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Emerging Trends in CAR Therapy for the Treatment of Cancer: A Literature Review

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Abstract
Cancer is the second leading cause of mortality worldwide, accounting for 1 in every 6 deaths over the years of 2020 and 2021. Immunotherapy has emerged as a pivotal treatment for cancer in recent decades. It focuses on engineering the body's immune system to target and attack cancer cells. Chimeric antigen receptor (CAR) T-cell therapy is a type of immunotherapy that utilizes genetically modified T lymphocytes to produce a specific CAR, enabling them to detect and destroy a patient's cancer cells possessing the cognate antigen. This therapy has shown to be effective against hematological cancers, but faces challenges in infiltrating solid tumors, toxicity due to excessive cytokine release, and other complications. To address these issues, various immune cells are being investigated for use in CAR therapy against cancer, including macrophages, natural killer cells, dendritic cells, and neutrophils. This literature review reports on new CAR therapies employing different immune cells in the treatment of blood and solid cancers. Clinical studies on CAR immunotherapies for cancer treatment published within the past 5 years (2019-2024) were chosen following multiple advanced searches of the PubMed database. Due to the limited number of clinical trials investigating treatments besides CAR T-cell therapy, the search was expanded to include pre-clinical studies and one ongoing clinical trial. This study's findings revealed that each of the five CAR immune cells examined (T-cells, natural killer cells, macrophages, dendritic cells, and neutrophils) demonstrated significant efficacy in inhibiting cancer growth, with unique benefits and weaknesses depending on cancer type, methods of preparation, additional treatment regimens, and side effects. T-cell and macrophage CAR therapies examined in clinical trials showed similar incidences of adverse events, while CAR natural killer cell therapy demonstrated fewer instances of cytokine release syndrome and neurotoxicity. CAR dendritic cells and neutrophils showed promise as specific carriers of cancer-killing elements, such as chemotherapy drugs or tumoricidal cytokines. Overall, this review underscores the need for further clinical study of CAR natural killer cells and the potential expansion of CAR technologies as tumoricidal instigators and/or targeted carriers of such therapies.

Keywords: cancer; chimeric antigen receptor; immunotherapy; t-cell; macrophage; natural killer cell; dendritic cell; neutrophil

Introduction

Cancer, defined as a disease in which the body's cells proliferate abnormally and uncontrollably, is the second leading cause of death worldwide [1]. Approximately 1 in 5 people will receive a cancer diagnosis in their lifetime [2]. The global cancer burden has sharply grown in recent decades, with a projected 35 million incident cancer cases to be seen worldwide in 2050 [3]. As such, the development of effective treatments for cancer has become increasingly urgent.

Immunotherapy is the modification of one's own immune system to better target and attack select invaders [4]. Key technologies in this field include immune checkpoint inhibitors, monoclonal antibodies, and cancer vaccines [4]. These methods have helped the immune system better detect cancer cells that have downregulated their transmembrane proteins targetable by the immune system [4].

What is CAR Therapy?

The chimeric antigen receptor (CAR) is a genetically engineered molecule that is constructed to have an antigen recognition domain, specific to a chosen protein [5]. The CAR transmembrane domain embeds the CAR within the cell membrane [5]. Finally, the CAR intracellular domain functions to kickstart signal transduction following the binding of an antigen to the receptor, activating the immune cell's response [5]. In its use as immunotherapy against cancer, a CAR can be designed to selectively bind a protein on the surface of a patient's cancer cells [5]. These tumor markers can be measured via blood test, biopsy, or urinalysis, allowing providers to identify specific types and stages of cancer present [6]. For example, human epidermal growth factor receptor 2 (HER2) is a tumor marker commonly found in a variety of solid cancers [7]. Engineered HER2-specific CAR immune cells will be able to detect cancer cells

expressing HER2 and activate an immune response against them.

There are several methods to generate a CAR-T cell, which differ in the methods by which they are extracted from the body and engineered. To decrease the risk of rejection following injection of these modified immune cells into the human body, a patient's own immune cells can be used, known as autologous T cell therapy [8]. Providers will draw a patient's blood and run it through an apheresis machine, centrifuging it into four separate layers: plasma, leukocytes, platelets, and erythrocytes [9]. Scientists can then enrich for T cells in the peripheral blood mononuclear cells, transduce CAR constructs that target tumor antigens using viral or non-viral methods, expand these engineered CAR T cells, and finally infuse these engineered cells back into the patient's circulation [8]. However, a limitation of the autologous approach is that patients have likely received rounds of lymphodepleting (LD) chemotherapy, reducing blood cell count and subsequent CAR product yield [8].

