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Translational Medicine

Killers at the crossroads: The use of innate immune cells in adoptive cellular therapy of cancer

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Abstract

Adoptive cell therapy (ACT) is an approach to cancer treatment that involves the use of antitumor immune cells to target residual disease in patients after completion of chemo/radiotherapy. ACT has several advantages compared with other approaches in cancer immunotherapy, including the ability to specifically expand effector cells in vitro before selection for adoptive transfer, as well as the opportunity for host manipulation in order to enhance the ability of transferred cells to recognize and kill established tumors. One of the main challenges to the success of ACT in cancer clinical trials is the identification and generation of antitumor effector cells with high avidity for tumor recognition. Natural killer (NK) cells, cytokine-induced killers and natural killer T cells are key innate or innate-like effector cells in cancer immunosurveillance that act at the interface between innate and adaptive immunity, to have a greater influence over immune responses to cancer. In this review, we discuss recent studies that highlight their potential in cancer therapy and summarize clinical trials using these effector immune cells in adoptive cellular therapy for the treatment of cancer.

KEYWORDS

adoptive cell therapy, cancer, cytokine-induced killer cells, immunotherapy, natural killer cells, natural killer T cells

1 | INTRODUCTION

Adoptive cell therapy (ACT) of cancer relies on the identification and generation of antitumor immune cells with high avidity for tumor recognition before infusion into patients. It was first described in 1988, with the use of tumor infiltrating lymphocytes (TILs) and interleukin (IL)-2 and achieved cancer regression in some patients with metastatic melanoma.[1] Since then, the transfer of immune cells with antitumor activity whether unmodified or following in vitro stimulation, expansion or genetic engineering has shown dramatic regressions in a variety of hematological and solid cancers. ACT has the advantage that large numbers of effector cells can be grown and activated in vitro before selection for specific antitumor functions. A critical

improvement in the efficacy of ACT-based cancer immunotherapy came in 2002 with the introduction of a lymphodepletion preparative regimen of chemotherapy and/or radiation prior to adoptive transfer, which enhances the ability of transferred cells to recognize and kill established tumors through elimination of host inhibitory factors and clonal repopulation of antitumor cells.[2,3] Much of the clinical focus has centered on adaptive T cells, with exciting reports from clinical trials on chimeric antigen receptor (CAR)-engineered T cells achieving impressive remission rates in patients with hematological malignancies, but there have also been some setbacks including patient experience of adverse side effects from infusion-related toxicity and cytokine release syndrome (CRS).[4,5] There is now a growing body of evidence suggesting that innate immune cells share many of the

attributes of adaptive immunity including antigen specificity, clonal expansion, and even memory.[6] Recent clinical studies have revived an interest in innate and innate-like effector cells as strong candidates for different immunotherapeutic strategies in cancer with a potentially safer therapeutic profile. Here, we focus on three key effector cells; natural killer (NK) cells, cytokine-induced killers (CIKs), and natural killer T cells (NKT), which exhibit direct antitumor activity by linking innate and adaptive immune responses. We summarize different approaches using these innate-immune effector cells in ACT-based immunotherapy of cancer, emphasizing those that have been clinically tested during this past decade.
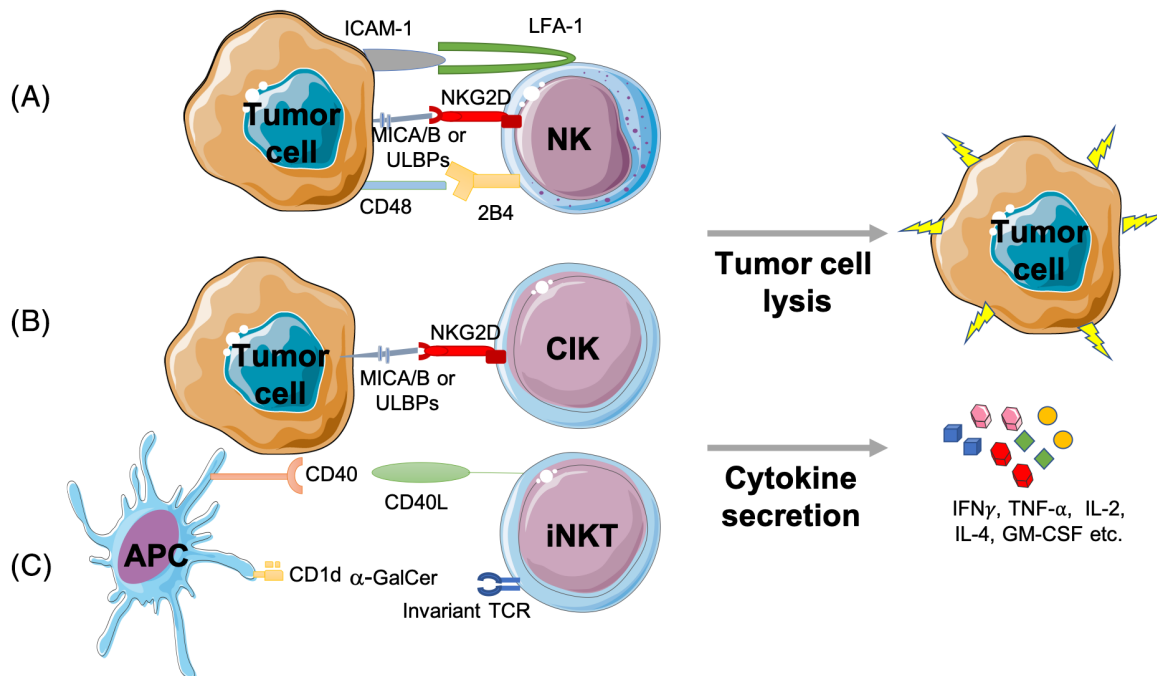
2 | NK CELLS

