalent and persists a few months following CAR-T cell injection (Fried et al. 2019; Neelapu 2019). Numerous biochemical markers, including lactate dehydrogenase, ferritin, C-reactive protein, inflammatory cytokines, GM-CSF, von Willebrand factor, and angiopoietin-2 (representing endothelial stimulation), and relatively low platelet count, were also linked to CRS and neurotoxicity complications, and yet a convincing developed model remains missing (Santomasso et al. 2018; Karschnia et al. 2019).

2.4 Improving CAR-T cell Efficacy

CAR-T cell treatments are considered particularly effective for the curative purposes of haematological cancers. Immunological and molecular biology innovations have enabled the development of the forthcoming generations of CAR-T cells endowed with various biological functions. Some include extra costimulatory motifs, safety switches, immune-checkpoint regulation, cytokine production, or deletion of therapy-interfering proteins. Deployment of contemporary CAR T-cells might enable circumventing present constraints on CAR-T therapy, reducing undesirable adverse reactions, and addressing other haematological cancers (Tomasik et al. 2022; Hu et al. 2022). Immune checkpoint protein molecules, such as PD-L1, commonly expressed in tumour cells, can inhibit CAR-T cell function. Experimental results indicate that incorporating CAR-T cells into established systemic checkpoint blockade antibodies significantly improves tumour destruction (Cherkassky et al. 2016; Moon et al. 2016). As a result, the administration of immune checkpoint inhibitors in conjunction with CAR-T cells is being investigated. The pairing of axicel and atezolizumab has been started testing in the ZUMA-6 experiment, with promising initial findings, and lisocel in pairing with some other anti-PD-L1 antibody, durvalumab, is now being examined in clients with R/R B-NHL (Jacobson et al. 2018; Hirayama et al. 2018; Siddiqi et al. 2019). Aside from the potential dangers regarding CAR-T cell-based therapy, it's also widely documented that cancer cells frequently establish methods to circumvent T-cell identification. T cells are activated and proliferated when antigens are presented to them in a human leucocytic antigen (HLA)-restricted manner. By decreasing the expression of HLA class-I compounds, malignant cells dissuade T-cell attention and killing (Chhabra and Kennedy 2021; van Vliet et al. 2021). NK cells, another prominent innate immune cell, have become an alternative possible target of CAR carriers for off-the-shelf product lines. Thus, there is a pressing need to develop efficient and safe targeted immunotherapies to treat advanced cancer. Unlike T cells, NK cells are a constituent of the innate immune system and could strike cancerous cells without previous sensitization; their operation relies upon the equilibrium of activating and inhibiting receptors located on cell surfaces (Karre 2002).

3 NK cell Adoptive transfer therapy

NK cells are cytotoxic lymphocytes that are essential for natural immunity. NK cells, which account for about 10% of blood leukocytes, are effective immune effectors linked to the control of tumorigenesis (Rey et al. 2009). Immune cells recognize MHC complexes represented on infective or unnaturally altered cell surface membranes to elicit cytokine production, resulting in immune system response and apoptotic cell death or lysis of target cells (Glienke et al. 2015). NK cells represent the sole immune system cells that can distinguish cancer cells without MHC and antibodies, resulting in an immediate immune system response. Thus, these cells have been referred to as "natural killers" because they can operate without being activated to dismantle cells lacking 'self' MHC class-I chemical biomarkers (Klingemann 2014). NK cells produce several cytokines, interleukins, and interferons, which act as immune suppressors (Jiang et al. 2014). Contrary to T lymphocytes, non-hematopoietic cells are not targeted by NK cells, implying that NK cell-mediated anticancer activity can be powered up in the non-appearance of graft-vs-host disease (GvHD) (Tang et al. 2018).

Transduced NK cells can identify malignant cells using their innate membrane proteins and CAR-specific target detection, lowering the risk of tumour expulsion (Quintarelli et al. 2019). Allogeneic cell lines could not induce GvHD as they do not imply HLA matching to identify targets. Furthermore, the relatively short lifetime of NK cells mitigates the likelihood of long-term adverse effects. In a murine model, NK cells designed and implemented to assert a CD19-directed CAR, release IL-15 to sustain ectopic proliferation and longevity, and evoke a suicide gene (i.e., inducible caspase-9) demonstrated destruction capacity (Liu et al. 2018). The preclinical trial outcomes recently explore the findings on the security and success of CD19-iCasp9-IL15 transduced umbilical cord-derived NK cells in individuals with R/R CD19-positive B-lymphoid leukaemias. Following lymphodepletion chemotherapeutics, 11 patients were given one CAR-NK injection. Eight of the eleven treated groups reacted immediately (within 30 days), with seven achieving CR, and no substantial complications, such as CRS, neurotoxic effects, or GvHD, were observed (Liu et al. 2020). In animal and *in vitro* studies, the CD19-CAR concept could be efficaciously expressed on the NK-92 cultured cells, imparting cytolytic functionality against initially impervious CD19-positive malignant cells (Romanski et al. 2016). Regrettably, before *in-vivo* activity, cells must be irradiated to prevent unnecessary cancer cell adhesion and proliferation, which may reduce their effectiveness.

Numerous investigations have been performed to assess the risks and benefits of adoptive NK cell treatment to cure haematological malignancies. But even so, since there is inadequate NK cell attachment and incursion into the tumour, applying NK cells to remediate solid tumours poses shortcomings. New therapeutic research is dedicated to enhancing NK cellular activities for adoptive transfer. Figure 4 depicts Contemporary NK cell therapy strategies that depend on autologous, allogeneic, or CAR-NK cells (Veluchamy et al. 2017).

