Review

Xiangyu Zhao, Minghao Lin and Xiaojun Huang*

Current status and future perspective of natural killer cell therapy for cancer

https://doi.org/10.1515/mr-2023-0031
Received July 20, 2023; accepted September 23, 2023;
published online October 24, 2023

Abstract: Natural killer (NK) cells possess innate abilities to effectively eliminate cancer cells. However, because of difficulties of proliferation and easy to be induced dysfunction in the setting of cancer post NK cell therapy, the curative effect of NK cell infusion has been constrained and not been widely applicable in clinical practice. The rapid development of biotechnology has promoted the development of NK cell therapy for cancer treatment. In this review, we will provide a comprehensive analysis of the current status and future prospects of NK cell therapy for cancer, focusing on the biological characteristics of NK cells, as well as strategies to enhance their targeting capabilities and overcome tumor immune suppression within the microenvironment.

Keywords: natural killer cell therapy; hematological malignancies; solid tumors; CAR-NK cell therapy

Introduction

Natural killer (NK) cells are a type of innate lymphoid cells (ILCs) and the third major subset of lymphocytes in the body in addition to T cells and B cells, accounting for approximately 10 %–15 % of the total peripheral blood (PB) lymphocytes. They are widely distributed in PB, liver, spleen, and other tissues, participating in forming the first line of defense in the human immune system. NK cells originate from CD34+ hematopoietic progenitor cells and undergo development into common lymphoid progenitor cells, eventually maturing into NK cells characterized by the upregulation of CD56 and downregulation of CD34 [1–3]. Unlike adaptive immune cells, NK cells possess the unique ability to rapidly respond to threats without requiring antigen stimulation or being restricted by major histocompatibility complex (MHC). Through the secretion of perforin and granzymes, as well as exerting antibody-dependent cell-mediated cytotoxicity (ADCC), NK cells have the ability to directly or indirectly eliminate target cells. Additionally, they can release a range of cytokines and chemokines, which play crucial roles in anti-tumor and anti-viral immune responses. In recent years, NK cell therapy has emerged as a promising approach in the field of anti-tumor treatment, with numerous preclinical trials and studies had been published or currently undergone [4, 5].

In this review, we will discuss the current status of biological research pertaining to NK cell anti-tumor activity, and address the challenges encountered by NK cells in the context of cancer treatment, particularly their susceptibility to dysfunction. Furthermore, we will provide a detailed overview of the application of NK cell-based therapies in the field of oncology, seen in Figure 1. These therapies encompass enhancing NK cell activity therapy and adoptive NK cell therapy, which includes purified NK cell therapy, expanded NK cell therapy, and chimeric antigen receptor (CAR)-NK cell therapy. By examining the existing literature and ongoing developments, we will shed light on the potential of NK cell-based approaches for combating cancer.

Biological study of anti-tumor effect of NK cells

NK cells participate in immune responses to tumors by killing target cells and producing cytokines [6]. NK cells express a variety of activating and inhibitory receptors, and the balance of signals mediated by these receptors determines the outcome of NK-cell activation [7]. Roles of NK cells were important for the tumor control, and prognostic values of NK cells were explored in different kinds of tumors,

***Corresponding author: Xiaojun Huang**, Peking University People's Hospital, Peking University Institute of Hematology, Beijing Key Laboratory of Hematopoietic Stem Cell Transplantation, National Clinical Research Center for Hematologic Disease, Beijing 100044, China,
E-mail: xjhrm@medmail.com.cn. https://orcid.org/0000-0002-2145-6643
Xiangyu Zhao and Minghao Lin, Peking University People's Hospital, Peking University Institute of Hematology, Beijing Key Laboratory of Hematopoietic Stem Cell Transplantation, National Clinical Research Center for Hematologic Disease, Beijing, China. https://orcid.org/0000-0002-8139-1773 (X. Zhao). https://orcid.org/0009-0003-9090-7959 (M. Lin)

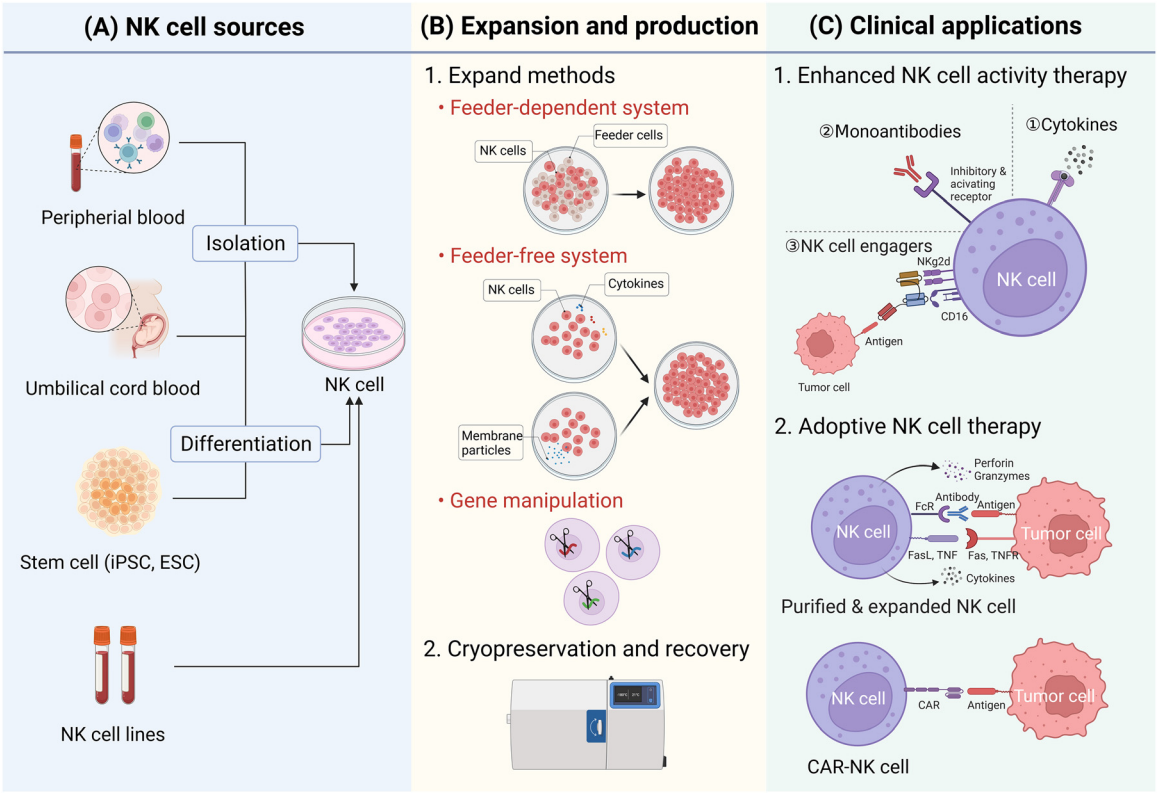

Figure 1: Natural killer (NK) cell therapy: an overview. (A) The main NK cell sources for NK cell therapy. (B) NK cells can be expanded *in vitro* through various methods. (C) Multiple therapeutic approaches based on NK cell therapy for anti-tumor treatment. iPSC, induced pluripotent stem cell; ESC, embryonic stem cell; CAR, chimeric antigen receptor (created with BioRender.com).

including hematological malignancies and solid tumors. Impaired NK cell activity and the capability of secretion cytokines, such as interleukin (IL)-1β, interferon (IFN)-γ and IL-2, have been associated with early relapse of acute leukemia [8]. In acute myeloid leukemia (AML), the rapid recovery of NK cells post chemotherapy has been linked to improved leukemia-free survival (LFS). AML blasts exhibit heterogeneous expression of NK receptor ligands (NKRLs), including 6 activating (MHC class I chain-related protein A and B (MICA, MICB), CD155, CD112, UL16 binding protein (ULBP)1, and ULBP2/5/6) and 3 inhibitory (HLA class I, programmed cell death ligand 1 (PD-L1), and PD-L2) NKRLs. Notably, ULBP1 expression was significantly associated with improved 2-year overall survival (OS), relapse-free survival (RFS) and reduced relapse rates [9]. Moreover, cytomegalovirus (CMV) infection induced the expansion of memory-like NK cells (CD56dimCD57+NKG2C+ NK cells) at 6 months post-transplantation, which independently correlated with a lower 2-year relapse risk [10]. Moreover, Nersesian et al. conducted the first meta-analysis on NK cell infiltration in solid tumors to assess its prognostic value for OS, and discovered a strong association between NK cell infiltration and a decreased risk of death [11]. In lung cancer, NK cell infiltration has shown a significant and positive association with tumor high Scarff-Bloom-Richardson (SBR) grade. In resected pulmonary adenocarcinoma, count of NK cells was significantly related to the regulation of tumor progression, involving T classification, N classification, and stage [12]. NK cells have also been significantly associated with human epidermal growth factor receptor 2 (HER2)-positive and triple-negative breast cancer subtypes. At a median follow-up of 5.5 years, high CD56 expression (≥5 cells/10 high-power field) was associated significantly with improved OS and disease-free survival (DFS) [13]. In addition, NK cells in the blood were an independent predictor of survival in colorectal cancer patients. A higher percentage of NK cells indicated a longer survival time compared to a lower percentage [14]. In patients with HER2+ advanced gastric cancer treated with first-line fluoropyrimidine-platinum doublet plus trastuzumab, low baseline NK cell activity was associated with worse progression-free survival (PFS) and OS than high baseline NK cell activity [15].