NK cells are the major cytotoxic effector cells of the innate immune system, known for their natural ability to lyse tumor cells in vitro without prior sensitization. They constitute 5% to 15% of circulating lymphocytes and belong to the recently identified group 1 innate lymphoid cells.[7] Their antitumor effector functions of cytotoxicity and cytokine secretion are determined by the balance of signals from both inhibitory and activating receptors expressed on their cell surface (Figure 1A).

Significance statement

This review article highlights the potential of innate and innate-like immune cells in cancer immunotherapy and summarizes recent clinical trials using these effector cells in adoptive cellular therapy for the treatment of cancer. It focuses on natural killer cells, cytokine-induced killer cells and invariant natural killer cells, which sit at the interface between innate and adaptive immunity to have a greater influence over immune responses to cancer. A better understanding of these killer cell responses and the optimal clinical conditions required for their full antitumor potential can help in utilizing their effector functions to advance clinical therapy of cancer.

Inhibitory receptors for major histocompatibility complex (MHC) class I molecules include killer immunoglobulin-like receptors (KIRs), which bind human leukocyte antigen (HLA)-A, B, and C, and the CD94-NKG2A heterodimer, which recognizes HLA-E. Downregulation of MHC Class I expression by tumor cells to escape T-cell immunity may lower the

FIGURE 1 Tumor recognition by natural killer cells, cytokine-induced killer cells, and invariant natural killer T cells. A, NK cell antitumor activity is determined by the balance of signals from inhibitory and activating receptors expressed at the cell surface to trigger cytokine secretion or direct tumor cell lysis, with the collective engagement of several activation receptors such as the combination of LFA-1, NKG2D, and 2B4 on NK cells inducing NK cell natural cytotoxicity against tumor targets. B, CIK cell cytokine secretion and tumor lysis are mainly mediated through NKG2D-signaling and the engagement of MHC-related ligands MICA/B and the ULBP family of ligands. C, iNKT cells express an invariant T-cell receptor that is specifically activated by α-GalCer loaded on CD1d molecules expressed by antigen-presenting cells to exert direct cytotoxicity against tumor targets or secrete large amounts of cytokines like IFN-γ or IL-4. α-GalCer; alpha-galactosylceramide, APC; antigen-presenting cell, GM-CSF; granulocyte-macrophage colony-stimulating factor, ICAM-1; intracellular adhesion molecule 1, IFN-γ; interferon-gamma, IL-2; interleukin-2, LFA-1; lymphocyte function-associated antigen 1 MICA/B; MHC class I chain-related protein A and B, NK; natural killer, iNKT; invariant natural killer T cell, TCR; T-cell receptor, TNF-α; tumor necrosis factor-alpha, ULBP; UL16 binding protein