To overcome this, allogeneic CAR therapy serves as an alternative. This treatment utilizes immune cells taken from a donor [8]. One risk is that the recipient's immune system may detect foreign proteins on the cell's surface and trigger a host immune response, known as graft vs. host disease (GVHD) [8]. This would attack the recently infused CAR cells and prevent them from reaching their cancer cell targets. Human leukocyte antigen (HLA) matching can address this problem. HLA is a protein that normal body tissues express to separate the body's own cells from foreign pathogens, and partially matching donor HLA to a patient's genotype can decrease chances of GVHD [10].

Incorporating CARs into a patient's own hematopoietic stem cells (HSCs), the precursors to all types of blood cells, is another promising method of CAR immunotherapy initiation. HSCs can be collected via apheresis, bone marrow extraction, or from umbilical cord blood [11]. Immune cells derived from these engineered stem cells will continually express CARs specific to the patient's cancer [12]. This will yield a sustained source of immunotherapy against tumor growth, decreasing one's chances of remission [12].

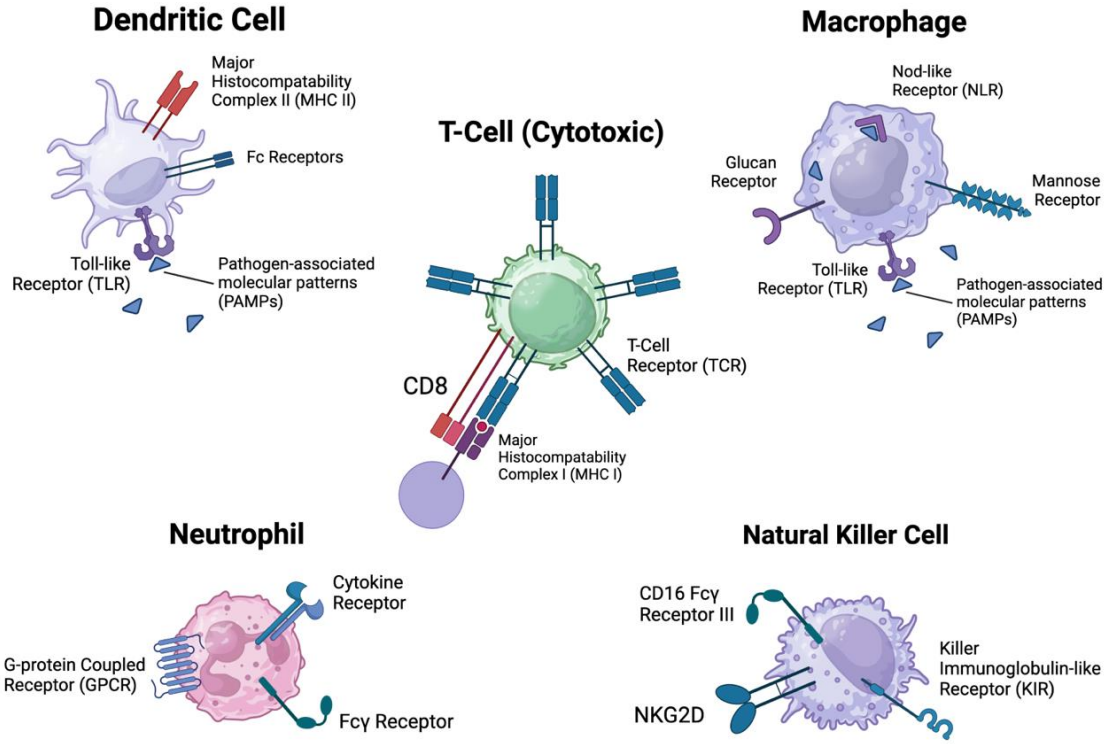

Figure 1. Key Receptors Expressed Within Each of the Five Immune Cells to Be Discussed Through the Lens of CAR Immunotherapy. Created With Biorender.com [36].

Types of CAR Immunotherapies

The first immune cell used for CAR treatment of cancer was the T-cell - more specifically, cytotoxic cluster of differentiation (CD) 8 T-cells, which are capable of binding to and recognizing their cognate antigen presented by the major histocompatibility complex (MHC) I and releasing cytotoxic granules to trigger apoptosis of a foreign cell [13].

Initial trials of CAR-T therapy in murine models were promising, slowing solid tumor growth and promoting anticancer activity [14]. However, this therapy was far less

effective when tested in humans with solid cancers [14]. T-cells function to circulate in the bloodstream and lymph tissue, explaining their weakened ability to infiltrate solid tumors through the vascular endothelium [15]. In contrast, CAR-T therapy showed significant anti-cancer effects in clinical trials against blood cancers, such as advanced chronic lymphocytic leukemia [16].