However, there are a lot of factors that influence the anti-tumor effect of NK cells, as depicted in Figure 2. Firstly,

the maturation status of NK cells plays a crucial role, as mature NK cells exhibit a stronger anti-tumor ability [16, 17]. The development and functional maturation of NK cells are complex process involving multiple cytokines (e.g., IL-15, IL-3 and IL-7), transcription factor (e.g., Ras–MEK–MAPK, JAK–STAT5, and PI3K–AKT–mTOR) and so on [6, 18–21]. For instance, Wang et al. performed conditional deletion of mechanistic/mammalian target of rapamycin complex 1 (mTORc1) and complex 2 (mTORc2) in mice, demonstrating that mTORc1 played a positive role in NK cell maturation and effector functions while mTORc2 negatively regulates NK cell cytotoxicity in a synergistic and non-redundant manner [20]. Meissl et al. disclosed the significance of signal transducer and activator of transcription 1 (STAT1) isoforms (STAT1α and STAT1β). In gene knockout mice, the absence of STAT1α rather than STAT1β was related to defects in IFN-γ signaling, leading to reduced surface levels of IL-15 receptor α in splenic dendritic cells, monocytes and macrophages and further impaired NK cell maturation [21]. Secondly, NK cell education correlated with enhanced anti-tumor effect. NK cell education refers to the interaction between inhibitory NK cell receptors (e.g., Killer Immunoglobulin-like receptors (KIR), NKG2A, and T cell immunoglobulin and ITIM domain [TIGIT]) and self-MHC-I molecules, allowing NK cells to obtain an immune response. Recently, some researches have shown that nonclassical MHC and non-MHC ligands also play an important role in NK cell education [22]. NK cell education is regulated by a combination of inhibitory signals, activating signals, and adhesion molecule signals, representing functional status of NK cells. Several models have been proposed to explain the mechanisms of this procedure [23, 24]. To investigate why educated NK cells have stronger function, our team prospectively enrolled 114 malignant hematological patients who underwent haploidentical hematopoietic stem cell transplantation (HSCT) to study the expression of activating receptors on the surface of reconstituted NK cells with the interaction of KIR with both donor HLA and recipient HLA [25]. We demonstrated that the expression of the activating receptor DNAX accessory molecule-1 (DNAM-1) was modulated by both donor and recipient KIR/MHC-I interaction. Additionally, *in vitro* studies have shown that compared to uneducated NK cells, educated NK cells are associated with high intensity expression of DNAM-1, NKP30, and NKG2D, resulting in stronger function. However, NK cell education is remolded by its environment factors, and phenotypic changes could not always consistently identify NK cell subsets and functional fate [26]. In addition, changes in cellular metabolism can distinguish NK cell education status in some way. A higher expression of glucose transporter and higher rate of glycolysis was observed in educated NK cells [27, 28]. Thirdly, the memory status of NK cells is another factor correlated with sustained anti-tumor effect of NK cells. Multiple studies have shown the existence of memory and memory-like functionality in NK cells after exposure to haptens, chronic viral infection, or cytokines [29, 30]. Cytokine-induced memory-like (CIML) NK cells can impart increased longevity and enhanced anti-cancer functionality through short priming of cytokine combination of IL-12, IL-15 and IL-18 *in vitro* [30]. Mature NK cells have a short lifespan of about 2 weeks post infusion, while memory NK cells induced by human CMV can survive for at least 7 months post-infusion [31]. By analyzing the phenotypic characteristics of CIML, it can be found that the expression of CD25 and semaphorin 7A are upregulated, while the expression of KIRs (e.g., KIR2DL2/L3, KIR2DL1 and KIR3DL1) and transforming growth factor (TGF)-β receptors are downregulated, which may help enhance anti-tumor response [32, 33]. Lastly, the dysfunction induced by tumor as well as immune suppressive microenvironment correlated with poor tumor control [34]. There are multiple factors contributing to NK cell dysfunction, and more detailed information will be elaborated in the following text. Therefore, in the future study, focused on the biological study of NK cells would be help to improve the curative effect of NK cell therapy.

NK cell dysfunction in the setting of cancer

NK cell dysfunction refers to a condition in which NK cells cannot function optimally or exhibit impaired activity. In new diagnosis AML patients, NK cell dysfunction was characterized by excessive maturation and downregulation of activating receptors (e.g., NKP30, NKP46, and NKG2D) [35–38]. Effective therapeutic response following chemotherapy correlated with NK function restoration. Refractory or relapsed (R/R) patients demonstrated even worse immune impairments [39]. Compared with the normal control, NK cells from patients with B- and T-cell acute lymphoblastic leukemia (B/T-ALL) were also exhausted with less cytotoxic but exhibit an activated signature that was characterized by high CD56, high CD69, production of activated NK cell-origin cytokines, which was likely caused by their chronic activation. Increased frequencies of activated cytokine-producing NK cells were associated with increased disease severity and independently predicted poor clinical outcome in patients with ALL [40].

There are multiple mechanisms that contribute to NK cell dysfunction. Higher levels of active TGF-β1 have been

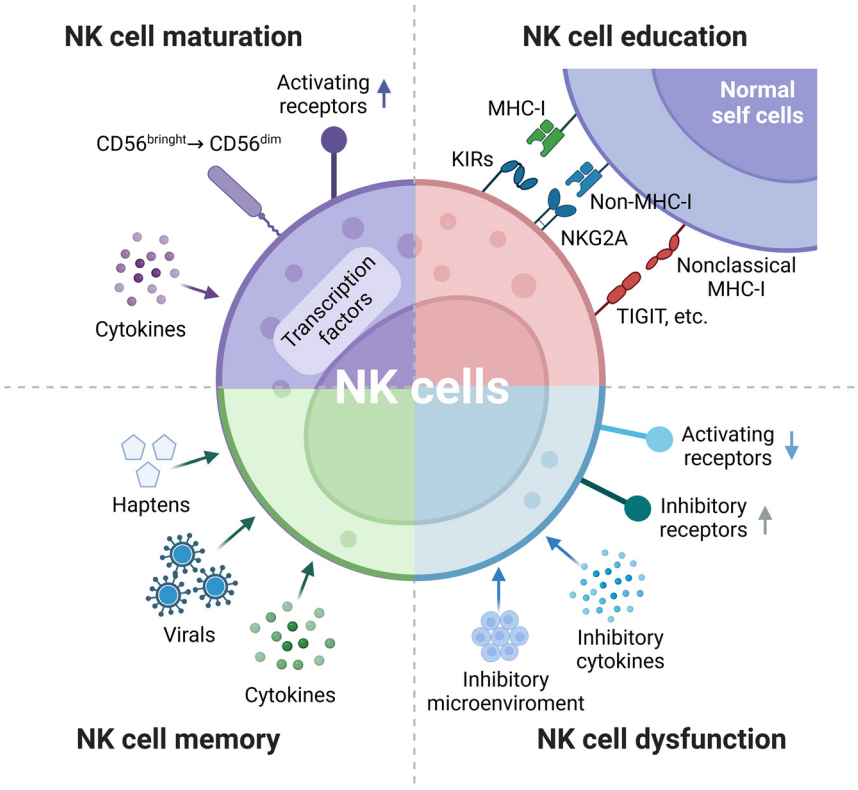

Figure 2: Schematic illustration of factors influencing the anti-tumor effect of natural killer (NK) cells. MHC, major histocompatibility complex; KIRs, killer inhibitory receptors; NKG2A, natural killer group 2, member A; TIGIT, T cell immunoreceptor with immunoglobulin and ITIM domains (created with BioRender.com).

linked to impaired effector function of bone marrow NK cells (BMNK) in AML patients. TGF-β1 activation was induced by the overexpression of glycoprotein A repetitions predominant (GARP), which was involved with a novel TGF-β processing pathway, on the surface of CD4+ T cells. Active TGF-β1 significantly suppressed mTORC1 activity, mitochondrial oxidative phosphorylation, the proliferation, and cytotoxicity of BMNK cells. Preclinical study has revealed that inhibition of the TGF-β1 pathway using galunisertib, a clinical inhibitor, would conversely restore mTORC1 activity, mitochondrial homeostasis, and cytotoxicity of NK cells *in vitro*, and improve the antitumor activity in a leukemia xenograft mouse model [41]. A similar relationship between TGF-β and NK cell dysfunction has been observed in breast cancer patients. There are also reduced IFN-γ production and cytotoxicity with clear metabolic deficits in PB NK cells and expression of GARP, and latency associated peptide (LAP) was increased on NK cells. Thus, blocking TGF-β and/or GARP can restore NK cell metabolism and function and is an important target for improving NK cell-based immunotherapies [42]. What's more, in the setting of glioblastoma multiforme (GBM), the most aggressive brain cancer, the glioblastoma stem cells (GSCs) are sensitive to lysis by healthy allogeneic NK cells *in vitro*. However, primary tumor samples revealed that GBM tumor-infiltrating NK cells acquired an altered phenotype associated with impaired lytic function relative to matched PB NK cells from patients with GBM or healthy donors. This immune evasion tactic to direct cell-to-cell contacts between GSCs and NK cells via αv integrin-mediated TGF-β activation. Treatment of GSC-engrafted mice with allogeneic NK cells in combination with inhibitors of integrin or TGF-β signaling or with *TGF-βR2* gene-edited allogeneic NK cells prevented GSC-induced NK cell dysfunction and tumor growth [43]. In addition, hypoxia is a common feature of solid tumors, resulting in upregulation of the transcription factor hypoxia-inducible factor (HIF)-1α in NK cells, which was also associated with NK cell dysfunction. Conditional deletion of HIF-1α in NK cells leaded to reduced tumor growth, elevated expression of activation markers, effector molecules, and an enriched NF-κB pathway in tumor-infiltrating NK cells. IL-18 from myeloid cells was required for NF-κB activation and the enhanced anti-tumor activity of HIF-1α (–/–) NK cells. Extended culture with an HIF-1α inhibitor increased human NK cell responses and could be exploited for cancer therapy [44].