threshold to trigger NK cell cytotoxicity through "missing-self" recognition.[8] Non-MHC-binding NK cell inhibitory receptors include carcinoembryonic-antigen-related-cell-adhesion molecule 1 (CEACAM1), NK-cell receptor protein 1 (NKRP1) family members, sialic-acid-binding immunoglobulin-like lectins (SIGLECs), and T-cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domain (TIGIT).[9,10] NK cell function also depends on the presence of activation signals through NK cell activation receptors including the low-affinity activating receptor FcγRIIIa (CD16) that binds the Fc portion of immunoglobulin G1 (IgG1) and mediates antibody-dependent cellular cytotoxicity, NKG2D, which binds MHC class I chain-related proteins A and B (MICA/B), UL16 binding proteins (ULBPs) and DNAM-1, which binds poliovirus receptor (PVR/CD155) and Nectin-2 (CD112).[11] Many other activation receptors have been characterized, including natural cytotoxicity receptors (NCRs) like NKp30, NKp46, NKp80, 2B4, CD2, lymphocyte function-associated antigen (LFA-1), and NK-T-B antigen (NTB-A), to play important costimulatory roles in NK cell recognition of tumors.[12] The collective engagement of several NK cell activation receptors such as the combination of LFA-1, NKG2D and 2B4 has been shown to define a minimal requirement for the induction of NK cell natural cytotoxicity against tumor targets (Figure 1A).[13] The use of NK cells in adoptive therapy is mainly hampered by the limited ability to generate large numbers of cells ex vivo as well as their short life span in vivo. Several protocols have been developed to overcome these challenges and expand large numbers of NK cells with strong antitumor functions, which are discussed elsewhere.[14]

The transfer of autologous NK cells for the treatment of different hematological malignancies and solid tumors has shown to be remarkably safe resulting in better engraftment and persistence of cells as well as a low risk of GvHD, but with limited clinical benefit.[15-17] A positive clinical outcome associated with large numbers of circulating antitumor NK cells was observed in hematological cancer patients in the early days following autologous hematopoietic stem cell transplantation (HSCT).[18] Conversely, the transfer of expanded autologous NK cells has not shown positive clinical responses in metastatic melanoma, renal-cell carcinoma, or advanced gastrointestinal cancer.[19,20] In many cases, circulating autologous NK cells demonstrate weak antitumor cytotoxicity without further in vitro or in vivo stimulation. Thus, recent and ongoing clinical trials focus on immunotherapeutic strategies that activate autologous NK cells with cytokines such as IL-2/−15, or target cells like K562-mb15-41BBL, to reach their full antitumor potential, in a combination setting with chemotherapy, cytotoxic T cells, or monoclonal antibodies (mAbs) (Table 1). In a recent phase I clinical trial, the transfer of activated and expanded NK cells (NKAE) co-cultured ex vivo with K562-mb15-41BBL before infusion into five patients with relapsed or refractory MM, showed disease stabilization in four patients and a long-term response (>1 year) in two patients, suggesting that ex vivo expansion of autologous NK cells can be tolerated in cancer patients to result in a clinical response.[21]

An earlier study by Ruggeri et al provided the first evidence that allogeneic NK cells can be more potent effectors against tumor targets, especially in the case of KIR-ligand incompatibility, in acute myeloid leukemia (AML) patients following allogeneic hematopoietic transplants from HLA-mismatched donors.[22] KIR-ligand incompatibility generates alloreactive NK cells that eliminate tumor cells through the absence of appropriate inhibitory KIRs on donor-derived NK cells to engage their respective MHC class I ligands on patient tumor cells. In an allogeneic setting, NK cells are thought to mediate antitumor responses following adoptive transfer with limited induction of graft-vs-host disease (GvHD).[23] Infusion of allogeneic NK cells has not been associated with GvHD in the majority of trials and NK cells have been shown to prevent GvHD through the elimination of T cells and dendritic cells.[24-26] However, conflicting reports have shown that NK cell expansion and activation methods can lead to the induction of GvHD through T-cell activation and the secretion of pro-inflammatory cytokines.[27-29] The transfer of IL-15/4-1BBL activated NK cells following allogeneic HSCT has also been associated with incidences of acute GvHD.[30] Allogeneic NK cell transfer with IL-2 infusions has shown some promising results in advanced cancer patients,[31,32] and numerous studies have reported similar results using allogeneic NK cells following HSCT.[33-39] A recent phase I clinical trial using allogeneic NK cells (MG4101) expanded ex vivo using IL-2 and an mAb against CD3 to treat 17 patients with malignant lymphoma or advanced recurrent solid tumors, reported no significant adverse events, and 8 patients showing stable disease and a reduction in regulatory T cells (Tregs) and myeloid-derived suppressive cells (MDSCs).[40] The combination of cryosurgery with infusions of KIR-mismatched allogeneic NK cells, activated and expanded ex vivo using irradiated K562-mb15-41BBL cells and other cultural additives showed enhanced immune function, improved quality of life, a higher response rate (RR) and disease control rate in a phase I clinical trial for the treatment of 60 patients with advanced non-small cell lung cancer (NSCLC).[41] In another recent study by Adotevi et al, allogeneic NK cells combined with high-dose IL-2 and cetuximab were used to treat liver metastasis of colorectal or pancreatic cancer, and clinical responses were observed in three of nine patients who were infused with cell products having one or two KIR ligand mismatches.[42] Similar to previous studies, IL-2 administration resulted in the expansion of FoxP3^{+} Treg cells and PD-1^{+} T cells.