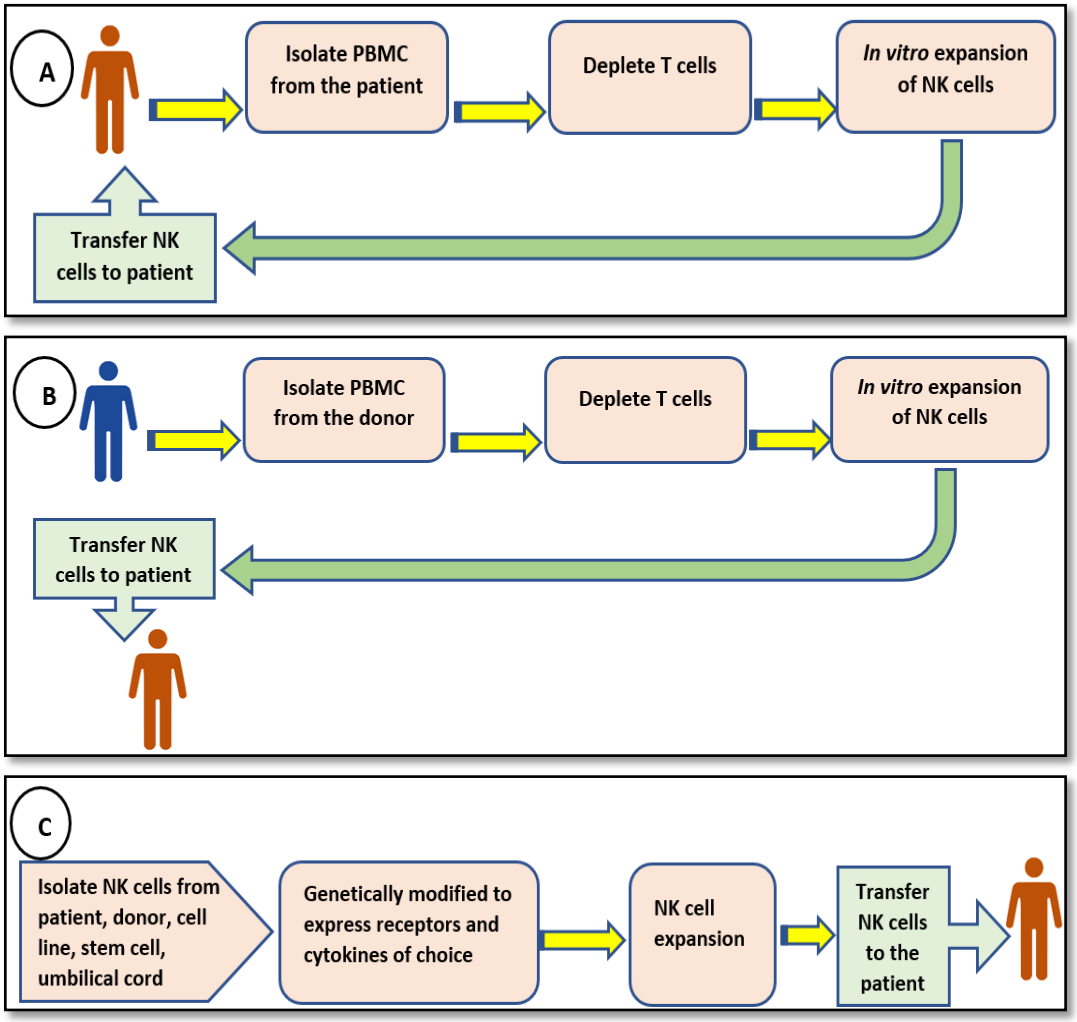

Figure 4 Schematic diagram for NK cell therapy (A) Autologous NK cell therapy, (B) Allogeneic NK cell therapy, and (C) CAR-NK therapy.

3.1 In autologous NK cell therapy

In autologous NK cell therapy, the patient's NK cells are collected, cultured, expanded outside the body, and reintroduced. The earlier experiments in NK adoptive transfer were designed to boost autologous NK cell antineoplastic actions by strengthening NK cell activity and multiplication with cytokines. When NK cells are prompted with cytokines like IL-2, IL-12, IL-15, and IL-18, they become powerful, with the elevated synthesis of adhesion molecules, activating receptors (NKp44), cytolytic protein perforin, granzymes, FasL, and TNF-related apoptosis-inducing ligand (TRAIL), along with improved cytokine release and cell multiplication (Smyth et al. 2004; Becknell and Caligiuri 2005; Davis et al. 2015). Some advantages of autologous NK cell therapy include minimizing the likelihood of system-wide immunogenicity, bio-incompatibility, and disease development affiliated with grafts or cell lines not fostered by the patient. Individuals who underwent adoptive transfer of autologous lymphokine-activated killer cells or more abundant NK cells augmented *ex vivo* in combined application with IL-2 execution had equivalent clinical outcomes (Parkhurst et al. 2011; Jiang et al. 2016). Furthermore, cytokine-induced autologous NK immune function advancement could be hampered by inhibitory activity via tumour-expressed self-MHC or prolonged immunodeficiency by the host tumour cells (Verneris 2013). With these considerations, the usefulness of allogeneic NK cells from relevant donors in the adoptive transfer was studied.