Besides dysfunction, NK cells can transform to regulatory cell and inhibit the anti-tumor effect of T cells through expression of immune checkpoint receptor CD73. These

CD73-positive NK cells undergo transcriptional reprogramming and upregulate IL-10 production via STAT3 transcriptional activity, suppressing CD4-positive T cell proliferation and IFN-γ production [45]. IL-22, a cytokine produced by T helper (Th) 17 cells, gamma delta (γδ) T cells, invariant natural killer T (iNKT) cells, and ILCs, is known to promote cancer cell growth, enhance migration, protect from apoptosis, induce epithelial-to-mesenchymal transition, and sustain stemness of malignant cells [46–48]. Meanwhile, IL-22 mediated immunosuppression in the metastatic niche by promoting the expression of CD155 on cancer cells, which was associated with decreased expression of CD226 on NK cells and reduced IFN-γ production. Therefore, an IL-22-CD155 axis triggers decreased expression of CD226 in NK cells and renders them inert in the tumor microenvironment, and blocking CD155 would be help for cancer control [49]. In hepatocellular carcinoma (HCC), dysfunction of both tumor-infiltrating NK cells and liver resident NK cells was mediated by T cell immunoglobulin and mucin domain 3 (Tim-3)-mediated PI3K/mTORC1 interference. Blockade of Tim-3 has shown to retard HCC growth in an NK cell-dependent manner, suggesting a potential strategy for immune checkpoint-based targeting [50].

In conclusion, how to avoid tumor induced NK cell dysfunction during NK cell therapy would be important to better improve the therapeutic effect of NK cell therapy in the future study.

Enhanced NK cell activity therapy for cancer

Enhancing the activity of NK cells can be achieved through several primary methods, including the use of exogenous activating cytokines, monoclonal antibodies targeting NK cell surface receptors, and NK cell engagers. Notably, exogenous cytokines such as IL-2, IL-15, and TGF-β have been extensively studied and proven to effectively induce NK cell proliferation and enhance their cytotoxic activity. In the realm of clinical research, exogenous cytokines are primarily employed through three distinct approaches. Firstly, they can be utilized to stimulate the ex vivo expansion of NK cells, which serves as the foundation for high-dose NK cell infusion therapy [51]. Secondly, cytokines can be administered intravenously to trigger the activation of NK cell activity *in vivo*. In a phase I multicenter trial led by Romee et al., the IL-15 superagonist complex ALT-803 was investigated in patients who experienced relapse after undergoing allogeneic HSCT for more than 60 days. The trial involved the administration of ALT-803 once a week to 33 patients, either intravenously or subcutaneously, for a total of 4 doses. The results of the study demonstrated that ALT-803 effectively stimulated the activation, proliferation, and expansion of NK cells and CD8+ T cells, while not increasing the levels of regulatory T cells. Additionally, a positive treatment response was observed in 19 % of the patients who were evaluated [52]. Lastly, cytokines can be expressed in NK cells through genetic modification. This approach involves introducing specific genes into NK cells to enable them to produce cytokines internally [53]. In addition, targeting the inhibitory and activating receptors on the surface of NK cells is a crucial component of tumor immunotherapy. Currently, monoclonal antibody therapies targeting PD-1/PD-L1 and NKG2A have been widely utilized in clinical practice [54, 55]. It is worth mentioning that in clinical research, monoclonal antibodies are often used in combination with cytokine therapy or adoptive NK cell transfer therapy.

NK cell engagers refer to engineered antibodies that are derived from the structure of monoclonal antibodies and have been modified to target multiple tumor antigens and NK cell receptors. These agents are composed of two essential components: a targeting moiety that binds to specific antigen on cancer cells, and an activating moiety that binds to NK cells. Some pre-clinical studies have demonstrated that NK cell engagers facilitate the selective destruction of cancer cells by stimulating the cytotoxic activity of NK cells by acting as a bridge between cancer cells and NK cells. For instance, in a study conducted by Arvindam et al. [56], a tri-specific killer engager (TriKE) molecule called CLEC12A TriKE was developed. This molecule consists of a humanized anti-CD16 heavy chain camelid single-domain antibody (sdAb) to activate NK cells, an IL-15 molecule to promote NK cell proliferation, activation, and survival, and a single-chain variable fragment (scFv) that specifically targets human CLEC12A, a myeloid antigen highly expressed on AML cells. The researchers demonstrated through *in vitro* experiments that CLEC12A TriKE effectively stimulated NK cell proliferation, enhanced NK cell activation, and induced the death of AML cell lines, while preserving the viability of healthy bone marrow cells. Moreover, CLEC12A TriKE demonstrated its potential in reducing tumor burden in preclinical mouse xenograft models [56]. Gauthier et al. reported the efficacy of TriKE in targeting NK cell activation receptors NKp46, CD16, and tumor antigens. The preclinical study involved *in vivo* experiments, which demonstrated that when used to treat solid tumors or invasive tumors, TriKE exhibited a remarkable capability to reduce tumor size. Notably, the anti-tumor effects of these engagers were found to be superior to those of tumor antigen-specific monoclonal antibodies [57]. In addition, a phase 2, open-label,

multicenter study (NCT04101331) conducted in 2019 evaluated the efficacy of AFM13, a bispecific CD30/CD16 antibody, in patients with R/R peripheral T cell lymphoma. A total of 108 patients were enrolled in the study and received weekly intravenous infusions of AFM13 at a dose of 200 mg. The median number of infusions administered was 9.0, ranging from 1 to 116. The study reported an overall response (OR) rate of 32.4 %, with a complete response (CR) rate of 10.2 %. The median duration of response, PFS, and OS were found to be 2.3, 3.5, and 13.8 months, respectively [58]. Furthermore, NK cell engagers are also commonly combined with NK cell adoptive transfer or other therapies to enhance their antitumor activity [59–61].

Purified NK cell therapy for cancer

Purified NK cells therapy for cancer comes from autologous or allogeneic NK cells. In an early clinical trial, 8 patients with metastatic melanoma or renal cell carcinoma were treated with adoptively transferred *in vitro* activated autologous NK cells with an average of 4.7×10^{10} ($\pm 2.1 \times 10^{10}$) NK cells after the patients received a lymphodepleting but nonmyeloablative chemotherapy regimen. The infused NK cells exhibited high levels of lytic activity *in vitro*. Although the adoptively transferred NK cells seemed to persist in the peripheral circulation of patients for at least one week post transfer and, in some patients, for several months, no clinical responses were observed [62]. This lack of efficacy may be attributed to the absence of NK cell inhibitory receptor mismatching with autologous tumor cells. To overcome this limitation, allogeneic NK cell infusions have become a promising approach. By 2005, Miller JS et al. reported the outcomes of patients receiving haploidentical, related-donor NK cell infusions after lower intensity regimens in a nontransplantation setting. This resulted in transient persistence but no *in vivo* expansion of donor NK cells. In contrast, when a more intense regimen of Hi-cyclophosphamide/fludarabine (Hi-Cy/Flu) was used, there was a marked rise in endogenous IL-15, leading to the expansion of donor NK cells and induction of complete hematologic remission in 5 of 19 poor-prognosis patients with AML. These findings suggest that haploidentical NK cells can persist and expand *in vivo* and may have a role in the treatment of selected malignancies used alone or as an adjunct to HSCT [63].

In order to enhance the efficacy of allogeneic NK cells infusions, *in vivo* expansion of haploidentical NK cell infusions with IL-2 can induce remission of refractory AML, but efficacy may be hampered by concurrent stimulation of host regulatory T cells [64, 65]. To overcome this limitation, 42 patients received either intravenous or subcutaneous recombinant human IL-15 (rhIL-15) after lymphodepleting chemotherapy and haploidentical NK cells. Escalating doses of rhIL-15 (0.3–1.0 μg/kg) were given on 12 consecutive days in a phase 1 trial. Of 26 patients, 36 % had robust *in vivo* NK-cell expansion at day 14, and 32 % achieved CR. Hypothesizing that subcutaneous dosing of rhIL-15 would be safer and better tolerated, 16 patients received 10 once per day doses of subcutaneous rhIL-15 at 2.0 μg/kg on a phase 2 trial. NK cell expansion at day 14 was seen in 27 % of the patients, and 40 % achieved remission. RhIL-15 induced better rates of *in vivo* NK cell expansion and remission compared with previous trials with IL-2, but it was associated with previously unreported cytokine release syndrome (CRS) after subcutaneous but not intravenous dosing. CRS was observed in 56 % of patients given stem cell (SC) rhIL-15 (with concurrent neurologic toxicity in 5 of 9 patients) and was responsive to steroids and tocilizumab. SC administration was associated with slower pharmacokinetic clearance and higher levels of IL-6 than IV dosing [66]. Maintenance of an exhausted T cell state at day +14 has been shown to permit haploidentical NK cell expansion, suggesting that selectively depleting recipient T cells or modulate their dysfunction would be help for NK cells expansion [67].

In a study involving 16 myelodysplastic syndrome (MDS) patients, fludarabine/cyclophosphamide (Flu/Cy) conditioning combined with total lymphoid irradiation was followed by adoptive immunotherapy with IL-2-activated haploidentical NK cells. The NK cell infusions were well-tolerated, with only transient adverse events observed in 16 patients. six patients achieved objective responses with CR, marrow CR, or partial remission (PR). Five of these patients proceeded to allogeneic HSCT. Three patients remain DFS over 3 years after treatment. All evaluable patients with objective responses (5/5 evaluable) had detectable donor NK cells at days 7/14 following infusion and displayed reduction of tumor cell clones, some of which carried poor prognosis mutations. This suggested that high-risk MDS was responsive to NK cell therapy and supported the use of haploidentical NK cell infusions as a bridge to HSCT in refractory patients. Responding patients displayed less pronounced activation of CD8+ T cells and lower levels of inflammatory cytokines following NK cell infusions, further highlighting the potential benefits of depleting recipient T cells or modulating their dysfunction to enhance the efficacy of NK cell therapy [68]. In another study involving 17 AML patient in first CR (median age 64 years, range 53–73), NK cells from haploidentical KIR-ligand mismatched donors were administered after Flu/Cy chemotherapy, followed by IL-2. All patients with molecular disease achieved molecular CR. A significantly higher number of donors alloreactive NK

cell clone was observed in responders over non-responders. The infusion of a higher number of alloreactive NK cells was associated with prolonged DFS [69].