To address the weaknesses of CAR-T therapy, including its limited efficacy against solid tumors and associated toxicities, cancer immunologists have been working to develop CAR therapies utilizing other immune cells (Figure 1). Innate immune cells are the body's first responders to foreign invaders and are equipped with receptors that facilitate migration to and survival within tissues [17]. For instance, upon recognizing pathogen-associated molecular patterns (PAMPs) on the surface of microbes, macrophages undergo a process known as phagocytosis to absorb and digest foreign substances while secreting cytokines [17]. Neutrophils carry out a similar function [17]. Dendritic cells (DCs) are antigen presenting cells (APCs) that work to take up antigens and display them on their cell surface for recognition by T-cells [17]. Finally, natural killer (NK) cells do not need prior receptor-mediated sensitization to an antigen, allowing them to kill any bodily cells that are diseased or infected [17].

Objective of the Study

This comprehensive literature review aims to evaluate the impact each CAR immunotherapy utilizing these cells has on cancer progression and quality of life (QoL), specifically in the context of solid versus hematologic cancers. Comparison of each therapy's adverse events (AEs), treatment regimens, and measurements of drug efficacy will draw new insights into the potentials of expanding the immune cell repertoire of CAR cancer therapy.

Methods

Each trial selected for review was found via multiple advanced searches of the PubMed database. Specific keywords included were each CAR immunotherapy's abbreviated name (e.g. "CAR-M") and full name (e.g. "Chimeric antigen receptor Macrophage") as well as "cancer". A publication date filter was applied so that each search result was published within the past 5 years (2019-2024), ensuring the inclusion of the most up-to-date knowledge and treatments in the rapidly evolving field of CAR immunotherapy. The initial search included an article type filter for clinical trials of any phase. However, due to the limited number of clinical trials investigating CAR immunotherapies besides CAR T-cell therapy, the search was expanded to include pre-clinical studies using animal models. Additionally, updates from an ongoing clinical trial of CAR-M therapy were incorporated into the review.

Results

The selected clinical studies evaluated the efficacy of CAR immunotherapy against many types of solid and hematologic cancers expressing a variety of tumor markers. These trials were in phase 1 or 2 (Table 1). Selected preclinical studies investigated CAR immunotherapies lacking complete clinical trials, namely CAR-M, CAR-Neutrophil, and CAR-DC, and their use in the treatment of solid cancers (Table 2).

Table 1. An Overview of the Clinical Studies Examined

Study Type	Clinical Trials							
Author and Year	**Phase**	**Participants**	**Type of Cancer**	**Type of Immuno therapy**	**Additional Treatment Regimen**	**Method of Administration**	**Measurement of Drug Efficacy**	**Side Effects**
Liu, et al. 2020 [20]	Phase 1 & 2	11 patients, male and female, ages 47-70. Each participant had failed between 3-11 previous lines of therapy for cancer.	Non-Hodgkin's lymphoma and chronic lymphocytic leukemia. Relapsed or refractory, CD19+.	CAR-Natural Killer Cell Therapy	Delivered 3 days of daily lymphodepleting chemotherapy with fludarabine and cyclophosphamide prior to CAR-NK Therapy.	One infusion of CAR-NK cells. Around 10^5 – 10^7 cells/kg used. Post-remission therapy 30 days after infusion was permitted. Median follow-up occurred at 13.8 months.	73% of patients had an objective response (OR) and 64% had a complete response (CR) via fluorodeoxyglucose (FDG) uptake on positron-emission tomography–computed tomography (PET-CT). Measured for B-cell aplasia,	No neurotoxicity, cytokine release syndrome (CRS), or GVHD despite HLA mismatch with two patients. Hematologic toxicity observed in every patient, likely associated with lymphodepleting chemotherapy.