3.2 In an allogeneic NK cell immunotherapy

In an allogeneic NK cell immunotherapy, NK cells are extracted from a donor with a matching HLA-type and reintroduced into the patient to suppress the cancer cells and restore the patient's immune cell activity. An allogeneic NK cell therapy differs from an autologous one, where the latter uses NK cells from the patient's body. After several stem cell transplantation and allogeneic infusions of isolated NK cells, allogeneic NK cell studies have revealed beneficial outcomes for many types of cancer, notably acute myeloid leukaemia (AML)(Geller and Miller 2011; Davis et al. 2015). Allogeneic NK cells can be obtained from the peripheral circulation, umbilical cord, or bone marrow; meanwhile, peripheral blood remains the most frequently employed type of cell line for stem cell transplantation (Shah et al. 2013). Another NK cell contributor, including NK cell lines, provides the advantage of being free of containing T and B cells, thereby lessening any alloreactive consequences and GvHD connected with blood-derived NK cells. Then again, NK cell lines, in addition, consistently show promise to be employed in adoptive transfer contexts, with several medical studies currently in progress. Consequently, difficulties in procuring and extracting those certain cells confine their pervasive clinical application in interventional NK cell therapies (Lupo and Matosevic 2019). NK cells derived from healthy and blood-related donors gain benefit from being guided in a non-immunosuppressive atmosphere and are, hence, completely operational. NK cells' contribution to GvHD progression after adoptive transfer into receivers has been hotly debated (Simonetta et al. 2017). Several research studies have indicated that in allogeneic adoptive transfer contexts, NK cells mitigate GvHD by restricting alloreactive T lymphocytes (Bachanova et al. 2014). The difficulties in obtaining NK cell supplementation exempt from alloreactive T cells, which are operationally developed and cannot facilitate GvHD, are apparent. Though some new clinical studies have revealed that NK cell adoptive transfer therapies are reliable and successful in treating cancer, the responsibility of NK cells in linking to GvHD must not be underestimated. Achievement in establishing NK cell therapeutic strategies will depend heavily on improvements in diverse cell sources, such as NK cell cultures and stem cell-derived NK cells, as well as understanding NK cell operational physiology (Lupo and Matosevic 2019).

3.3 CAR-NK cells

CAR-NK cells have been gaining popularity due to the advancement of CAR-engineered NK cells for cancer immunotherapy. The tumour microenvironment (TME) is complex, and immune escape is needed for cancerous cells to advance and invade nearby tissues. According to an article, T-cell immunotherapy's limited success considers the notoriety of burgeoning numerous revolutionary immunotherapeutics, particularly NK-cell-based treatment options. Biological NK cells have become the most important innate immune specialized cells against cancerous cells, and they are extremely diverse inside this TME (Basar et al. 2020). CAR-NK cell lines outperform CAR-T cells in safety parameters (e.g., total lack or relatively limited CRS and GvHD, activation of diverse measures for strengthening cytolytic activity, and greater achievability for 'off-the-shelf' large-scale production). All those adaptive immune cells might be adjusted to detect specific antigens, maximize *in vivo* expansion and intensity, increase infiltration into tumour tissues, and subdue impervious TME, culminating in the preferred antineoplastic reaction. More pertinently, CAR-NK responses are considered antigen receptors for TAAs, rerouting immunoregulatory NK cells and assisting with tumour-related immunotherapy (Marofi et al. 2021).

Many animal model experiments have examined the effectiveness of CAR-NK cells against a wide range of targeted antigens for leukaemia and lymphoma along with solid tumours, constructed on the insights obtained with CAR-Tcells. To improve malignant cell identification, NK cells could be phenotypically manipulated to produce a CAR that acknowledges TAAs (Zhao et al. 2015; Chen

et al. 2016; Park et al. 2017). CAR-T's new treatment is steeply advancing. Meanwhile, CAR-NK cell advancement is struggling to keep up due to being generally restricted to early clinical trials (Mehta and Rezvani 2018). Even with their potential benefits, NK cells have downsides that might constrain their usefulness. These involve a limited lifetime of between a week and two in the case of lack of cytokine intervention, limited cell quantities that frequently necessitate *ex vivo* growth and stimulation, and high susceptibility to the immunosuppressive TME, which could hinder their tumour invasion and effective killing (Basar et al. 2020).

3.4 Future Directions in NK Cell Therapy

CAR-NK therapy could be improved by investigating the significance of optimizing the immunochemical characteristics of the CAR model, comprehending variables that contribute to cell product persistence, improving transporting of transferred cells to the tumour, facilitating metabolic wellness of the newly delivered product category, and finding ways for minimizing tumour escape via antigen destruction as well as trogocytosis (Kilgour et al. 2023; Raftery et al. 2023). NK cell therapy can be improved further by implementing different approaches, as shown in Figure 5. By initiating transformation using the C-X-C motif chemokine receptor (CXCR1), NK cells can be channelled to the tumour site. TME immunosuppressive influence could be thwarted at the cancer tissue by antagonists designed to target immunosuppressive features such as transforming growth factor-beta (TGF-β). Combined treatment could potentiate cytotoxic effects, such as hindering checkpoint blockers (i.e., NKG2A) or employing killer engagement proteins, such as bi/tri-specific antibodies. One further method for increasing immune cell cytotoxic activity is to modify NK cells to over-express NK receptors like NKG2D genetically or to modify NK cells with CARs that target TAAs (van Vliet et al. 2021; Laskowski et al. 2022).

4 Conclusion and Future Prospects

Immunotherapy has gained much attention nowadays due to its promising results in clinical trials. CAR-T treatment is promising; however, the adverse effects are the primary concerns and must be overcome. CAR-T immunotherapy has progressed from a novel concept at the turn of the century to an immensely successful treatment with effective therapeutic prospects in B-cell neoplasms. Then again, CAR-T immunotherapy is still in its early stages, along with its numerous obvious benefits above other kinds of anticancer medications, such as in vivo expansion and long-term intensity. The prevailing deficiencies under these therapeutic interventions are already being addressed by implementing new operating systems for CAR construction, such as CAR-NK cells. Although NK cell adoptive transfer therapy showed lesser toxicity than CAR-T, the clinical trials are still ongoing and have yet to be approved by the FDA. Elaborating on the pivotal variables that influence NK cell effectiveness and consistency would be critical as the realm moves towards emerging strategies for dealing with unique challenges on every disease evidence. Eventually, creating and incorporating optimal NK proliferation, differentiation, and cryopreservation methods will be vital to maintaining high production efficiency. More scrutiny and innovations on CAR-NK cells' origin, modification of gene transfer strategies, and manufacturing for clinical use should also be considered.