As previous mentioned, NK cells exhibit memory like properties with an enhanced recall response after cytokine pre-activation [70]. CIML NK cellular therapy has been shown to be safe and induces remissions in AML patients, with the potential for long-lasting effects [71]. Mass cytometry revealed an *in vivo* memory-like NK-cell phenotype, where NKG2A served as a dominant checkpoint, and CD8α was associated with treatment failure after CIML NK cell therapy [72]. Meanwhile, PB NK cells from allogeneic healthy donor or patients with advanced melanoma can be differentiated into ML NK cells for use as a novel immunotherapeutic treatment for advanced melanoma, which warrants testing in early-phase clinical trials [73].

Expanded NK cell therapy for cancer

Besides purified NK cell therapy, the rapid development of ex vivo NK cells expansion has facilitated the clinical study of clinical grade expanded NK cells for cancer treatment. Expanded NK cells can be derived from a wide range of sources, including PB, umbilical cord blood (UCB), NK cell lines, as well as stem cells, such as human embryonic stem cell (hESC) and induced pluripotent stem cell (iPSC). Each source of NK cells has its own unique advantages and limitations, as summarized in Table 1. And there are several methods that can be used to expand NK cells derived from various cellular sources, including cytokine stimulation, co-culture with feeder cells, magnetic bead selection and genetic modification [74].

The most efficient way to expand NK cell is based on feeder cells. In our previous study, we demonstrated that expansion for 2 and 3 weeks produced approximately 4 and 8×10^9 NK cells from 2×10^7 PB mononuclear cells with by co-culture with K562 cells transfected with 4-1BBL and membrane-bound IL-21 (mbIL-21/4-1BBL NK). The NK cell products generated through this expansion method exhibited higher levels of CD107a and TNF-α production in response to AML cell lines and primary blasts compared to resting NK cells. When the 2-week expanded NK cell products were xenografted into immunodeficient mice with leukemia, they were persistently detected in the BM, spleen, liver, lung, and PB for at least 13 days. Furthermore, these expanded products reduced AML burden *in vivo*. Compared with matched AML patients with persistent or relapsed minimal residual disease positive (MRD (+) who underwent regular consolidation therapy, MRD (+) patients who underwent NK treatment had better OS and showed no major adverse events. Meanwhile, repeated infusions of mbIL-21/4-1BBL NK cells every 7 days showed a stronger anti-leukemia effect, providing valuable insights for the design of further clinical studies [75]. NK cells from healthy donors and myeloma patients could expand a median of 804- and 351-fold, respectively by co-culture with K562 cells transfected with 4-1BBL and membrane-bound IL-15. These expanded NK cells could kill both allogeneic and autologous primary myeloma cells avidly via a perforin-mediated mechanism in which the activating receptor NKG2D, natural cytotoxicity receptors, and DNAM1 played a central role. Furthermore, when transferred into the body, these expanded NK cells continued to proliferate *in vivo* in an IL-2 dose-dependent manner and could persist for up to 4 weeks. They were readily detectable in the human BM and had been shown to inhibit myeloma growth while also protecting the bone from myeloma-induced osteolysis [76].

In addition to feeder cells, another commonly used method for expanding NK cells is pure cytokine culture. Tschan-Plessl et al. conducted a study where they demonstrated that NK cells, expanded with IL-2 and IL-15 under Good Manufacturing Practice (GMP) conditions, exhibited a remarkable 56.0-fold increase in proliferation (ranging from 37.4- to 75.5-fold). In their study, 10 myeloma patients

Table 1: Advantages and limitations of natural killer (NK) cell sources.

NK cell source	Advantages	Limitations
PB	– Mature phenotype – High tumor-killing ability – High safety and low toxicity	– Need for cell isolation and expansion *in vitro* – Potentially immunosuppression (auto-PB)
UCB	– Easy to collect and store – High proliferation and persistence potential	– Immature phenotype – Low tumor-killing ability
NK cell lines	– Easy to obtain (NK92, KHYG-1, NKL, YT) – High tumor-killing ability due to low expression of inhibitory receptors – Homogeneous cell composition	– Requires irradiation owing to possible tumorigenicity – Short lifespan – Impaired ADCC activity due to low or no expression of CD16
hESC	– Integrated the advantages of primary NK cells and NK cell lines	– High technical difficulty – Uncertain safety and clinical efficacy
iPSC	– Integrated the advantages of primary NK cells and NK cell lines – Better availability than hESC	– High technical difficulty – Uncertain safety and clinical efficacy

PB, peripheral blood; UCB, umbilical cord blood; hESC, human embryonic stem cell; iPSC, induced pluripotent stem cell.

were treated with induction therapy followed by high-dose melphalan (200 mg/m^2) at day 1, autologous stem cell transplantation (ASCT) at day 0 and escalating doses of NK cells (1.5×10^6, 1.5×10^7, and multiple doses of 1.0×10^8 cells/kg body weight) from day +1 to day +30 after ASCT. Donor NK cells were detectable in the PB, peaking 1 h after each dose, with up to 90 % of the NK cells being of donor origin. Importantly, this treatment was found to be safe and well tolerated, with no evidence of graft-versus-host disease (GVHD). And comparison with a control group of patients who received ASCT without NK cells infusions showed no significant difference in relapse, PFS and OS [77]. Similarly, Heinze et al. investigated the cytotoxic capabilities of IL-activated NK cells compared to cytokine-induced killer (CIK) cells for treating neuroblastoma (NB) [78]. The separated NK cells from PB mononuclear cells (PBMCs) were expanded with various cytokines (e.g., IL-2, IL-15, and/or IL-21) in feeder-cell free conditions. CIK cells were generated by stimulating PBMCs with IFN-γ, IL-2, OKT-3, and IL-15. After 10–12 days of expansion, NK cell preparations exhibited significantly higher median cytotoxicity against NB cells compared to CIK cells [78].

In contrast to obtaining NK cells from PB, there are two distinct approaches for acquiring a significant quantity of NK cells from UCB. The first approach involves directly isolating and expanding NK cells from UCB. The second approach involves inducing the differentiation of CD34 hematopoietic stem and progenitor cells (HSPCs) derived from cord blood into NK cells, followed by subsequent expansion of the NK cells [79]. Additionally, when working with stem cells, it is necessary to utilize a differentiation system to generate NK cells, which may involve the use of a feeder system or a feeder-free system [80]. However, the primary method for expanding NK cell lines still relies on cytokine-dependent culture methods, particularly IL-2 [81]. Importantly, studies have shown that expanded NK cells derived from UCB, stem cells and NK cell lines were effective to kill tumor cells [82, 83]. More research is imperative to elucidate the variances in cytotoxic functions and the underlying factors among NK cells derived from different sources within diverse tumor environments, which will provide a solid groundwork for improved implementation of NK cell therapy in clinical settings.

CAR-NK cell therapy for cancer

CAR-NK cell therapy is a promising approach in which NK cells are artificially constructed NK cell activation signaling pathways through gene modification to specifically enhance their anti-tumor capabilities. Compared to CAR-T cell therapy, CAR-NK cell therapy offers superior safety, a broader range of cell sources, multiple killing pathways, and wider indications. It also holds significant potential for development as off-the-self product.

Construction of CAR-NK cell system

CAR structure are antibody-based hybrid receptors engineered to recognize specific ligands on the surface of target cells. All CAR constructs consist of an extracellular antigen binding region, transmembrane region, and intracellular signal region [84]. The ectodomain comprises a signal peptide and a scFv, typically derived from heavy and light chains of a monoclonal antibody. The scFv endows CAR-NK cells with the ability to specifically recognize and bind to target antigens in a manner independent of MHC. So far, a variety of scFv-recognized antigens have been designed according to the characteristics of different tumor types. The transmembrane domain anchors the CAR structure on the surface of effector cells, commonly using sequences from CD28, CD8 or CD3ζ. Upon recognition and activation by the specific antigens, the intracellular signal region will be activated, resulting in downstream processes that facilitate tumor cells killing. The structure of intracellular signal region determines the generation of CAR, and it has been developed to the fourth generation. The first generation of CAR-NK cells contains only a signaling domain (e.g., CD3ζ, DAP-10, DAP-12, or FcεRLγ), while the second and third generations carry one and two additional co-stimulatory signals, respectively (e.g., CD28, 4-1BB, or 2B4). The fourth generation of CAR is further modified with a constitutive or inducible expression cassette for a transgenic protein, such as cytokine, to address the inherent limitations of immune cell therapies [85].

Application of CAR-NK cell therapy in tumor control

The effectiveness of CAR-NK cells against malignant tumors has been confirmed in numerous *in vitro* and *in vivo* preclinical studies, laying the foundation for the initiation of further clinical trials. At this stage, preclinical studies on CAR-NK cells are focused on developing CAR structures and improving therapeutic efficacy without compromising the safety.