Study Type	Clinical Trials							
Author and Year	**Phase**	**Participants**	**Type of Cancer**	**Type of Immuno therapy**	**Additional Treatment Regimen**	**Method of Administration**	**Measurement of Drug Efficacy**	**Side Effects**
							serum cytokines, and AEs.	
Riess, et al. 2022 [28]	Phase 1 (in progress)	9 patients in group 1. 6 female, 3 male. Median age of 58. Each participant had received between 2-11 prior lines of therapy for cancer.	Locally advanced or metastatic HER2+ solid tumors. Group 1 cancers included breast, esophageal, cholangiocarcinoma, ovarian, and parotid gland cancers.	CAR-Macrophage Therapy	No previous chemotherapy administered. Treatment with filgrastim stimulated hematopoietic stem cells out of the bone marrow and into the bloodstream for monocyte collection by apheresis.	Group 1 received partial doses of CAR-M infusion on treatment days 1, 3, and 5.	Pre- and post-treatment blood samples and solid tumor biopsies taken. Four of seven participants evaluated had stable disease without further tumor advancement. Observed changes in tumor microenvironment (TME) and T-cell activity against tumors.	Grade 1-2 CRS and/or infusion reaction observed in 7 participants. No grade 3-4 CRS or neurotoxicity. All 5 recorded severe adverse events (SAEs) that were related to treatment were due to hospitalization for these grade 2 CRS or infusion reactions.
Xiao, et al. 2019 [17]	Phase 1	3 patients, male and female, aged 48-51. Participants failed at least two lines of systemic therapy for cancer.	Metastatic colorectal cancer confirmed via pathological examinations and adequate radiological imaging.	CAR-Natural Killer Cell Therapy	No previous chemotherapy or treatment administered.	CAR-NK Therapy was injected directly into the peritoneal cavity. Patients 1 & 2 received 2-6 injections of escalating amounts of CAR-NK cells, ranging from $2x10^7$ to $7x10^8$ cells. Patient 3 had ultrasound-directed percutaneous injection of CAR-NK alongside intraperitoneal (IP) infusion.	Patients 1 & 2 had a significant reduction of epithelial cell adhesion molecule (EpCAM)-positive cancer cells in abdominal fluid. Stable disease detected in peritoneal target lesions by computed tomography (CT) scan. Patient 3 PET-CT imaging revealed decreased FDG uptake in liver metastases following treatment. Fewer adenocarcinoma cells	Most common side effects were fever, fatigue, and anorexia. One case of grade 1 CRS reported. No neurologic symptoms, SAEs, or GVHD in patients treated with haploidentical (partially HLA matched) CAR-NK cells.

Study Type	Clinical Trials							
Author and Year	Phase	Participants	Type of Cancer	Type of Immuno therapy	Additional Treatment Regimen	Method of Administration	Measurement of Drug Efficacy	Side Effects
							visible in CAR-NK injected intestinal site.	
Adusumilli, et al. 2021 [18]	Phase 1	27 patients, male and female, aged 53-77. All patients received more than one line of therapy for cancer.	Histologically proven malignant pleural diseases. 25 participants with malignant pleural mesothelioma (MPM), 1 with metastatic lung cancer, and 1 with metastatic breast cancer.	CAR-T Cell Therapy	At least 3 doses of pembrolizumab, an anti-programmed cell death immune checkpoint inhibitor. Cyclophosphamide preconditioning only administered in patients 4-27.	Single dose of mesothelin-targeted CAR-T cells administered IP, either through pleural catheter or CT/ultrasound-guided imaging. 4 patients received a second infusion at time of disease progression, 7-24 months after first dose.	22 patients with MPM had a significant decline in serum-soluble mesothelin-related protein (SMRP), which is a biomarker of MPM. Of patients receiving CAR-T Therapy with pembrolizumab, 12.5% had partial response, 56.3% had stable disease, and 31.3% had progressive disease, measured by CT.	No CRS or neurotoxicity over grade 2, no on-target off-tumor toxicity. 26% of participants experienced neurotoxicity and grade 1-2 CRS. Grade 3 SAEs included constipation, dysphagia, dyspnea, and febrile neutropenia. Grade 4 SAEs were associated with cyclophosphamide lymphodepleting chemotherapy.
Hu, et al. 2022 [21]	Phase 1	12 patients, male and female, aged 8-66. Median age was 34. All patients received between 2-7 lines of therapy for cancer.	Measurable T-cell leukemia/lymphoma and acute myeloid leukemia, CD7+.	CAR-T Cell Therapy	Delivered 5 days of daily lymphodepleting chemotherapy with fludarabine, cyclophosphamide, and etoposide.	Single infusion of CD7-targeted, NK cell inhibitory receptor and common cytokine receptor incorporated CAR-T cells. 3 dosage groups of $1x10^7$, $2x10^7$, and $3x10^7$ cells/kg were established.	7 of 11 surviving patients achieved CR with or without hematological recovery, while 9 of 11 achieved OR at day 28 following infusion. 4 OR patients had disease relapse or progression at 59-103 days after infusion. Disease states monitored by PET-CT and bone marrow examination.	Grade 1-2 CRS occurred in 83% of patients. SAEs occurring in every patient included neutropenia and decreased platelet count. No severe CRS grade 3 or above. No GVHD or neurotoxicity observed. Epstein-Barr virus-associated B-cell lymphoproliferation observed in one patient.