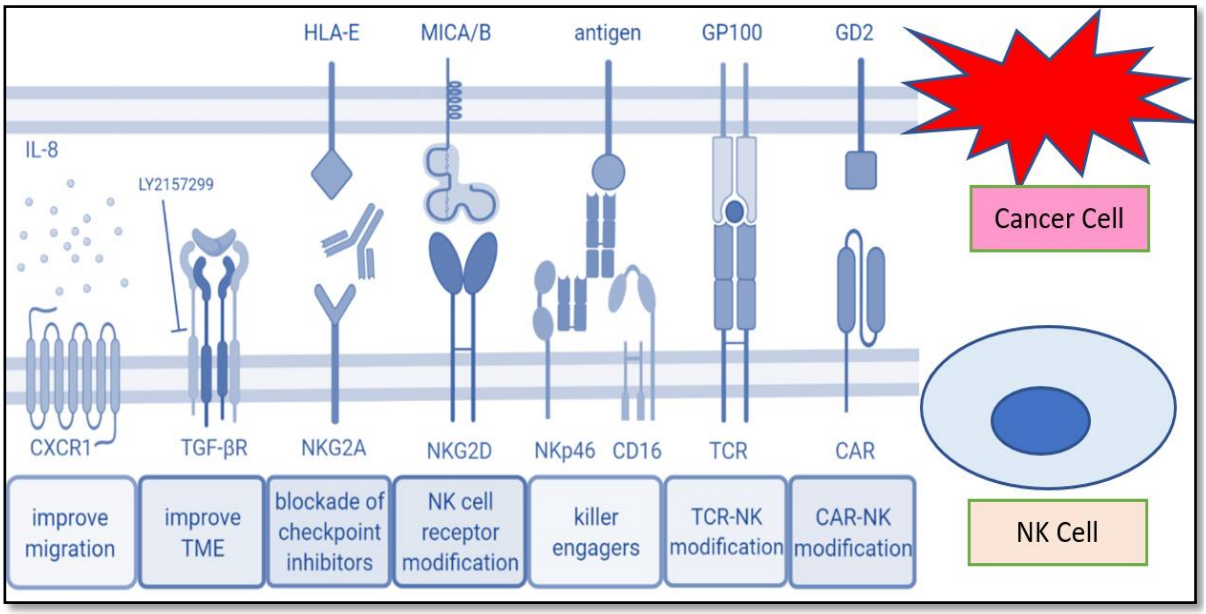

Figure 5 The schematic diagram for the strategies to improve NK cell therapy (Adapted from van Vliet et al., 2021). MICA/B =MHC class I chain-related protein A/B, GP= Glycoprotein, GD= disialoganglioside, TCR= T cell receptor, NKG2A/2D= NK cell receptor group-2A/2D

Advanced techniques, which include clustered, regularly interspaced short palindromic repeats (CRISPR) gene processing and editing, should be assessed to help combat tumour-initiated immunosuppressive strategies. When such gene-editing techniques are likely to succeed, immune-cell-mediated cancer treatment will become a standard therapy instead of a secondary choice. Operationally, for evolving NK immunotherapy implementation to be successful, interdisciplinary team systems comprised of research scientists, physicians, and regulatory bodies will be required to depict a multifaceted direction to therapeutic applications cooperatively. Combined therapy seemed promising, but more experiments are necessary to achieve the most reliable result and appropriate combination. Since tumour cells downregulate MHC-I to eschew being recognized from T lymphocytes, MHC-I negative cells are readily identified and killed by NK cells. Thus, trials comprising the alternate use of CAR-T and allogenic NK cell therapy should be considered to achieve better outcomes, especially in preventing remission and relapse of various cancers.

Conflict of Interest

All authors claim that they have no competing interests.

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References

Alabanza, L., Pegues, M., Geldres, C., Shi, V., et al. (2017). Function of novel Anti-CD19 chimeric antigen receptors with human variable regions is affected by hinge and transmembrane domains. *Molecular Ther*apeutics, *25*(11), 2452–65.

Bachanova, V., Cooley, S., Defor, T.E., Verneris, M.R., et al. (2014). Clearance of acute myeloid leukemia by haploidentical natural killer cells is improved using IL-2 diphtheria toxin fusion protein. *Blood, 123*(25), 3855-63. doi: 10.1182/blood-2013-10-532531.

Basar, R., Daher, M., & Rezvani, K. (2020). Next-generation cell therapies: the emerging role of CAR-NK cells. *Blood Advances*, *4*(22), 5868–76.

Becknell, B., & Caligiuri, M.A. (2005). Interleukin-2, interleukin-15, and their roles in human natural killer cells. *Advance inImmunol*ogy, *86*, 209–39.

Bouchkouj, N., Kasamon, Y.L., de Claro, R.A., George, B., et al. (2019). FDA Approval Summary: Axicabtagene Ciloleucel for Relapsed or Refractory Large B-cell Lymphoma. *Clinical Cancer Research*, *25*(6),1702-1708. doi: 10.1158/1078-0432.CCR-18-2743.