Early clinical trials investigating the transfer of allogeneic NK cells from haploidentical donors combined with chemotherapy and IL-2 infusions reported complete remissions in AML patients with poor prognosis.[33] More recently, haploidentical NK cells used in combination with 3F8 mAb in a phase I study for the treatment of resistant high-risk neuroblastoma, showed complete remission in 3 out of 20 patients without GvHD or myeloablation reported.[43] Although haploidentical transplants are associated with high rates of GvHD, in a recent phase I study using IL-2-activated haploidentical NK cells for the treatment of high-risk AML, myelodysplastic syndromes (MDS) or chronic myeloid leukemia (CML) patients enhanced the outcome of the hematopoietic transplantation without inducing GvHD, but patients receiving KIR-mismatched NK cells did not show higher survival rates.[44] In another phase II study investigating the efficacy of haploidentical NK cell and IL-2 infusions following allogeneic HCT in eight MDS/AML patients, no incidence of GvHD was observed, two

patients showed a complete response but relapsed by 1.8 months, and one patient showed stable disease for 65 months.[45] In two studies using infusions of NKAE cells from haploidentical donors with low-dose IL-2 for the treatment of relapse or refractory acute leukemia

after rescue chemotherapy, complete remission was achieved in 13 out of 18 patients, without GvHD or other serious adverse events.[46] In two phase I/II trials for the treatment of advanced AML, 42 patients received intravenous (IV) or subcutaneous (SC) recombinant human

TABLE 1 Summary of NK-, CIK-, and iNKT-cell-based clinical trials for adoptive cellular therapy of cancer completed on or after 1 January 2010

Approach/treatment	Disease	Patients (total)	Trial identifier
NK			
Autologous NK cell transfer (+/− chemotherapy, IL-2/−15, K562-mb15-41BBL, CTLs, and/or monoclonal antibodies)	Pediatric cancers, neuroblastoma, sarcoma, brain and solid tumors, HCC, MM, prostate cancer, glioblastoma, and B-cell lymphoma	139	NCT01875601, NCT0114738, NCT01884688, NCT02481934, NCT01313897, NCT00891345, NCT01422850, NCT01588769, NCT02843061
Allogeneic NK cell transfer (+/− chemotherapy, HSCT, IL-2, and/or cryosurgery)	Advanced biliary tract cancer, liver metastases of colorectal or pancreatic cancers, lymphomas, leukemias, ovarian cancer, fallopian tube carcinoma and primary peritoneal cancer, breast cancer, NSCLC, neuroblastoma, and HCC	207	NCT03358849, NCT02845999, NCT01212341, NCT01853358, NCT01287104, NCT01105650, NCT01181258, NCT00303667, NCT00383994, NCT02843815, NCT02008929
Haploidentical NK cell infusions (+/− chemotherapy, HSCT, IL-2/IL-15, 41BBL, CNDO-109, genetic modification, KIR-ligand mismatch, and/or monoclonal antibodies)	NSCLC, neuroblastoma, lymphoma, leukemia, MDS, myeloma, ovarian cancer, fallopian tube carcinoma, primary peritoneal cancer, MM, neuroblastoma, rhabdomyosarcoma, and melanoma	526	NCT03366064, NCT02130869, NCT00640796, NCT00697671, NCT00660166, NCT01947322, NCT02118285, NCT01385423, NCT01795378, NCT00995137, NCT00089453, NCT00823524, NCT00187096, NCT02074657, NCT02395822, NCT00877110, NCT01576692, NCT01386619, NCT00846833, NCT00526292, NCT01593670, NCT00402558, NCT01390402, NCT01520558
NK cell line NK-92 (+/− CAR)	Leukemia, lymphoma, MM, Hodgkin's disease, and solid tumors	21	NCT00990717, NCT00900809, NCT03027128
Unspecified NK cell transfer + cryosurgery	Advanced kidney cancer, advanced breast cancer, liver cancer, and esophageal cancer	200	NCT02843607, NCT02844335, NCT02849015, NCT02843802, NCT02843581
CIK			
Autologous CIK cell transfer (+/− surgery, stem cell transplant or RFA)	HCC, hematological malignancies, CRCLM	499	NCT00769106, NCT01749865, NCT00477035, NCT00394381, NCT02419677
Autologous DC-CIK (+/− surgery, chemotherapy, or γδ T cells)	Pancreatic, liver, colorectal, prostatic, renal, lung, gastric, and breast cancer	799	NCT02406846, NCT02416635, NCT02450422, NCT02450435, NCT02450448, NCT02412384, NCT02450357, NCT02425735, NCT02418481, NCT02425748, **NCT01781520, NCT01783951, NCT01232062, NCT01395056**
CIK cell agent following curative resection (PEIT, RFA, or surgery)	HCC	230	NCT00699816
Allogeneic CIK cell transfer following allogeneic stem cell transplantation	MDS, MPD, and hematologic malignancies	142	NCT01392989, NCT01186809, NCT00460694
iNKT			
Autologous iNKT cells	Malignant melanoma	9	NCT00631072