Table 2. An Overview of the Pre-Clinical Studies Examined

Study Type	**Pre-Clinical Trials**						
Author and Year	**Animal Model**	**Type of Cancer**	**Type of Immunotherapy**	**Additional Treatment Regimen**	**Method of Administration**	**Measurement of Drug Efficacy**	**Side Effects**
Klichinsky, et al. 2021 [29]	Two ovarian cancer xenograft mouse models used, strain NOD-scid IL2Rg$_{null}$-3/GM/SF. Humanized immune system (HIS) mouse models used, combining NSGS + human CD34+ hematopoietic stem cells.	Ovarian cancer positive for HER2 expression, cell line SKOV3.	CAR-Macrophage Therapy	No previous chemotherapy or treatment administered.	First model involved a single intravenous (IV) injection of anti-HER2 human CAR-Ms, while the second model utilized a single IP injection. HIS mouse model utilized 1x10^7 anti-HER2 human macrophages injected intratumorally, 19 days after SKOV3 tumor xenograft.	Anti-HER2 CAR-Ms caused a marked reduction in metastatic tumor burden observed by bioluminescent imaging, alongside prolonged survival. CAR-Ms took on a pro-inflammatory M1 phenotype, which was not affected by other phenotype-inducing cells in HIS. Pro-inflammatory tumor microenvironment measured by scRNAseq.	Limited discussion of predicted side effects in human models. Treatments were not associated with significant toxicity and did not phagocytose normal tissue.
Chang, et al. 2023 [32]	Glioblastoma (GBM) xenograft mouse model used, strain NOD.Cg-RAG^{1tm1Mom}IL2rg^{tm1Wjl}/SzJ (NRG).	GBM, an aggressive form of solid brain cancer originating from glial cells.	CAR-Neutrophil Therapy	Prepared rough silica nanoparticles (R-SiO2 NP) loaded with tirapazamine (TPZ), a hypoxia-responsive anticancer drug.	Weekly IV injections containing 5x10^6 R-SiO2-TPZ NP-loaded, anti-GBM chlorotoxin (CLTX), CAR-neutrophils	R-SiO2-TPZ NP-loaded CAR-neutrophils had the highest anti-tumor activity, determined by bioluminescent imaging over time. Also had the greatest lifespan extending effects in tumor-bearing mice when injections were administered 6 times.	R-SiO2-TPZ NP-loaded CAR-neutrophils had decreased human cytokine production (e.g. tumor necrosis factor alpha (TNFα), interleukin-6) compared to unmodified neutrophils, implying a decreased chance of CRS. No noticeable abnormalities or damage to major organs upon histological evaluation.
Duan, et al. 2024 [33]	Breast cancer xenograft mouse model used, strain NOD.Cg-Prkdc^{scid}	Breast cancer positive for Mucin 1 (MUC1) expression.	CAR-Dendritic Cell Therapy	Some mice were treated with 3 mg/kg SM-164, an antagonist of inhibitor of apoptosis proteins, twice	Single subcutaneous injection of 2x10^5 anti-MUC1 DCs expressing TNFα local to the tumor site.	Anti-MUC1 CAR-DCs expressing TNFα combined with SM-164 caused a significant reduction in tumor size and	DCs are inactive, meaning they should not produce many cytokines besides TNFα, which it is specifically programmed to overexpress. As such,

Study Type	Pre-Clinical Trials						
Author and Year	**Animal Model**	**Type of Cancer**	**Type of Immunotherapy**	**Additional Treatment Regimen**	**Method of Administration**	**Measurement of Drug Efficacy**	**Side Effects**
	Il2rg^{tm1Wjl}/ SzJ.			a day for 10 days.		weight. SM-164 enables TNFα to induce cancer cell death.	chances of CRS should be low. Prolonged high levels of TNFα could cause inflammation and cancer growth.

Participant Selection Process

Participants across all studies were diagnosed cancer patients that had failed more than one line of therapy. Male and female patients were included, with ages generally ranging from the late 40s to 70s. Cancers in clinical trials were advanced stage. Participants' diagnoses were confirmed via imaging or staining.