Brudno, J.N., & Kochenderfer, J.N. (2016). Toxicities of chimeric antigen receptor T cells: recognition and management. *Blood, 127*(26), 3321–30.

Cerrano, M., Ruella, M., Perales, M. A., Vitale, C., Faraci, D. G., et al. (2020). The Advent of CAR T-Cell Therapy for Lymphoproliferative Neoplasms: Integrating Research Into Clinical Practice. *Frontiers in immunology*, *11*, 888. https://doi.org/10.3389/fimmu.2020.00888

Chen, K.H., Wada, M., Firor, A.E., Pinz, K.G., et al. (2016). Novel anti-CD3 chimeric antigen receptor targeting of aggressive T cell malignancies. *Oncotarget*, *7*(35), 56219–32.

Cherkassky, L., Morello, A., Villena-Vargas, J., Feng, Y., et al. (2016). Human CAR T cells with cell-intrinsic PD-1 checkpoint blockade resist tumor-mediated inhibition. *Journal of Clinical Investigation,126*(8), 3130-44. doi: 10.1172/JCI83092.

Chhabra, N., & Kennedy, J. (2021). A Review of Cancer Immunotherapy Toxicity II: Adoptive cellular therapies, kinase inhibitors, monoclonal antibodies, and oncolytic viruses. *Journal of Medical Toxicology*, *18*(1), 43-55. doi: 10.1007/s13181-021-00835-6

Davis, Z.B., Felices, M., Verneris, M.R., & Miller, J.S. (2015). Natural Killer Cell Adoptive Transfer Therapy. *The Cancer Journal*, *21*(6), 486–91.

Dholaria, B.R., Bachmeier, C.A., & Locke, F. (2018). Mechanisms and Management of Chimeric Antigen Receptor T-Cell Therapy-Related Toxicities. *BioDrugs*, *33*(1), 45–60.

Dholaria, B.R., Bachmeier, C.A., & Locke, F. (2019). Mechanisms and Management of Chimeric Antigen Receptor T-Cell Therapy-Related Toxicities. *BioDrugs*, *33*(1), 45-60. doi: 10.1007/s40259-018-0324-z.

Du, H., Hirabayashi, K., Ahn, S., Kren, N.P., et al. (2019). Antitumor Responses in the Absence of Toxicity in Solid Tumors by Targeting B7-H3 via Chimeric Antigen Receptor T Cells. *Cancer Cell*, 11, 35(2), 221-237.e8. doi: 10.1016/j.ccell.2019.01.002.

Fischer, J. W., & Bhattarai, N. (2021). CAR-T Cell Therapy: Mechanism, Management, and Mitigation of Inflammatory Toxicities. *Frontiers in immunology*, *12*, 693016. https://doi.org/10.3389/fimmu.2021.693016

Franks, S. E., Wolfson, B., & Hodge, J. W. (2020). Natural Born Killers: NK Cells in Cancer Therapy. *Cancers*, *12*(8), 2131. https://doi.org/10.3390/cancers12082131

Fried, S., Avigdor, A., Bielorai, B., Meir, A., et al.(2019). Early and late hematologic toxicity following CD19 CAR-T cells. *Bone Marrow Transplant*, *54*(10), 1643-1650. doi: 10.1038/s41409-019-0487-3.

Gardner, R. A., Ceppi, F., Rivers, J., Annesley, C., Summers, C., et al. (2019). Preemptive mitigation of CD19 CAR T-cell cytokine release syndrome without attenuation of antileukemic efficacy. *Blood*, *134*(24), 2149–2158. https://doi.org/10.1182/blood.2019001463.

Geller, M.A., & Miller, J.S. (2011). Use of allogeneic NK cells for cancer immunotherapy. *Immunotherapy*, *3*(12), 1445–59.

Giavridis, T., van der Stegen, S.J.C., Eyquem, J., Hamieh, M., et al. (2018). CAR T cell-induced cytokine release syndrome is mediated by macrophages and abated by IL-1 blockade. *Nature Medicine*, *24*(6), 731-738. doi: 10.1038/s41591-018-0041-7.

Gleichmann, N. (2020). Innate Vs Adaptive Immunity. From Technology Networks. Retrieved from: https://www.technologynetworks.com/immunology/articles/innate-vs-adaptive-immunity-335116

Glienke, W., Esser, R., Priesner, C., Suerth, J. D., Schambach, A., et al. (2015). Advantages and applications of CAR-expressing natural killer cells. *Frontiers in pharmacology*, *6*, 21. https://doi.org/10.3389/fphar.2015.00021

Grigore, A. (2017). Plant Phenolic Compounds as Immunomodulatory Agents. In M., Soto-Hernandez, M., Palma-Tenango, & M. R., Garcia-Mateos (Eds.) *Phenolic Compounds - Biological Activity*. IntechOpen. http://dx.doi.org/10.5772/66112.

Guedan, S., Posey, A.D. Jr., Shaw, C., Wing, A., et al. (2018). Enhancing CAR T cell persistence through ICOS and 4-1BB costimulation. *JCI Insight*, *3*(1), e96976. doi: 10.1172/jci.insight.96976.

Gust, J., Hay, K.A., Hanafi, L.A., Li, D., et al.(2017). Endothelial Activation and Blood-Brain Barrier Disruption in Neurotoxicity after Adoptive Immunotherapy with CD19 CAR-T Cells. *Cancer Discovery*,*7*(12), 1404-1419. doi: 10.1158/2159-8290.

Hay, K. A., Hanafi, L. A., Li, D., Gust, J., Liles, W. C., Wurfel, M. M., et al. (2017). Kinetics and biomarkers of severe cytokine release syndrome after CD19 chimeric antigen receptor-modified T-cell therapy. *Blood*, *130*(21), 2295–2306. https://doi.org/10.1182/blood-2017-06-793141.