Abbreviations: CIK; cytokine-induced killer, CRCLM; colorectal cancer liver metastases, CTL; cytotoxic T lymphocyte, HCC; hepatocellular carcinoma, HSCT; hematopoietic stem cell transplantation, iNKT; invariant natural killer, IL; interleukin, KIR; killer cell immunoglobulin-like receptors, MDS; myelodysplastic syndromes, MM; multiple myeloma, MPD; myeloproliferative disorders, NK; natural killer, NSCLC; non-small cell lung cancer.

IL-15 (rhIL-15) infusions following chemotherapy and haploidentical NK cell transfer.[47] Although 32% and 40% of patients achieved complete remission after IV and SC dosing, respectively, cytokine release syndrome was associated with SC but not IV dosing. Two phase I studies using NK cells generated from related HLA haploidentical donors, activated using a tumor cell lysate in the absence of cytokine stimulation, were also shown to be well tolerated with no dose-limiting toxicities in AML patients, with the infused NK cells surviving and expanding in vivo and diapedesing into the bone marrow to exert antileukemia effects.[48,49] Contrasting results from different studies may be explained by variations in T-cell depletion, which can have a great influence on GvHD risk, or the nature of the transferred NK cells and the method of their activation.[50]

An alternative approach is the use of NK cell lines, induced-pluripotent stem cells (iPSCs), human embryonic stem cells (hESCs), or umbilical cord blood (CB)-derived NK cells for adoptive transfer.[51] The NK-92 cell line, which lacks inhibitory receptor expression, shows high cytotoxicity against a variety of tumor targets, is easy to transfect and can be expanded under good manufacturing practice conditions using recombinant IL-2, has been investigated in numerous clinical studies, some of which have been completed in the last 10 years (Table 1). The first clinical trial using NK92 cell reported initial signs of antitumor activity,[52] and this was followed by other clinical studies reporting that unmodified NK-92 cells can be safely infused into patients with advanced treatment-resistant malignancies to show encouraging antitumor responses in patients with lung cancer.[53] A recent phase I clinical trial in AML investigated the safety of activated NK-92 (aNK) cells and reported no grade 3 to 4 toxicities related to cell infusion and some signs of clinical activity, with two out of seven patients showing stable disease and one patient showing a reduction in blast percentage from 70% to 48%.[54] In another recent phase I study, irradiated NK-92 cells were used to treat 12 lymphoma or MM patients, and the cell infusions were also well tolerated, with 2 patients achieving complete response, 2 showing minor responses, and 1 reporting clinical improvement.[55] Boyiadzis et al recently reported that the adoptive transfer of NK92 cells for the treatment of refractory/relapsed AML patients resulted in three out of six patients showing transient clinical responses.[56] To explore other sources for adoptive NK cell transfer, the first clinical trial exploring the safety and efficacy of NK cells derived from iPSCs (FT500 NK cells) in combination with immune checkpoint inhibitors for the treatment of 64 patients with advanced solid tumors including lymphoma, melanoma, gastric, colorectal, lung, cervical, and breast cancer is currently ongoing and open for recruitment (NCT03841110). Similarly, a first-in-human study investigating the use of umbilical CB-derived NK cells for the treatment of 12 MM patients undergoing high-dose chemotherapy and autologous HSCT has been reported, with no infusional toxicities or GvHD observed and 8 patients achieving near complete responses.[57] Although the widespread use of these alternative approaches is currently hampered by challenges in procurement and sourcing, encouraging results from ongoing trials, especially from NK cell lines used in adoptive transfer, is likely to result in a noticeable increase in their clinical development over the next decade.