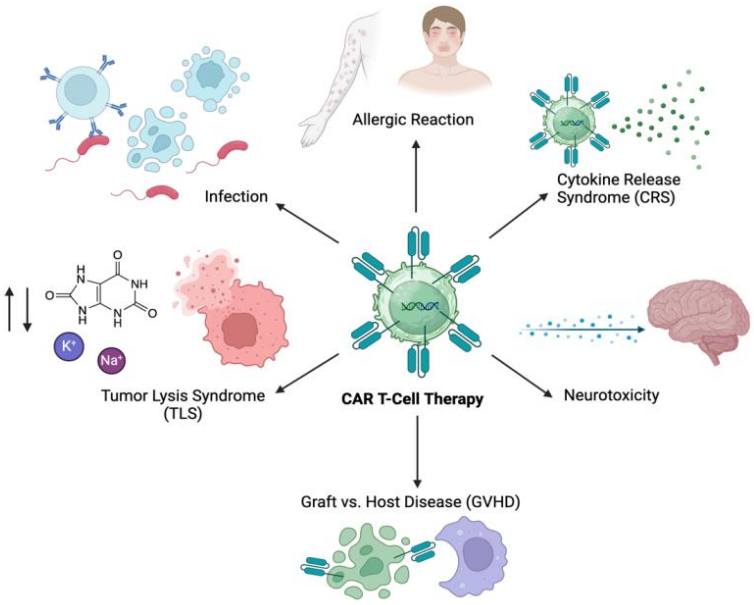

Figure 2. A Diagram of Possible Adverse Events Associated With CAR-T Cell Therapy [12]. Created With Biorender.com [37].

Discussion

CAR-T vs. CAR-NK in Solid Cancer

Measuring the presence of cancer cell markers in areas of solid tumor growth allows investigators to quantify a CAR treatment's anti-cancer activity. Both the CAR-T trial by Adusumilli et al. and the CAR-NK trial by Xiao et al. showed significant declines in their respective cancer biomarkers, soluble mesothelin-related protein (SMRP) and epithelial cell adhesion molecule (EpCAM) [18, 19]. Both studies were evaluated by computed tomography (CT) visualization of solid tumors and found to primarily result in stable disease, alongside reduction of malignant ascites in the CAR-NK trial [18, 19].

Severe adverse events (SAEs) were commonly associated with any LD chemotherapy administered in preparation for CAR immunotherapy (Figure 2). LD prior to CAR therapy serves to eliminate a patient's own T-cells, preventing them from recognizing and attacking CAR-expressing cells. In Adusumilli et al.'s CAR-T trial, 56% of participants experienced a grade 4 hematological SAE likely attributed to LD, alongside 26% of the study population experiencing neurotoxicity symptoms (i.e. headache, confusion, delirium) and grade 1-2 cytokine release syndrome (CRS) [19]. In comparison, Xiao et al.'s CAR-NK trial that did not involve the use of LD described no cases of neurotoxicity and one case of CRS, but small sample size (n=3) makes it difficult to draw translatable conclusions about incidence [18].

Given their similar efficacies of anti-cancer activity, yet this CAR-NK therapy's promising advantage of not requiring chemotherapy or other treatments preceding CAR

infusion, future studies should be directed towards the development of CAR-NK therapies against solid tumors.

The severe hematological toxicities associated with LD may lead to serious infections, loss of blood coagulation ability, and anemia [20]. If possible, CAR therapies excluding an additional treatment regimen of LD should be sought out for this reason. Additionally, larger-scale studies on CAR-NK's ability to eliminate solid tumors should be conducted to assess the true associated risk of CRS and neurotoxicity.

CAR-T vs. CAR-NK in Blood Cancer

CAR-T therapy has been widely regarded as an effective treatment for those with hematological malignancies, such as leukemia or lymphoma [16]. However, CAR-NK is also showing great promise in the clinical setting.

The CAR-NK trial by Liu et al. analyzed the efficacy of anti-CD19 targeting by measuring peripheral blood B-cells expressing CD19 [21]. Two out of three patients had depletions in CD19-positive B cells [21]. Hu et al.'s CAR-T trial revealed that 7 of 9 patients achieving objective responses (ORs) had declines in CD7+ T-cells 28 days post infusion [22]. The presence of CAR-T and CD7+ T-cells were measured using flow cytometry, while the magnitude of response was determined using PET-CT and bone marrow examination [22]. In a similar manner, CAR-NK treatment response was measured via fluorodeoxyglucose (FDG) uptake on positron emission tomography-computed tomography (PET-CT) [21]. With CAR-NK, 73% had OR and 64% had complete response (CR) within 30 days after infusion. With CAR-T, 82% had OR and 64% had CR 28 days post infusion. Their efficacies are nearly equal [21, 22].

Hematologic toxicity was seen in every patient following LD in this CAR-NK trial, yet there were no cases of CRS or GVHD throughout all 11 patients, despite some HLA mismatch [21, 22]. All participants in the CAR-T trial also experienced hematological toxicity following LD, specifically declines in neutrophil and platelet counts. In striking contrast, 83% of all participants in this CAR-T trial had grade 1-2 CRS [21, 22].