Hirayama, A.V., Gauthier, J., Hay, K.A., Sheih, A., et al. (2018). Efficacy and Toxicity of JCAR014 in Combination with Durvalumab for the Treatment of Patients with Relapsed/Refractory Aggressive B-Cell Non-Hodgkin Lymphoma. *Blood*,*132*(Supplement 1), 1680–0.

Hu, Y., Feng, J., Gu, T., Wang, L., et al. (2022). CAR T-cell therapies in China: rapid evolution and a bright future. *The Lancet Haematology*, *9*(12), e930–e941. https://doi.org/10.1016/S2352-3026(22)00291-5

Jacobson, C.A., Locke, F.L., Miklos, D.B., Zheng, L., et al. (2018). End of phase 1 results from Zuma-6: Axicabtagene Ciloleucel (Axi-Cel) in combination with atezolizumab for the treatment of patients with refractory diffuse large B Cell Lymphoma. *Blood*, *132*(Supplement 1), 4192–2.

Jiang, H., Zhang, W., Shang, P., Zhang, H., Fu, W., et al. (2014). Transfection of chimeric anti-CD138 gene enhances natural killer cell activation and killing of multiple myeloma cells. *Molecular oncology*, *8*(2), 297–310. https://doi.org/10.1016/j.molonc.2013.12.001

Jiang, T., Zhou, C., & Ren, S. (2016). Role of IL-2 in cancer immunotherapy. *Oncoimmunology*, *5*(6), e1163462. https://doi.org/10.1080/2162402X.2016.1163462.

Karre, K. (2002). NK Cells, MHC Class I Molecules and the Missing Self. *Scandinavian Journal of Immunology*, *55*(3), 221–8.

Karschnia, P., Jordan, J. T., Forst, D. A., Arrillaga-Romany, I. C., Batchelor, T. T., et al. (2019). Clinical presentation, management, and biomarkers of neurotoxicity after adoptive immunotherapy with CAR T cells. *Blood*, *133*(20), 2212–2221. https://doi.org/10.1182/blood-2018-12-893396.

Kilgour, M. K., Bastin, D. J., Lee, S. H., Ardolino, M., McComb, S., & Visram, A. (2023). Advancements in CAR-NK therapy: lessons to be learned from CAR-T therapy. *Frontiers in immunology*, *14*, 1166038. https://doi.org/10.3389/fimmu.2023.1166038.

Klingemann, H. (2014). Are natural killer cells superior CAR drivers?. *Oncoimmunology*, *3*, e28147. https://doi.org/10.4161/onci.28147

Laskowski, T. J., Biederstädt, A., & Rezvani, K. (2022). Natural killer cells in antitumour adoptive cell immunotherapy. *Nature reviews. Cancer*, *22*(10), 557–575. https://doi.org/10.1038/s41568-022-00491-0

Lawler, S. E., Speranza, M. C., Cho, C. F., & Chiocca, E. A. (2017). Oncolytic Viruses in Cancer Treatment: A Review. *JAMA oncology*, *3*(6), 841–849. https://doi.org/10.1001/jamaoncol.2016.2064

Lee, D.W., Santomasso, B.D., Locke, F.L., Ghobadi, A., et al. (2019). ASTCT Consensus Grading for Cytokine Release Syndrome and Neurologic Toxicity Associated with Immune Effector Cells. *Biology of Blood Marrow Transplant,25*(4), 625-638. doi: 10.1016/j.bbmt.2018.12.758

Leyfman, Y.(2018). Chimeric antigen receptors: unleashing a new age of anticancer therapy. *Cancer Cell International*, *18*, 182. doi: 10.1186/s12935-018-0685-x.

Liu, E., Tong, Y., Dotti, G., Shaim, H., et al. (2018). Cord blood NK cells engineered to express IL-15 and a CD19-targeted CAR show long-term persistence and potent antitumor activity. *Leukemia*, *32*(2), 520-531. doi: 10.1038/leu.2017.226.

Liu, S., Deng, B., Yin, Z., Pan, J., et al. (2020). Corticosteroids do not influence the efficacy and kinetics of CAR-T cells for B-cell acute lymphoblastic leukemia. *Blood Cancer Journal,10*(2), 15. doi: 10.1038/s41408-020-0280-y.

Locke, F.L., Ghobadi, A., Jacobson, C.A., Miklos, D.B., et al. (2019). Long-term safety and activity of axicabtagene ciloleucel in refractory large B-cell lymphoma (ZUMA-1): a single-arm, multicentre, phase 1-2 trial. *Lancet Oncology*, *20*(1), 31-42. doi: 10.1016/S1470-2045(18)30864-7

Lupo, K.B., & Matosevic, S.(2019). Natural Killer Cells as Allogeneic Effectors in Adoptive Cancer Immunotherapy. *Cancers*, *11*(6), 769.

Marofi, F., Al-Awad, A.S., Sulaiman, H.R., Markov, A., et al. (2021). CAR-NK Cell: A New Paradigm in Tumor Immunotherapy. *Frontiers in Oncology,11,* 673276. doi: 10.3389/fonc.2021.673276.

Martino, M., Alati, C., Canale, F. A., Musuraca, G., Martinelli, G., & Cerchione, C. (2021). A Review of Clinical Outcomes of CAR T-Cell Therapies for B-Acute Lymphoblastic Leukemia. *International journal of molecular sciences*, *22*(4), 2150. https://doi.org/10.3390/ijms22042150.