3 | CIK CELLS

CIK cells are a heterogeneous population of CD3$^+$CD56$^-$, CD3$^-$CD56$^+$, and CD3$^+$CD56$^+$ cells, which are expanded in vitro from peripheral blood mononuclear cells to demonstrate cytolytic activity against a wide range of tumor targets in a non-MHC-restricted manner.[58] The main antitumor effector cell population comprises mostly CD3$^+$CD56$^+$ cells, which share characteristics with NK cells and T lymphocytes. CIK cell lysis of tumors is mediated through NKG2D-signaling and the engagement of MHC-related ligands MICA/B and the ULBP family of ligands, the expression of which is frequently upregulated in hematologic or solid cancers (Figure 1B).[59-61] Several protocols for the generation of CIK cells have been reported, and typically involve the sequential incubation of peripheral blood lymphocytes with interferon (IFN)-γ, anti-CD3 mAb, and IL-2.[62] The use of IL-15 in the presence or absence of IL-2 has also been shown to induce faster expansion of CIK cells to exhibit enhanced cytotoxicity, with the additional advantage of inducing fewer numbers of suppressive Treg cells.[63,64] CIK cells exhibit stronger cytolytic activity compared to lymphokine-activated killer (LAK) cells, which are generated by incubation with interleukins. Enhanced function of CIK cells relative to LAK cells has been attributed to a higher proliferative capacity and an increase in lytic units.[64,65]

The first phase I trial using CIK cells was conducted in the early 1990s by Schmidt-Wolf and colleagues and demonstrated high cytotoxicity in patients with metastatic renal cancer, colorectal cancer, and lymphoma.[66] At the time of writing this manuscript, a total of 117 clinical trials using CIK cells for the treatment of cancer were registered on ClinicalTrials.gov. The majority of clinical trials completed within the last 10 years used autologous CIK cells in a combination setting, with the highest number of patients enrolled in autologous, combinatorial dendritic cell (DC)-CIK therapy trials (Table 1). DCs are important APCs that capture and process tumor antigens, express co-stimulatory molecules, and secrete cytokines to activate adaptive and innate immune responses, making them good candidates for cancer immunotherapy. In a recent study by Jiang et al, DC-CIK infusions combined with chemotherapy resulted in favorable overall survival (OS) (212 vs 141 days) and progression-free survival (PFS) (136 vs 92 days) relative to chemotherapy alone, without reported grade 3 or 4 toxicities in a total of 47 patients with clinical advanced pancreatic cancer.[67] Similarly, clinical trials for the treatment of MM and advanced gastric cancer using DC-CIK combined with chemotherapy also provided favorable survival rates, without serious adverse events reported.[68,69] Although DC-CIK infusions combined with chemotherapy for the treatment of 23 patients with negative metastatic breast cancer resulted in enhanced survival rates, serious adverse events including neutropenia and anemia were commonly observed.[70]

The use of autologous and allogeneic CIK cells in combination with curative therapy has thus far yielded mixed responses. For the treatment of 60 patients with colorectal liver metastases, autologous CIK cell transfer 1 week following RFA showed higher median PFS (23 vs 18.5 months), 3-year progression-free rates (20.3% vs 13.3%),

and 3-year survival rates (81.0% vs 64.6%) relative to the control group not receiving CIK cell transfer.[71] In a phase 3 clinical trial for the treatment of hepatocellular carcinoma (HCC), autologous CIK cell transfer after liver resection did not improve disease-free survival (DFS) or OS, but prolonged the median time in therapeutic range (TTR) in a total of 200 patients (13.6 vs 7.8 months).[72] Another phase 3 trial treating 230 HCC patients with autologous CIK cells after RFA, surgery or percutaneous ethanol injection, demonstrated higher median RFS (44 vs 30 months), but a higher number of patients receiving CIK cell infusions experienced adverse events (62% vs 41%) relative to the control group.[73] In an allogeneic setting, a one-time infusion of CIK cells (1×10^8/kg) administered after nonmyeloablative allogeneic transplantation for patients with myeloid neoplasms did not significantly change relapse or survival rates, compared to data from a retrospective cohort of 100 patients.[74] In another phase I study using allogeneic CIK cells to treat 11 patients relapsing from AML, ALL, MDS, or Hodgkin's disease after allogeneic HSCT, a median number of two CIK cell infusions at 12.4×10^6/kg were administered, with GvHD observed in four cases.[75] Otherwise, three patients achieved complete responses, one had stable disease, and one patient showed hematologic improvement. Similarly, in five leukemia patients relapsing after cord blood transplantation, treatment with cord blood-derived CIK cells ex vivo expanded using the washouts of cord blood units remaining at the end of the transplant, one partial response was observed who also developed acute grade II GvHD.[76] Overall the data show potential for clinical responses achieved from adjuvant CIK cell therapy and feasibility of using cord blood CIK cells for patients who could not otherwise benefit from donor lymphocyte infusion, but not without concern for toxicity. The next decade is likely to see novel CIK-based combination strategies in the clinic based on in vitro studies using CIK cells with the addition of other cytokines, CARs, immune checkpoint inhibitors, or antibodies.[62]