In the context of hematological malignancies, multiple targeted antigens have been designed and conducted based on the phenotypic characteristics of tumor cells, including CD19, CD20, and CD22 for ALL, B cell maturation antigen (BCMA), CD38, and CD138 for multiple myeloma (MM), and

CD33, CD123, and FLT3 for AML. In a preclinical trial, Caruso et al. developed an off-the-shelf product derived from PB NK cells, and engineered it with the second-generation CD123--CAR incorporating 4-1BB as costimulatory domain. Researchers confirmed that CD123-CAR-NK cells had significant anti-leukemia ability through an animal model of human AML-bearing immune-deficient mice. Moreover, they evaluated the efficacy and safety of CD123-CAR-T cells vs. CD123-CAR-NK cells. No difference in OS was observed in mice treated with CAR-T or CAR-NK cells therapy, but CAR-NK cells had safer off-tumor/on-target profile. They infused human hematopoietic cells from UCB to immunodeficient mice that were treated with either CAR-T, CAR-NK, or unmodified T or NK cells 10 weeks after transplantation. The results reflected that all mice that received CAR-T cells infusion developed acute toxicity and died 5 days after treatment, while OS rate of other mice was 100 % at day 15 [86]. Significantly, high expression of inhibitory receptors on the surface of NK cells will also limit the clinical effects of therapy [87]. Therefore, it is worthwhile to combine treatment of CAR-NK cells and inhibitory receptor blocking antibodies or knock out some inhibitory receptors in NK cells by gene editing [88, 89].

In terms of solid tumors, preclinical data has mainly focused on glioblastoma, breast cancer, ovarian cancer, and pancreatic cancer [90–95]. PD-1, which is expressed in various cell types, plays a role in negative regulation of immune response to tumors when it interacts with PD-L1. HER2 is overexpressed in several tumors, such as breast cancer, esophageal cancer, ovarian cancer, and glioblastoma, and is associated with poor long-term survival [96, 97]. At this point, Xia et al. designed a new HER2-CAR-NK cell derived from parental NK-92 cells that co-expressed soluble PD-1 (sPD-1-CAR-NK cells) to block PD-1/PD-L1 interaction. *In vitro* studies revealed that sPD-1-CAR-NK cells enhanced cytotoxicity toward HER2-and PD-L1-positive breast cancer cells compared to NK-92 cells. Animal experiments further revealed that sPD-1-CAR-NK cells had a superior anti-tumor ability compared to HER2-CAR NK cells plus sPD-1 therapy [95].

There are currently no commercially available CAR-NK cell products. As of July 1, 2023, 73 clinical studies have been registered on Clinicaltrials.gov to evaluate the safety and effectiveness of CAR-NK cell therapy against malignant tumors. However, only 3 clinical trials have been completed, and 47 clinical trials are ongoing among them, which are summarized in Tables 2 and 3. Unfortunately, all CAR-NK cell clinical trials are in phase I and II.

At present, a subset of clinical trials has published encouraging clinical results that highlight the potential of CAR-NK cell therapy as a promising anti-tumor approach, particularly in the field of hematological malignancies [98–102]. In 2020, Liu et al. reported the results of the first clinical trial of CAR-NK cells (NCT03056339) in the United States. The CAR construct designed in this trial expressed the encoded anti-CD19 structural region, as well as IL-15 and inducible caspase 9 as safety switches to ensure the proliferation, persistence, and safety of CAR-NK cells derived from UCB. 11 R/R CD19-positive chronic lymphocytic leukemia (CLL) or non-Hodgkin's lymphoma (NHL) patients, with a median age of 60 years, received a single dose at one of three dose levels (1×10^5, 1×10^6, or 1×10^7 CAR-NK cells per kilogram of body weight) after lymphodepletion. Out of these patients, 8 patients responded to this immunotherapy within 30 days of treatment, and 7 patients achieved CR after a median follow-up of 13.8 months. Importantly, none of the patients developed CRS or neurotoxicity [98]. In addition, the biopharmaceutical company Nkarta, Inc. has provided updates on the clinical progress of NKX101, targeting NKG2D ligands (NCT04623944), and NKX019, targeting CD19 (NCT05020678), which are well tolerated and highly active in heavily pre-treated R/R AML and R/R NHL patients, respectively [99, 100, 103]. For example, NKX019 is a cryopreserved, allogeneic CD19-targeting CAR-NK cell product, which is derived from healthy donor NK cells and contains CD3 zeta and OX40 costimulatory domains, as well as a separate membrane-bound IL-15 for activation. An initial phase I clinical trial of NKX019 included 19 patients with R/R B-cell malignancies, with a median age of 59 years. These patients received three infusions of CAR-NK cells at day 0, 7, and 14 of a 28-day cycle, with doses ranging from 3×10^8 to 1.5×10^9 CAR-NK cells per infusion. During the follow-up period, no treatment-related adverse reactions leading to discontinuation of NKX019 were observed, and there were no dose-limiting toxicities (DLTs), neurotoxicity, or GvHD reported. Among the 14 NHL patients, 8 achieved CR, while 3 experienced relapsed after a remission period of more than 6 months [99]. Furthermore, Zhang et al. reported the phase I clinical results of off-the-shelf CD33-CAR NK cell therapy for R/R AML (NCT05008575). They enrolled 10 R/R AML patients aged between 18 and 65 years-old who underwent CD33-CAR NK cells infusion (6.0×10^8, 1.2×10^9 or 1.8×10^9 cells per round) after preconditioning, and recorded the long-term safety and curative effect. During the follow-up period, only 1 patient developed grade 2 CRS and was subsequently in remission with intravenous glucocorticoid administration. In contrast, 6 patients achieved MRD CR at day 28 assessment [101].

While there is limited information available on clinical trials of CAR-NK cells in solid tumors compared to hematological malignancies, the published data also suggests its superior efficacy in treating solid tumors. Xiao et al. conducted

Table 2: Ongoing clinical trials of chimeric antigen receptor natural killer (CAR-NK) cell therapies against hematological malignancies.

NCT number	Start date	Disease	Target antigen	NK source	Status	Phase	Country
NCT04245722	2020/3/19	B-cell lymphoma, CLL	CD19	iPSC	No yet recruiting	I	America
NCT04623944	2020/9/21	ALL, MDS	NKG2D	PB	Recruiting	I	America
NCT05215015	2020/11/30	AML	CD33/CLL1	NA	Recruiting	Early I	China
NCT04639739	2020/12/17	NHL	CD19	NA	Not yet recruiting	I	China
NCT04796675	2021/4/10	B cell malignancies	CD19	UCB	Recruiting	I	China
NCT04887012	2021/5/1	B-NHL	CD19	PB	Recruiting	I	China
NCT05020678	2021/8/20	Hematological malignancies	CD19	PB	Recruiting	I	America
NCT05008536	2021/10/1	MM	BCMA	UCB	Recruiting	Early I	China
NCT05379647	2021/11/4	B cell malignancies	CD19	iPSC	Recruiting	I	China
NCT05020015	2021/11/22	R/R B-NHL	CD19	UCB	Recruiting	II	America
NCT05182073	2021/11/24	MM	BCMA	iPSC	Recruiting	I	America
NCT05008575	2021/12/23	AML	CD33	NA	Recruiting	I	China
NCT05410041	2022/5/25	ALL, CLL, NHL	CD19	NA	Recruiting	I	China
NCT05472558	2022/9/10	B-NHL	CD19	UCB	Recruiting	I	China
NCT05574608	2022/10/1	AML	CD123	PB	Recruiting	I	China
NCT05487651	2022/10/1	B-cell malignancies	CD19	NA	Recruiting	I	America
NCT05601466	2022/10/28	AML	CD33	iPSC	Recruiting	I	China
NCT05092451	2022/11/1	B-cell lymphoma, MDS, AML	CD70	UCB	Recruiting	I/II	America
NCT05652530	2022/11/13	MM	BCMA	NA	Recruiting	Early I	China
NCT05645601	2022/12/1	R/R B-cell hematological malignancies	CD19	NA	Recruiting	I	China
NCT05654038	2022/12/8	B cell malignancies	CD19	NA	Recruiting	I/II	China
NCT05667155	2022/12/15	B-NHL	CD19/CD70	UCB	Recruiting	I	China
NCT05665075	2022/12/24	AML	CD33	iPSC	Recruiting	I	China
NCT05673447	2023/1/1	DLBCL	CD19	NA	Not yet recruiting	Early I	China
NCT05842707	2023/1/18	R/R B-NHL	CD19/CD70	UCB	Recruiting	I/II	China
NCT05336409	2023/1/24	R/R CD19-positive B-cell malignancies	CD19	iPSC	Recruiting	I	America
NCT05739227	2023/3/1	B cell malignancies	CD19	NA	Recruiting	Early I	China
NCT05734898	2023/3/1	AML	NKG2D	NA	Recruiting	NA	China
NCT05618925	2023/4/15	NHL	CD19	NA	Not yet recruiting	I	America
NCT05110742	2023/6/30	Hematological malignancies	CD5	UCB	No yet recruiting	I/II	America

CLL, chronic Lymphocytic Leukemia; iPSC, induced pluripotent stem cell; ALL, acute lymphocytic leukemia; MDS, myelodysplastic syndrome; PB, peripheral blood; AML, acute myelogenous leukemia; CLL1, c-type lectin-like molecule 1; NHC, non-Hodgkin's lymphoma; MM, multiple lymphoma; BCMA, B cell maturation antigen; NA, not available; UCB, umbilical cord blood; R/R, relapsed or refractory; DLBCL, diffuse large B cell lymphoma.

a pilot clinical study involving 3 patients with chemotherapy-refractory metastatic colorectal cancer who received NKG2D-CAR-NK cells infusions (NCT03415100). The NKG2D receptor, mainly expressed by human NK cells, interacts with NKG2D ligands that are upregulated in tumor cells. The study demonstrated that the volume of ascites of enrolled patients was reduced significantly and disease remained stable within 2 weeks of target therapy. Unfortunately, the current status of this clinical trial is unknown, and further results have not been reported [104]. Besides, CAR-NK cells combined with other therapies is also an area of active research. Serry et al. reported the early results of a novel combination immunotherapy protocol called QUILT 88, which involves PD-L1-targeted high-affinity NK cell (PD-L1 t-haNK) therapy, low-dose chemoradiation and an IL-15 cytokine superagonist (NCT04390399). Fifty five patients that had received third line or greater treatments was enrolled to explore the efficacy and safety of QUILT 88 against pancreatic cancer, with a median age of 22 years. The OS rate reached 81.8 % among the 44 patients evaluable at 3 months to date, and the disease control rate of 47 evaluable patients is 36.2 %. There was no treatment-related deaths occurred, indicating the potential efficacy and safety of QUILT 88 [105].