Maude, S. L., Laetsch, T. W., Buechner, J., Rives, S., Boyer, M., et al. (2018). Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia. *The New England journal of medicine*, *378*(5), 439–448. https://doi.org/10.1056/NEJMoa1709866.

Mehta, R. S., & Rezvani, K. (2018). Chimeric Antigen Receptor Expressing Natural Killer Cells for the Immunotherapy of Cancer. *Frontiers in immunology*, *9*, 283. https://doi.org/10.3389/fimmu.2018.00283.

Moon, E. K., Ranganathan, R., Eruslanov, E., Kim, S., Newick, K., et al. (2016). Blockade of Programmed Death 1 Augments the Ability of Human T Cells Engineered to Target NY-ESO-1 to Control Tumor Growth after Adoptive Transfer. *Clinical cancer research : an official journal of the American Association for Cancer Research*, *22*(2), 436–447. https://doi.org/10.1158/1078-0432.CCR-15-1070.

Morgan, R.A., Yang, J.C., Kitano, M., Dudley, M.E., Laurencot, C.M., & Rosenberg, S.A.(2010). Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. *Molecular Therapeutics,18*(4), 843–51.

Mostafa, A. A., & Morris, D. G. (2014). Immunotherapy for Lung Cancer: Has it Finally Arrived?. *Frontiers in oncology*, *4*, 288. https://doi.org/10.3389/fonc.2014.00288

Neelapu, S.S., Locke, F.L., Bartlett, N.L., Lekakis, L.J., et al. (2017). Axicabtagene Ciloleucel CAR T-Cell Therapy in Refractory Large B-Cell Lymphoma. *New England Journal Medicine*, *377*(26), 2531-2544. doi: 10.1056/NEJMoa1707447

Neelapu, S.S. (2019). Managing the toxicities of CAR T-cell therapy. *Haematological Oncology*, 37(S1):48–52.

Norelli, M., Camisa, B., Barbiera, G., Falcone, L., et al. (2018). Monocyte-derived IL-1 and IL-6 are differentially required for cytokine-release syndrome and Neurotoxicity due to CAR T cells. *Nature Medicine*, *24*(6), 739-748. doi: 10.1038/s41591-018-0036-4.

Obstfeld, A.E., Frey, N.V., Mansfield, K., Lacey, S.F., et al .(2017). Cytokine release syndrome associated with chimeric-antigen receptor T-cell therapy: clinicopathological insights. *Blood*, *130*(23), 2569-2572. doi: 10.1182/blood-2017-08-802413.

Park, H., Awasthi, A., Ayello, J., Chu, Y., et al.(2017). ROR1-Specific chimeric antigen receptor (CAR) NK cell immunotherapy for high-risk neuroblastomas and sarcomas. *Biology of Blood and Marrow Transplant*, *23*(3), S136–7.

Parkhurst, M.R., Riley, J.P., Dudley, M.E., & Rosenberg, S.A. (2011). Adoptive Transfer of Autologous Natural Killer Cells Leads to High Levels of Circulating Natural Killer Cells but Does Not Mediate Tumor Regression. *Clinical Cancer Research*, *17*(19), 6287–97.

Quintarelli, C., Sivori, S., Caruso, S., Carlomagno, S., et al. (2019). Efficacy of third-party chimeric antigen receptor modified peripheral blood natural killer cells for adoptive cell therapy of B-cell precursor acute lymphoblastic leukemia. *Leukemia*, *34*(4), 1102-1115. doi: 10.1038/s41375-019-0613-7.

Rafiq, S., Hackett, C.S., & Brentjens, R.J. (2020). Engineering strategies to overcome the current roadblocks in CAR T cell therapy. *Nature Review Clinical Oncology*, *17*(3), 147–67.

Raftery, M. J., Franzén, A. S., & Pecher, G. (2023). CAR NK Cells: The Future is Now. *Annual Reviews of Cancer Biology*, *7*(1). https://doi.org/10.1146/annurev-cancerbio-061521-082320

Rey, J., Veuillen, C., Vey, N., Bouabdallah, R., & Olive, D. (2009). Natural killer and γδ-T cells in haematological malignancies: enhancing the immune effectors. *Trends in Molecular Medicine*, *15*(6), 275–84.

Robert C. (2020). A decade of immune-checkpoint inhibitors in cancer therapy. *Nature communications*, *11*(1), 3801. https://doi.org/10.1038/s41467-020-17670-y

Romanski, A., Uherek, C., Bug, G., Seifried, E., et al. (2016). CD19-CAR engineered NK-92 cells are sufficient to overcome NK cell resistance in B-cell malignancies. *Journal of Cellular and Molecular Medicine*, *20*(7), 1287-94. doi: 10.1111/jcmm.12810.

Santomasso, B.D., Park, J.H., Salloum, D., Riviere, I., et al. (2018). Clinical and Biological Correlates of Neurotoxicity Associated with CAR T-cell Therapy in Patients with B-cell Acute Lymphoblastic Leukemia. *Cancer Discovery*, *8*(8), 958-971. doi: 10.1158/2159-8290.CD-17-1319.

Saxena, M., van der Burg, S.H., Melief, C.J.M.,& Bhardwaj, N. (2021). Therapeutic cancer vaccines., *Nature Review Cancer*, *21*(6), 360–78.

Schuster, S.J., Bishop, M.R., Tam, C.S., Waller, E.K., et al. (2019). Tisagenlecleucel in Adult Relapsed or Refractory Diffuse Large B-Cell Lymphoma. *New England Journal Medicine*, *380*(1), 45-56. doi: 10.1056/NEJMoa1804980.

Shah, N., Martin-Antonio, B., Yang, H., Ku, S., et al. (2013). Antigen presenting cell-mediated expansion of human umbilical cord blood yields log-scale expansion of natural killer cells with anti-myeloma activity. *PLoS One*, *8*(10), e76781. doi: 10.1371/journal.pone.0076781.