CRS occurs when introduced CAR immune cells release an excess of cytokines, resulting in rapid activation of other immune cells and potentially life-threatening damage to organ systems [23]. CRS is a systemic inflammatory response, graded on a scale of 1-4 in terms of increasing symptom severity. Grade 1 CRS typically involves fever, chills, and/or fatigue [24]. Grade 4 CRS is life-threatening, involving significant hypotension, hypoxia, and/or organ failure [24]. Minimizing risk of CRS is crucial, especially in the face of LD and its associated high risk of infection [20]. This gives CAR-NK a potential advantage in the treatment of hematological cancers paired with chemotherapy, achieving a similar reduction in cancer burden while minimizing the physical and psychological challenges associated with CRS. This comparison reinforces the importance of prioritizing advancements in CAR-NK therapy, specifically for hematological cancers.

Burden of Treatment Preparation and Administration

The major differences in health related QoL between CAR immunotherapies can be attributed to their associated AEs, such as CRS and toxicity from LD. Both CRS and LD can cause extremely uncomfortable physical and psychological symptoms. LD is additionally considered very invasive due its non-specific cytotoxic nature, killing any cells that can rapidly proliferate [25]. This results in the death of hair follicular cells, gastrointestinal cells, and skin cells [25]. For these reasons, CAR therapies minimizing risk of CRS and LD use should be prioritized for further study.

Pairing chemotherapy with CAR immunotherapy can also create a large financial burden for patients. While the examined clinical trials typically required only 3-5 days of lymphodepleting chemotherapy, systematic review and meta-analysis of global chemotherapy administration reveals the hourly cost of administration to be $125-150 USD per hour [26]. CAR therapy alone is extremely expensive, with a single infusion of CAR-T costing anywhere from than $373,000-$475,000 USD [27].

Additionally, certain CAR immune cells can cost far less to manufacture and administer. As performed in Liu et al.'s CAR-NK trial, cord blood-derived cells are significantly easier to obtain and store, making them more cost-effective [28]. Finally, treatment for CRS can cost anywhere from $36,000-$56,000 USD per patient [29]. This can give CAR-NK therapy, which appears to have a reduced associated risk of CRS, a lower financial burden on patients overall.

CAR-M Preclinical and Clinical Trials

Due to the absence of concluded CAR-M clinical trials for cancer treatment, an ongoing clinical trial and a completed preclinical study were reviewed. Both involved the engineering of anti-HER2 CARs into macrophages for the treatment of solid HER2+ cancers [30, 31]. In Klichinsky et al.'s preclinical research, anti-HER2 CAR-Ms were able to selectively phagocytose a HER2-expressing ovarian cancer cell line and had no effects on normal human tissue cells [31]. Mouse models of ovarian cancer who received these CAR-Ms intraperitoneally had significant reduction of tumor burden 100 days post-injection via bioluminescence, prolonged survival, and increased T-cell activation [31].

A recent update in Reiss et al.'s CAR-M clinical trial seems to convey similar results – 89% of participants had high anti-HER2 CAR-M detection in the tumor microenvironment and resulting T-cell infiltration, activation, and proliferation [32]. 57% of participants evaluated achieved stable disease, and the trial reported a 78% incidence of grade 1-2 CRS and/or infusion reaction [32]. These are notably similar results to Adusumilli et al.'s CAR-T trial against solid cancers, with 56% of participants reaching stable disease and 83% having grade 1-2 CRS [19].

However, this CAR-M trial has not reported on partial response or progressive disease outcomes yet.

Based on these preliminary results, CAR-M may offer a treatment for solid cancers comparable to CAR-T. Yet, it is likely not going to eliminate the issue of cytokine toxicity as it is engineered currently. Macrophages in the M1 form, as used in CAR-M, largely function in the release of pro-inflammatory chemokines and cytokines [33]. As such, a cytokine storm remains a plausible risk. It is important to note that this impression could change as the study continues.

CAR-Neutrophils and Dendritic Cells as Drug Carriers

The CAR-Neutrophil and CAR-DC preclinical trials examined in this review utilized a different approach, designing these immune cells to act primarily as cancer-specific carriers of tumoricidal elements rather than acting as the killing agents themselves.