In conclusion, CAR-NK cell therapy has shown great potential in tumor control based on published results from preclinical studies and early clinical trial. Further research and clinical trials are needed to explore the full potential of CAR-NK cell therapy.

iPSC-derived CAR-NK cells therapy for cancer

Although CAR-NK cells can derive from a wide range of sources, iPSC has the advantages both from primary NK cells

Table 3: Ongoing clinical trials of chimeric antigen receptor natural killer (CAR-NK) cell therapies against solid tumors.

NCT number	Start date	Disease	Target antigen	NK source	Status	Phase	Country
NCT03383978	2017/12/1	Glioblastoma	HER2	NK-92	Recruiting	I	German
NCT03656705	2018/9/29	NSCLC	PD-1	NK-92	Enrolling by invitation	I	China
NCT03692663	2018/12/1	Metastatic castration-resistant prostate cancer	PSMA	iPSC	Recruiting	Early I	China
NCT04390399	2020/07/21	Pancreatic cancer	PD-L1	NK-92	Recruiting	II	China
NCT05213195	2021/12/10	Refractory metastatic colorectal cancer	NKG2D	NA	Recruiting	I	China
NCT04847466	2021/12/14	GEJ cancers, HNSCC	PD-L1	NK-92	Recruiting	II	America
NCT05194709	2021/12/30	Solid tumor	5T4 oncofoetal antigen	NA	Recruiting	Early I	China
NCT05395052	2022/5/31	Solid tumor	MICA/MICB	iPSC	Active not recruiting	I	America
NCT05410717	2022/6/1	Ovarian cancer, testis cancer	Claudin6	PB	Recruiting	I/II	China
NCT05507593	2022/9/1	SCLC	DLL3	NA	Recruiting	I	China
NCT05686720	2023/2/1	Advanced triple negative breast cancer	Mesothelin	NA	Not yet recruiting	Early I	China
NCT05776355	2023/3/1	Ovarian cancer	NKG2D	NA	Recruiting	NA	China
NCT05703854	2023/3/29	Renal cell carcinoma, mesothelioma, osteosarcoma	CD70	UCB	Recruiting	I	America
NCT05845502	2023/5/4	Hepatocellular carcinoma	Glypican 3	NA	Not yet recruiting	NA	China
NCT05856643	2023/6/1	Ovarian epithelial carcinoma	Mesothelin	NA	Not yet recruiting	Early I	China
NCT05678205	2023/8/1	Breast cancer, gastric cancer, GEJ adenocarcinoma	HER2	UCB	Not yet recruiting	I/II	America
NCT05922930	2023/12/31	Ovarian cancer, mesonephric-like adenocarcinoma, pancreatic cancer	TROP2	UCB	Not yet recruiting	I/II	America

HER2, human epidermal growth factor receptor 2; NSCLC, non-small cell lung cancer; PD-1, programmed cell death receptor 1; PSMA, prostate-specific membrane antigen; iPSC, induced pluripotent stem cell; GEJ, gastroesophageal junction; HNSCC, head and neck squamous cell carcinoma; PD-L1, programmed cell death ligand 1; NA, not available; MICA/MICB, MHC class I chain-related protein A and B; PB, peripheral blood; SCLC, small cell lung cancer; DLL3, delta-like ligand 3; UCB, umbilical cord blood. TROP2, trophoblast antigen 2.

and NK cell lines, and has become a research hotspot. Similar to primary NK cells, iPSC-NK cells maintain high cytotoxicity and can expand well *in vivo* after cryopreservation [106]. Meanwhile, iPSC-NK cells exhibit clonal growth, high *in vitro* expansion, and differentiation capabilities, enabling the production of a large number of homogeneous NK cells with reduced tumorigenic risk compared to NK cell lines [107]. More importantly, the efficiency of transduction or transfection for iPSC-NK cells is high, allowing them to be better genetically engineered through viral vectors, transposons, and CRISPR-Cas9 to create more lethal NK cells [108–110]. Several laboratories are devoted to developed the differentiation process of NK cells from iPSC, and they commonly follow the differentiation route of hematopoietic precursor cells [93, 111].

One notable study by the Kaufman group involved the engineering of iPSC with a CAR targeting the tumor-associated antigen mesothelin, named meso-CAR-iPSC-NK cells. Mesothelin, a membrane surface glycoprotein, is highly expressed in ovarian cancer but less expressed in normal mesothelial tissue. They revealed that meso-CAR-iPSC-NK cells were able to more effectively inhibit tumor growth and prolong survival in an ovarian cancer xenograft model, compared to conventional PB NK cell therapy, iPSC-NK cell therapy and CAR-T cell therapy. Furthermore, they tested multiple CAR constructs to identify the optimal combination, and found that CAR containing the transmembrane domain of NKG2D, the 2B4 co-stimulatory domain, and the CD3ζ signaling domain maximized the anti-tumor ability [112]. Apart from CAR design, researchers have also employed various modification to iPSC in many ways, including gene knockout and gene knockin, to improve *in vivo* persistence and efficacy of CAR-iPSC-NK cells [113]. Wang et al. developed CD33-CAR-iPSC-iNK cells (QN-023a) that showed superior antitumor activity among the candidates both *in vitro* and in NOG mouse model [113]. QN-023a exhibited 4 anti-tumor modalities: (1) a proprietary CD33-targeting CAR; (2) a high-affinity, non-cleavable CD16 (hnCD16) to enhance antibody-dependent cellular cytotoxicity (ADCC) and mitigate antigen escape; (3) an IL-15 receptor fusion (IL-15RF) to enable NK cell persistence without the need for exogenous cytokine support; and (4) a CD38 knockout to effectively avoid fratricide of CAR-NK cells.

At present, the number of clinical studies on iPSC-NK cells is increasing year by year, and some clinical data on iPSC-CAR-NK cell therapy has been disclosed, including data

on FT596 and FT576. FT596 is an off-the-shelf, CD19-directed CAR-NK cell derived from iPSC, and is generated with a hnCD16Fc receptor and IL-15RF. Phase I clinical trial of FT596 in patients with R/R B-cell lymphomas and chronic lymphocytic leukemia was reported at the 2021 American society of hematology (ASH) annual meeting (NCT 04245722). Researchers evaluated the safety and efficacy of FT596 as a monotherapy or in combination with anti-CD20 monoclonal antibody at 3 different dose levels. The effect of FT596 was dose-dependent, and 5 of the 6 patients (83 %) reached OR at single-dose levels of 300 million cells [114]. Further, the clinical-stage biopharmaceutical company Fate Therapeutics, Inc. presented the outcomes of phase I trial of FT576 in patients with R/R MM at the 2022 ASH annual meeting to assess safety and tolerability (NCT05182073) [102]. FT576 is a first-of-kind, multi-engineered BCMA CAR-NK cell therapy derived from a clonal master engineered iPSC. Besides the construct of hnCD16 Fc receptor and IL-15RF, FT576 is engineered to knock out CD38 gene. Nine R/R MM patients have been treated and evaluated, and it is encouraging that nobody developed CRS and neurotoxicity. Three patients had a decrease in their myeloma disease burden (38 %–97 % decrease), with 2 subjects with a confirmed objective response.

Future perspective

NK cells, as crucial effector cells in innate immunity, possess potent anti-tumor functions, making them promising candidates for cancer treatment. The continuous development of biotechnology has unveiled the tremendous therapeutic potential of NK cell-based therapies in the field of anticancer treatment. However, it is important to acknowledge the current challenges and difficulties associated with NK cell therapy. On one hand, there is a limited sample size in preclinical research and clinical trials conducted so far, along with the relatively short follow-up periods. These limitations hinder the comprehensive validation of the safety and efficacy of NK cell therapy. On the other hand, it is necessary to be mindful of the numerous technical issues in the field of NK cell therapy. Determining the optimal sources of NK cells, developing effective NK cell expansion methods, and determining the duration of NK cell therapy are crucial considerations. Moreover, reducing immune evasion to prevent immune exhaustion in the tumor microenvironment is of utmost importance. For CAR-NK cell therapy, the design of target antigens and signaling domains, as well as strategies to enhance NK cell activity and persistence, such as combination therapy with other drugs, are anticipated to be research hotspots in the future of cellular therapy.

Furthermore, despite the promising anti-tumor effects demonstrated in preclinical and clinical research, particularly with CAR-NK cell therapy, NK cell therapy still faces significant ethical challenges in its clinical translation. Firstly, there is a lack of clear benefit assessment criteria for NK cell therapy, especially the genetic editing techniques used to enhance its anti-tumor efficacy requiring further research to investigate its long-term effects and safety. Additionally, with more clinical trials of iPSC-NK therapy, more attention should be pay to the safety issues. Moreover, with the growing number of clinical trials for NK cell therapy, there is a need for further refinement of admission criteria for clinical research platforms and relevant regulatory frameworks.

In summary, future basic research should focus on elucidating the key factors that determine the efficacy and persistence of NK cells, which enable to optimize and design the next generation of NK-based therapeutic product. By addressing these issues and conducting further research, we can unlock the full potential of NK cell-based therapies and pave the way for innovative and effective cancer treatments.

Ethical approval: The local Institutional Review Board deemed the study exempt from review.
Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.
Competing interests: Xiaojun Huang is a member of the editorial board of Medical Review.
Research funding: This study was supported by the Key Program of the National Natural Science Foundation of China (81530046, 81930004), National Natural Science Foundation of China (81870140, 82070184, 82270228), Peking University People's Hospital Research and Development Funds (RDL2021-01), Beijing Nova Program (20220484235), and Sclence, Technology & Innovation Project of Xiongan New Area (2023XACX0004).