Shimabukuro-Vornhagen, A., Gödel, P., Subklewe, M., Stemmler, H.J., et al. (2018). Cytokine release syndrome. *Journal of Immunotherapy and Cancer*, *6*(1), 56. doi: 10.1186/s40425-018-0343-9.

Siddiqi, T., Abramson, J.S., Lee, H.J., Schuster, S., Hasskarl J.,et al. (2019). Safety of lisocabtagene maraleucel given with durvalumab in patients with relapsed/refractory aggressive b-cell non-Hodgkin lymphoma: first results from the platform study. *Haematological Oncology*, *37*, 171–2.

Simon, B., & Uslu,U. (2018). CAR-T cell therapy in melanoma: A future success story? *Experimental Dermatology*, *27*(12), 1315–21.

Simonetta, F., Alvarez, M., & Negrin, R. S. (2017). Natural Killer Cells in Graft-versus-Host-Disease after Allogeneic Hematopoietic Cell Transplantation. *Frontiers in immunology*, *8*, 465. https://doi.org/10.3389/fimmu.2017.00465

Smyth, M.J., Cretney, E., Kershaw, M.H.,& Hayakawa, Y. (2004). Cytokines in cancer immunity and immunotherapy. *Immunological Reviews*, *202*, 275–93.

Sterner, R.C., & Sterner, R.M. (2021). CAR-T cell therapy: current limitations and potential strategies. *Blood Cancer Journal*,*11*(4), 1–11.

Sterner, R.M., Sakemura, R., Cox, M.J., Yang, N., et al. (2019). GM-CSF inhibition reduces cytokine release syndrome and neuroinflammation but enhances CAR-T cell function in xenografts. *Blood*, *133*(7), 697-709. doi: 10.1182/blood-2018-10-881722.

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 Cancer Journal of Clinician*, *71*(3), 209-249. doi: 10.3322/caac.21660

Tang, X., Yang, L., Li, Z., Nalin, A. P., Dai, H., et al. (2018). First-in-man clinical trial of CAR NK-92 cells: safety test of CD33-CAR NK-92 cells in patients with relapsed and refractory acute myeloid leukemia. *American journal of cancer research*, *8*(6), 1083–1089.

Taraseviciute, A., Tkachev, V., Ponce, R., Turtle, C.J., et al. (2018). Chimeric Antigen Receptor T Cell-Mediated Neurotoxicity in Nonhuman Primates. *Cancer Discovery*, *8*(6), 750-763. doi: 10.1158/2159-8290.

Tariq, S.M., Haider, S.A., Hasan, M., Tahir, A., Khan, M., Rehan, A., & Kamal, A. (2018). Chimeric Antigen Receptor T-Cell Therapy: A Beacon of Hope in the Fight Against Cancer. *Cureus,10*(10), e3486. doi: 10.7759/cureus.3486.

Tomasik, J., Jasiński, M., & Basak, G. W. (2022). Next generations of CAR-T cells - new therapeutic opportunities in hematology? Frontiers in Immunology, *13*, 1034707. https://doi.org/10.3389/fimmu.2022.1034707

van Vliet, A.A., Georgoudaki, A-M., Raimo, M., de Gruijl, T.D., & Spanholtz, J. (2021). Adoptive NK cell therapy: A promising treatment prospect for metastatic melanoma. *Cancers*, *13*(18), 4722.

Veluchamy, J. P., Kok, N., van der Vliet, H. J., Verheul, H. M. W., de Gruijl, T. D., & Spanholtz, J. (2017). The Rise of Allogeneic

Natural Killer Cells As a Platform for Cancer Immunotherapy: Recent Innovations and Future Developments. *Frontiers in immunology*, *8*, 631. https://doi.org/10.3389/fimmu.2017.00631

Verneris M. R. (2013). Natural killer cells and regulatory T cells: how to manipulate a graft for optimal GVL. *Hematology. American Society of Hematology. Education Program*, *2013*, 335–341. https://doi.org/10.1182/asheducation-2013.1.335

World Health Organization (2020). Palliative Care. Available from: https://www.who.int/news-room/fact-sheets/detail/palliative-care

World Health Organization (2022). Cancer. Available from: https://www.who.int/news-room/fact-sheets/detail/cancer

Zahavi, D., & Weiner, L. (2020). Monoclonal antibodies in cancer therapy. *Antibodies*, *9*(3), 34.

Zhang, G., Wang, L., Cui, H., Wang, X., Zhang, G., et al. (2014). Anti-melanoma activity of T cells redirected with a TCR-like chimeric antigen receptor. *Scientific reports*, *4*, 3571. https://doi.org/10.1038/srep03571

Zhang, Q., Ping, J., Huang, Z., Zhang, X., Zhou, J., Wang, G., Liu, S., & Ma, J. (2020). CAR-T Cell Therapy in Cancer: Tribulations and Road Ahead. *Journal of immunology research*, *2020*, 1924379. https://doi.org/10.1155/2020/1924379

Zhang, T., Jou, T.H., Hsin, J., Wang, Z., et al. (2023). Talimogene Laherparepvec (T-VEC): A Review of the Recent Advances in Cancer Therapy. *Journal of Clinical Medicine,12*(3):1098. doi: 10.3390/jcm12031098.

Zhao, Q., Ahmed, M., Tassev, D.V., Hasan, A., et al. (2015). Affinity maturation of T-cell receptor-like antibodies for Wilms tumour 1 peptide greatly enhances therapeutic potential. *Leukaemia*, *29*(11), 2238–47.