4 | NKT CELLS

Natural killer T (NKT) cells are a heterogeneous subset of lymphocytes that share properties of both NK cells and T lymphocytes by co-expressing NK cell lineage markers and the T-cell receptor (TCR). The NKT cell receptor recognizes lipid antigens in the context of antigen-presenting nonclassical MHC class I molecule CD1d expressed on B cells, DCs, monocytes, and macrophages. There are three types of NKT cells, type-I NKT cells, type II NKT cells, and NKT-like cells. Type I NKT cells are also known as invariant NKT (iNKT) cells because of their highly restricted TCR repertoire. iNKT cells are the most prevalent type of NKT cells with the highest levels of antitumor activity and will thus be the focus of this review. Human iNKT cells express the invariant Vα24-Jα18 chain coupled with Vβ11 chain and are specifically activated by the glycolipid α-galactosylceramide (GalCer).[77,78] Once activated, iNKT cells can exert direct cytotoxicity against CD1d-expressing tumors or indirectly initiate antitumor responses by secreting large amounts of cytokines like IFN-γ, tumor necrosis factor (TNF)-α,

granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-2, −6, −10, −17, and −21 to affect a broad spectrum of innate and adaptive immune cells, including macrophages, dendritic cells, NK cells, B cells, and T cells (Figure 1C).[79] Activated iNKT cells can also induce DC maturation through direct engagement of CD40 on DCs to its ligand CD40L on iNKT cells. In turn, mature DCs secrete IL-12 and upregulate their expression of costimulatory molecules such as CD40, CD70, CD80, CD86, and NKG2D ligands to enhance antitumor immune responses.[80] Studies have reported iNKT dysfunction in cancer as evidenced by decreased iNKT cell proliferation and lower levels of IFN-γ secretion.[81-83] A reduction in circulating iNKT cells has also been reported in a wide range of cancers, including MDS, MM, and prostate cancer, and high numbers of circulating iNKT cells is associated with better clinical outcome in advanced cancer patients.[84-87] This has led to different therapeutic strategies aiming to reconstitute or activate the deficient iNKT cell population in clinical trials for the treatment of cancer.

At the time of writing this manuscript, seven clinical trials using iNKT cells in cancer were registered on ClinicalTrials.gov, with only one completed in the last 10 years (Table 1). Early clinical trials administering synthetic α-GalCer (KRN-7000) to activate iNKT cells in patients with solid tumors showed that it can be tolerated over a wide range of doses (50-4800 μg/m^2) to increase serum cytokine levels of TNF-α and GM-CSF and transiently enhance NK cell numbers and cytotoxicity.[88] Another approach that has been the focus of clinical trials using iNKT cells in the treatment of cancer is the use of α-GalCer-pulsed APCs to activate innate and adaptive immune responses. In a phase I clinical trial, Nieda et al reported enhanced NK and T-cell function as well as increased serum levels of IL-12 and IFN-γ using α-GalCer-pulsed DCs.[89] Chang et al also showed a 100-fold expansion of iNKT cells and increased levels of IL-12p40 and IP-10 in advanced cancer patient sera following injection with α-GalCer-pulsed DCs.[90] Expanded iNKT cells could still be detected 6 months following vaccination, and memory cytomegalovirus-specific CD8+ T cells were also expanded in patients treated with α-GalCer-pulsed DCs, but not unpulsed DCs. The use of α-GalCer-pulsed APCs in patients with lung cancer resulted in better infiltration and activation of iNKT cells in the tumor microenvironment.[91] In patients with advanced non-small lung cancer, α-GalCer-pulsed APCs from PBMCs cultured with GM-CSF/IL-2 induced an increase in the numbers of NKT and NK cells and higher levels of IFN-γ in peripheral blood, which was significantly associated with prolonged median survival times in 10 out of 17 patients.[92] ex vivo expansion of autologous NKT cells using IL-2 and CD3 mAb before infusion induced in vivo expansion of NKT cells and enhanced IFN-γ production in melanoma patients.[93] The combination of NKT expansion and α-GalCer-pulsed APCs used in a phase II clinical trial for the treatment of head and neck squamous cell carcinoma activated iNKT cells to induce antitumor functions associated with positive clinical outcomes, with 5 of 10 patients achieving tumor regression.[94] Studies in mice have shown that iPSCs can be differentiated into NKT cells to suppress tumor growth in vivo, an approach that is yet to be tested for NKT cell-based therapy in humans.[95]