References

1. Herberman RB, Nunn ME, Holden HT, Lavrin DH. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors. II. Characterization of effector cells. Int J Cancer 1975;16: 230–9.
2. Abel AM, Yang C, Thakar MS, Malarkannan S. Natural killer cells: development, maturation, and clinical utilization. Front Immunol 2018;9:1869.
3. Mace EM. Human natural killer cells: form, function, and development. J Allergy Clin Immunol 2023;151:371–85.
4. Imai K, Matsuyama S, Miyake S, Suga K, Nakachi K. Natural cytotoxic activity of peripheral-blood lymphocytes and cancer incidence: an 11-year follow-up study of a general population. Lancet 2000;356:1795–9.

5. Vivier E, Tomasello E, Baratin M, Walzer T, Ugolini S. Functions of natural killer cells. Nat Immunol 2008;9:503–10.
6. Wang X, Zhao XY. Transcription factors associated with IL-15 cytokine signaling during NK cell development. Front Immunol 2021;12:610789.
7. Montaldo E, Del Zotto G, Della Chiesa M, Mingari MC, Moretta A, De Maria A, et al. Human NK cell receptors/markers: a tool to analyze NK cell development, subsets and function. Cytometry 2013;83: 702–13.
8. Tajima F, Kawatani T, Endo A, Kawasaki H. Natural killer cell activity and cytokine production as prognostic factors in adult acute leukemia. Leukemia 1996;10:478–82.
9. Mastaglio S, Wong E, Perera T, Ripley J, Blombery P, Smyth MJ, et al. Natural killer receptor ligand expression on acute myeloid leukemia impacts survival and relapse after chemotherapy. Blood Adv 2018;2: 335–46.
10. Cichocki F, Cooley S, Davis Z, DeFor TE, Schlums H, Zhang B, et al. CD56dimCD57+NKG2C+ NK cell expansion is associated with reduced leukemia relapse after reduced intensity HCT. Leukemia 2016;30: 456–63.
11. Nersesian S, Schwartz SL, Grantham SR, MacLean LK, Lee SN, Pugh-Toole M, et al. NK cell infiltration is associated with improved overall survival in solid cancers: a systematic review and meta-analysis. Transl Oncol 2021;14:100930.
12. Takanami I, Takeuchi K, Giga M. The prognostic value of natural killer cell infiltration in resected pulmonary adenocarcinoma. J Thorac Cardiovasc Surg 2001;121:1058–63.
13. Bouzidi L, Triki H, Charfi S, Kridis WB, Derbel M, Ayadi L, et al. Prognostic value of natural killer cells besides tumor-infiltrating lymphocytes in breast cancer tissues. Clin Breast Cancer 2021;21: e738–47.
14. Tang YP, Xie MZ, Li KZ, Li JL, Cai ZM, Hu BL. Prognostic value of peripheral blood natural killer cells in colorectal cancer. BMC Gastroenterol 2020;20:31.
15. Cho H, Ryu MH, Lee HE, Kim HD, Kang YK. Prognostic value of natural killer cell activity for patients with HER2 + advanced gastric cancer treated with first-line fluoropyrimidine-platinum doublet plus trastuzumab. Cancer Immunol Immunother 2022;71: 829–38.
16. Bi J, Cheng C, Zheng C, Huang C, Zheng X, Wan X, et al. TIPE2 is a checkpoint of natural killer cell maturation and antitumor immunity. Sci Adv 2021;7:eabi6515.
17. Wang X, Sun R, Hao X, Lian ZX, Wei H, Tian Z. IL-17 constrains natural killer cell activity by restraining IL-15-driven cell maturation via SOCS3. Proc Natl Acad Sci U S A 2019;116:17409–18.
18. Stabile H, Fionda C, Santoni A, Gismondi A. Impact of bone marrow-derived signals on NK cell development and functional maturation. Cytokine Growth Factor Rev 2018;42:13–19.
19. Wang D, Malarkannan S. Transcriptional regulation of natural killer cell development and functions. Cancers 2020;12:1591.
20. Wang F, Meng M, Mo B, Yang Y, Ji Y, Huang P, et al. Crosstalks between mTORC1 and mTORC2 variagate cytokine signaling to control NK maturation and effector function. Nat Commun 2018;9:4874.
21. Meissl K, Simonović N, Amenitsch L, Witalisz-Siepracka A, Klein K, Lassnig C, et al. STAT1 isoforms differentially regulate NK cell maturation and anti-tumor activity. Front Immunol 2020;11:2189.
22. He Y, Tian Z. NK cell education via nonclassical MHC and non-MHC ligands. Cell Mol Immunol 2017;14:321–30.
23. Boudreau JE, Hsu KC. Natural killer cell education and the response to infection and cancer therapy: stay tuned. Trends Immunol 2018;39: 222–39.
24. Wagner AK, Kadri N, Snäll J, Brodin P, Gilfillan S, Colonna M, et al. Expression of CD226 is associated to but not required for NK cell education. Nat Commun 2017;8:15627.
25. Shang QN, Yu XX, Xu ZL, Cao XH, Liu XF, Zhao XS, et al. Functional competence of NK cells via the KIR/MHC class I interaction correlates with DNAM-1 expression. J Immunol 2022;208:492–500.
26. Poznanski SM, Ashkar AA. What defines NK cell functional fate: phenotype or metabolism? Front Immunol 2019;10:1414.
27. Schafer JR, Salzillo TC, Chakravarti N, Kararoudi MN, Trikha P, Foltz JA, et al. Education-dependent activation of glycolysis promotes the cytolytic potency of licensed human natural killer cells. J Allergy Clin Immunol 2019;143:346–58 e6.
28. Pfeifer C, Highton AJ, Peine S, Sauter J, Schmidt AH, Bunders MJ, et al. Natural killer cell education is associated with a distinct glycolytic profile. Front Immunol 2018;9:3020.
29. Fehniger TA, Cooper MA. Harnessing NK cell memory for cancer immunotherapy. Trends Immunol 2016;37:877–88.
30. Tarannum M, Romee R. Cytokine-induced memory-like natural killer cells for cancer immunotherapy. Stem Cell Res Ther 2021;12:592.
31. Rückert T, Lareau CA, Mashreghi MF, Ludwig LS, Romagnani C. Clonal expansion and epigenetic inheritance of long-lasting NK cell memory. Nat Immunol 2022;23:1551–63.
32. Ewen EM, Pahl JHW, Miller M, Watzl C, Cerwenka A. KIR downregulation by IL-12/15/18 unleashes human NK cells from KIR/HLA-I inhibition and enhances killing of tumor cells. Eur J Immunol 2018;48:355–65.
33. Ghofrani J, Lucar O, Dugan H, Reeves RK, Jost S. Semaphorin 7A modulates cytokine-induced memory-like responses by human natural killer cells. Eur J Immunol 2019;49:1153–66.
34. Myers JA, Miller JS. Exploring the NK cell platform for cancer immunotherapy. Nat Rev Clin Oncol 2021;18:85–100.
35. Stringaris K, Sekine T, Khoder A, Alsuliman A, Razzaghi B, Sargeant R, et al. Leukemia-induced phenotypic and functional defects in natural killer cells predict failure to achieve remission in acute myeloid leukemia. Haematologica 2014;99:836–47.
36. Chretien AS, Fauriat C, Orlanducci F, Galseran C, Rey J, Bouvier Borg G, et al. Natural killer defective maturation is associated with adverse clinical outcome in patients with acute myeloid leukemia. Front Immunol 2017;8:573.
37. Chretien AS, Fauriat C, Orlanducci F, Rey J, Borg GB, Gautherot E, et al. NKp30 expression is a prognostic immune biomarker for stratification of patients with intermediate-risk acute myeloid leukemia. Oncotarget 2017;8:49548–63.
38. Martner A, Rydström A, Riise RE, Aurelius J, Brune M, Foà R, et al. NK cell expression of natural cytotoxicity receptors may determine relapse risk in older AML patients undergoing immunotherapy for remission maintenance. Oncotarget 2015;6:42569–74.
39. Tang L, Wu J, Li CG, Jiang HW, Xu M, Du M, et al. Characterization of immune dysfunction and identification of prognostic immune-related risk factors in acute myeloid leukemia. Clin Cancer Res 2020;26: 1763–72.
40. Duault C, Kumar A, Taghi Khani A, Lee SJ, Yang L, Huang M, et al. Activated natural killer cells predict poor clinical prognosis in high-risk B- and T-cell acute lymphoblastic leukemia. Blood 2021;138:1465–80.
41. Wang D, Sun Z, Zhu X, Zheng X, Zhou Y, Lu Y, et al. GARP-mediated active TGF-beta1 induces bone marrow NK cell dysfunction in AML patients with early relapse post-allo-HSCT. Blood 2022;140:2788–804.
42. Slattery K, Woods E, Zaiatz-Bittencourt V, Marks S, Chew S, Conroy M, et al. TGFbeta drives NK cell metabolic dysfunction in human metastatic breast cancer. J Immunother Cancer 2021;9:e002044.