CAR-Neutrophil therapy designed by Chang et al. shows great promise in the treatment of solid cancers [34]. Neutrophils have an innate ability to cross biological barriers, such as the blood-brain barrier [34]. This translates well into the infiltration of solid tumors, a major limitation of CAR-T therapy [14]. The introduction of anti-glioblastoma chlorotoxin CARs into the neutrophil membrane enabled targeted migration to brain tumors, rather than general migration to sites of inflammation as typically observed [34]. On top of neutrophils' ability to detect and phagocytose glioblastoma, Chang et al. incorporated tirapazamine within the cell to be released and selectively kill hypoxic tumor cells [34]. This diminishes chances of on-target, off-tumor toxicity. The study found no AEs and noted a reduced production of cytokines by CAR-neutrophils compared to unmodified neutrophils [34].

CAR-DC therapy designed by Duan et al. also proposes a unique method of solid cancer treatment [35]. As inactive dendritic cells were engineered to express anti-Mucin 1 (MUC1) CARs and tumor necrosis factor α (TNFα), these CAR-DCs will only release one kind of cytokine upon MUC1 binding to CAR. Paired with SM-164, an inhibitor of TNFα inhibitors, these CAR-DC cells can selectively deliver substantial amounts of tumoricidal TNFα to MUC1-expressing cancer masses [35]. Both studies potentially address the issues of CRS, neurotoxicity, and LD-associated hematological toxicity commonly seen in current clinical trials.

Future Directions

While CAR-T is the first and only CAR immunotherapy to be FDA approved for treatment of hematological cancers [14], other CAR immune cells have vast potential for clinical use in both solid and blood cancers. For CAR neutrophils and DCs, preclinical studies evaluating their efficacy in other types of solid cancers and blood cancers should be piloted. Additionally, larger-scale research on the optimization of their preparation and dosage should be conducted, establishing standards for future clinical trials. Studies on the prevention of CRS and other AEs following CAR infusions are also necessary.

Limitations of this Study

A considerable challenge in this literature review was the lack of available clinical trials on CAR therapies besides CAR-T. An ongoing clinical trial was used to discuss CAR-M in humans, but incomplete results made it difficult to fully compare with CAR-M preclinical studies and other completed clinical trials. There was a significant lack of similarity between studies and variability of patients' histories with past treatments, ages, and demographics. Crucially, these phase 1-2 clinical trials had small sample sizes, making it difficult to generalize these literature results to larger populations.

Conclusions

This literature review presents an up-to-date assessment of the efficacies and limitations of present CAR immune cell therapies in the treatment of solid and hematological cancers. Each of the 5 types of CAR immunotherapies discussed showed significant cancer responses to treatment, but differed in their methods of preparation, administration, additional therapeutic regimens, and AEs. Of the clinical trials reviewed, CAR-NK therapies demonstrated promising cancer responses with fewer instances of CRS and neurotoxicity. Paired with their feasibility of manufacture and reduced financial burden, expanded phase testing of CAR-NK in clinical trials is essential. Additionally, the use of CAR immune cells as delivery agents of tumoricidal therapies should be investigated further. As cancer incidence continues to rise, prioritizing CAR immunotherapy research aimed at significantly reducing cancer growth while minimizing toxicities and preserving QoL is crucial.

List of Abbreviations Used

AE: adverse event
APC: antigen presenting cell
CAR: chimeric antigen receptor
CAR-DC: CAR-dendritic cell
CAR-M: CAR-macrophage
CAR-NK: CAR-natural killer
CD: cluster of differentiation
CLTX: chlorotoxin
CR: complete response
CRS: cytokine release syndrome
CT: computed tomography
DC: dendritic cell
EpCAM: epithelial cell adhesion molecule
FDG: fluorodeoxyglucose
GBM: glioblastoma
GVHD: graft vs. host disease
H&E: hematoxylin and eosin
HER2: human epidermal growth factor 2
HIS: humanized immune system

HLA: human leukocyte antigen
HSC: hematopoietic stem cell
IHC: immunohistochemistry
IP: intraperitoneal
IV: intravenous
LD: lymphodepletion
MHC: major histocompatibility complex
MPM: malignant pleural mesothelioma
MUC1: mucin 1
NK: natural killer
OR: objective response
PAMP: pathogen-associated molecular pattern
PET-CT: positron-emission tomography-computed
QoL: quality of life
SAE: severe adverse event
SMRP: serum-soluble mesothelin-related protein
TME: tumor microenvironment
TNFα: tumor necrosis factor alpha
TPZ: tirapazamine

Conflicts of Interest
The author declares that they have no conflict of interests.

Ethics Approval and/or Participant Consent
No ethics/participant consent was needed to complete this study.

Authors' Contributions
CFG: made contributions to the design of the study, collected and analysed data, drafted the manuscript, and gave final approval of the version to be published.

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