5 | GENETIC MODIFICATION OF KILLER CELLS FOR ADOPTIVE CELL THERAPY

The genetic modification of killer cells can induce more sustained changes to their function for adoptive transfer. CAR-modified NK cell development has been largely limited to the preclinical stage, with studies showing promising results for the use of CAR-NK cells in vitro and in animal models against some hematological malignancies and solid tumors as well as potential advantages in CAR-NK cell production compared to CAR-T cells.[96-105] One clinical trial assessing anti-CD19 CAR-NK cells for the treatment of patients with relapsed or refractory B-lineage ALL has been completed, awaiting results (NCT00995137). In this study, a second-generation CAR design with 4-1BB co-stimulatory domain linked to the CD3 zeta chain was used, which is identical to CAR constructs used in other clinical trials currently underway (eg, NCT01974479).[106] Third-generation CAR designs incorporating CD28 and 4-1BB costimulatory domains linked to the CD3 zeta chain are also being tested on different targets, including CD7, CD33, and MUC1-positive tumors (eg, NCT02742727, NCT02944162, NCT02839954). The safety and efficacy of umbilical and cord-blood-derived CAR-NK cell infusions administered after chemotherapy in stem cell transplant patients with lymphoid malignancies will be tested in a clinical trial that is currently recruiting (NCT03056339).

CAR-modified NK cells may provide several advantages compared to their T-cell counterparts. NK cells have a shorter life span and secrete a safer cytokine profile compared to T cells, which might lower the risk and severity of adverse effects relating to autoimmunity and CRS.[107] A shorter life span might also negate the need for a suicide or off-switch to clear the cells after infusion. Their limited time in vivo however, also raises concern about their potential efficacy. Other concerns relating to the use of CAR-NK cells in cancer therapy include uncertainty about their ability to migrate to tumor sites, their sensitivity to cryopreservation, low transfection efficiency, and the challenge of acquiring large numbers in vitro.[108] Some of these challenges can be addressed using NK cell lines for genetic modification. The delivery of IL-2, IL-12, IL-15, and stem cell factor (SCF) genes into human NK cell lines like NK-92, results in cells with a higher capacity for proliferation, cytokine production and cytolysis against a wider range of targets compared to parental cells in the absence of exogenous cytokines in vitro and in animal models.[109-114] CAR-modified NK92 cells have shown promising results in preclinical studies.[115,116] Anti-CD19 CAR-NK-92 cells were shown to exert enhanced cytotoxicity against CD19 positive leukemic cell lines and primary leukemia cells.[117] Preclinical studies in mice on receptor tyrosine-protein kinase ErbB2 (HER2)-CAR-modified NK-92 cells showed encouraging results against solid tumors like glioblastoma.[118-120] Since NK-92 cells require irradiation to render them replication incompetent prior to administration, a recent study assessed the effects of irradiation on genetically modified NK-92 cells and demonstrated that ErbB2-CAR NK cells tested in a mouse model for lung metastasis retain their cytolytic capability after irradiation. Similarly, NK-92 cells do not express CD16 to mediate antibody-dependent cellular cytotoxicity (ADCC) or IL-2, which is necessary for their proliferation and survival. Thus, a clinical study using NK-92 cells genetically modified to express CD16 and retain IL-2, high-affinity NK (haNK) cells combined with monoclonal antibody therapy is currently underway (NCT03027128). Several clinical studies using CAR-NK92 cells are ongoing, and the next decade should yield important clinical results that will help us assess their overall potential for efficacy against cancer.

Preclinical studies investigating the antitumor activity of CAR-CIK cells have shown promising results against different cancer targets.[121-125] Preclinical evaluation of allogeneic CD19 specific CAR-CIK cells modified with a nonviral gene delivery approach showed encouraging results for the treatment of ALL,[126] which is now being tested in an ongoing phase I/II clinical trial treating ALL patients after allogeneic stem cell transplant (NCT03389035). Preclinical CAR-NKT cell studies have also yielded promising results on the potential of these cells to be used as autologous and allogeneic off-the-shelf cancer treatment, but no clinical trials involving CAR-NKT cells have been conducted to date.[127]

6 | CONCLUSION

ACT-based clinical trials involving killer cells have delivered remarkable clinical outcomes in some cancer patients particularly in hematological malignancies over the last few decades, and recent reports on novel approaches using NK-, CIK-, and NKT-cell-based therapies have shown encouraging signs of clinical efficacy. The genetic modification of killer cells, particularly CAR-NK cells has attracted much attention based on their potential ability to overcome some of the shortcomings of T-cell-based toxicities. Still, there remain many challenges in identifying the therapeutic conditions that harness the full antitumor potential of these effector cells in vivo and render them more optimal "off-the-shelf" products to a wider range of donors. The coming decade is likely to see accelerated clinical development of combination strategies and therapeutic conditions that influence the tumor microenvironment, as well as new cell sources that mitigate some of the challenges of blood-derived killer cells. Ultimately, the results of these studies will give important insights that will hopefully lead to novel therapeutic options that prevent recurrence and improve survival and quality of life in cancer patients.

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