43. Shaim H, Shanley M, Basar R, Daher M, Gumin J, Zamler DB, et al. Targeting the alphav integrin/TGF-beta axis improves natural killer cell function against glioblastoma stem cells. J Clin Invest 2021;131: e142116.
44. Ni J, Wang X, Stojanovic A, Zhang Q, Wincher M, Bühler L, et al. Single-cell RNA sequencing of tumor-infiltrating NK cells reveals that inhibition of transcription factor HIF-1α unleashes NK cell activity. Immunity 2020;52:1075–87.e8.
45. Neo SY, Yang Y, Record J, Ma R, Chen X, Chen Z, et al. CD73 immune checkpoint defines regulatory NK cells within the tumor microenvironment. J Clin Invest 2020;130:1185–98.
46. Kryczek I, Lin Y, Nagarsheth N, Peng D, Zhao L, Zhao E, et al. IL-22(+) CD4(+) T cells promote colorectal cancer stemness via STAT3 transcription factor activation and induction of the methyltransferase DOT1L. Immunity 2014;40:772–84.
47. Chen X, Wang Y, Wang J, Wen J, Jia X, Wang X, et al. Accumulation of T-helper 22 cells, interleukin-22 and myeloid-derived suppressor cells promotes gastric cancer progression in elderly patients. Oncol Lett 2018;16:253–61.
48. Zeng H, Liu Z, Wang Z, Zhou Q, Qi Y, Chen Y, et al. Intratumoral IL22-producing cells define immunoevasive subtype muscle-invasive bladder cancer with poor prognosis and superior nivolumab responses. Int J Cancer 2020;146:542–52.
49. Briukhovetska D, Suarez-Gosalvez J, Voigt C, Markota A, Giannou AD, Schübel M, et al. T cell-derived interleukin-22 drives the expression of CD155 by cancer cells to suppress NK cell function and promote metastasis. Immunity 2023;56:143–61 e11.
50. Tan S, Xu Y, Wang Z, Wang T, Du X, Song X, et al. Tim-3 hampers tumor surveillance of liver-resident and conventional NK cells by disrupting PI3K signaling. Cancer Res 2020;80:1130–42.
51. Ma S, Caligiuri MA, Yu J. Harnessing IL-15 signaling to potentiate NK cell-mediated cancer immunotherapy. Trends Immunol 2022;43: 833–47.
52. Romee R, Cooley S, Berrien-Elliott MM, Westervelt P, Verneris MR, Wagner JE, et al. First-in-human phase 1 clinical study of the IL-15 superagonist complex ALT-803 to treat relapse after transplantation. Blood 2018;131:2515–27.
53. Christodoulou I, Ho WJ, Marple A, Ravich JW, Tam A, Rahnama R, et al. Engineering CAR-NK cells to secrete IL-15 sustains their anti-AML functionality but is associated with systemic toxicities. J Immunother Cancer 2021;9:e003894.
54. Wan C, Keany MP, Dong H, Al-Alem LF, Pandya UM, Lazo S, et al. Enhanced efficacy of simultaneous PD-1 and PD-L1 immune checkpoint blockade in high-grade serous ovarian cancer. Cancer Res 2021;81:158–73.
55. André P, Denis C, Soulas C, Bourbon-Caillet C, Lopez J, Arnoux T, et al. Anti-NKG2A mAb is a checkpoint inhibitor that promotes anti-tumor immunity by unleashing both T and NK cells. Cell 2018;175:1731–43 e13.
56. Arvindam US, van Hauten PMM, Schirm D, Schaap N, Hobo W, Blazar BR, et al. A trispecific killer engager molecule against CLEC12A effectively induces NK-cell mediated killing of AML cells. Leukemia 2021;35:1586–96.
57. Gauthier L, Morel A, Anceriz N, Rossi B, Blanchard-Alvarez A, Grondin G, et al. Multifunctional natural killer cell engagers targeting NKp46 trigger protective tumor immunity. Cell 2019;177:1701–13 e16.
58. Kim WS, Shortt J, Zinzani PL, Mikhaylova N, Marin-Niebla, Radeski D, et al. Abstract CT024: REDIRECT: a Phase 2 study of AFM13 in patients with CD30-positive relapsed or refractory (R/R) peripheral T cell lymphoma (PTCL). Cancer Res 83;2023:CT024.
59. Kerbauy LN, Marin ND, Kaplan M, Banerjee PP, Berrien-Elliott MM, Becker-Hapak M, et al. Combining AFM13, a bispecific CD30/CD16 antibody, with cytokine-activated blood and cord blood-derived NK cells facilitates CAR-like responses against CD30(+) malignancies. Clin Cancer Res 2021;27:3744–56.
60. Nieto Y, Banerjee PP, Kaur I, Griffin L, Ganesh C, Kerbauy L, et al. Innate cell engager AFM13 combined with preactivated and expanded cord blood-derived NK cells for patients with double refractory CD30+ lymphoma. Blood 2022;140:415–6.
61. Arulanandam A, Lin L, Chang HM, Cerutti M, Choblet S, Gao P, et al. Derivation and preclinical characterization of CYT-303, a novel NKp46-NK cell engager targeting GPC3. Cells 2023;12:996.
62. Parkhurst MR, Riley JP, Dudley ME, Rosenberg SA. Adoptive transfer of autologous natural killer cells leads to high levels of circulating natural killer cells but does not mediate tumor regression. Clin Cancer Res 2011;17:6287–97.
63. Miller JS, Soignier Y, Panoskaltsis-Mortari A, McNearney SA, Yun GH, Fautsch SK, et al. Successful adoptive transfer and in vivo expansion of human haploidentical NK cells in patients with cancer. Blood 2005; 105:3051–7.
64. Lundqvist A, Yokoyama H, Smith A, Berg M, Childs R. Bortezomib treatment and regulatory T-cell depletion enhance the antitumor effects of adoptively infused NK cells. Blood 2009;113:6120–7.
65. Bachanova V, Cooley S, Defor TE, Verneris MR, Zhang B, McKenna DH, et al. Clearance of acute myeloid leukemia by haploidentical natural killer cells is improved using IL-2 diphtheria toxin fusion protein. Blood 2014;123:3855–63.
66. Cooley S, He F, Bachanova V, Vercellotti GM, DeFor TE, Curtsinger JM, et al. First-in-human trial of rhIL-15 and haploidentical natural killer cell therapy for advanced acute myeloid leukemia. Blood Adv 2019;3: 1970–80.
67. Williams RL, Cooley S, Bachanova V, Blazar BR, Weisdorf DJ, Miller JS, et al. Recipient T cell exhaustion and successful adoptive transfer of haploidentical natural killer cells. Biol Blood Marrow Transplant 2018; 24:618–22.
68. Björklund AT, Carlsten M, Sohlberg E, Liu LL, Clancy T, Karimi M, et al. Complete remission with reduction of high-risk clones following haploidentical NK-cell therapy against MDS and AML. Clin Cancer Res 2018;24:1834–44.
69. Curti A, Ruggeri L, Parisi S, Bontadini A, Dan E, Motta MR, et al. Larger size of donor alloreactive NK cell repertoire correlates with better response to NK cell immunotherapy in elderly acute myeloid leukemia patients. Clin Cancer Res 2016;22:1914–21.
70. Romee R, Schneider SE, Leong JW, Chase JM, Keppel CR, Sullivan RP, et al. Cytokine activation induces human memory-like NK cells. Blood 2012;120:4751–60.
71. Romee R, Rosario M, Berrien-Elliott MM, Wagner JA, Jewell BA, Schappe T, et al. Cytokine-induced memory-like natural killer cells exhibit enhanced responses against myeloid leukemia. Sci Transl Med 2016;8:357ra123.
72. Berrien-Elliott MM, Cashen AF, Cubitt CC, Neal CC, Wong P, Wagner JA, et al. Multidimensional analyses of donor memory-like NK cells reveal new associations with response after adoptive immunotherapy for leukemia. Cancer Discov 2020;10:1854–71.
73. Marin ND, Krasnick BA, Becker-Hapak M, Conant L, Goedegebuure SP, Berrien-Elliott MM, et al. Memory-like differentiation enhances NK cell responses to melanoma. Clin Cancer Res 2021;27:4859–69.
74. Fang F, Xie S, Chen M, Li Y, Yue J, Ma J, et al. Advances in NK cell production. Cell Mol Immunol 2022;19:460–81.

75. Zhao XY, Jiang Q, Jiang H, Hu LJ, Zhao T, Yu XX, et al. Expanded clinical-grade membrane-bound IL-21/4-1BBL NK cell products exhibit activity against acute myeloid leukemia in vivo. Eur J Immunol 2020; 50:1374–85.
76. Garg TK, Szmania SM, Khan JA, Hoering A, Malbrough PA, Moreno-Bost A, et al. Highly activated and expanded natural killer cells for multiple myeloma immunotherapy. Haematologica 2012;97: 1348–56.
77. Tschan-Plessl A, Kalberer CP, Wieboldt R, Stern M, Siegler U, Wodnar-Filipowicz A, et al. Cellular immunotherapy with multiple infusions of in vitro-expanded haploidentical natural killer cells after autologous transplantation for patients with plasma cell myeloma. Cytotherapy 2021;23:329–38.
78. Heinze A, Grebe B, Bremm M, Huenecke S, Munir TA, Graafen L, et al. The synergistic use of IL-15 and IL-21 for the generation of NK cells from CD3/CD19-depleted grafts improves their ex vivo expansion and cytotoxic potential against neuroblastoma: perspective for optimized immunotherapy post haploidentical stem cell transplantation. Front Immunol 2019;10:2816.
79. Domogala A, Blundell M, Thrasher A, Lowdell MW, Madrigal JA, Saudemont A, et al. Natural killer cells differentiated in vitro from cord blood CD34(+) cells are more advantageous for use as an immunotherapy than peripheral blood and cord blood natural killer cells. Cytotherapy 2017;19:710–20.
80. Knorr DA, Ni Z, Hermanson D, Hexum MK, Bendzick L, Cooper LJ, et al. Clinical-scale derivation of natural killer cells from human pluripotent stem cells for cancer therapy. Stem Cells Transl Med 2013;2:274–83.
81. Nowakowska P, Romanski A, Miller N, Odendahl M, Bonig H, Zhang C, et al. Clinical grade manufacturing of genetically modified, CAR-expressing NK-92 cells for the treatment of ErbB2-positive malignancies. Cancer Immunol Immunother 2018; 67:25–38.
82. Reina-Ortiz C, Constantinides M, Fayd-Herbe-de-Maudave A, Présumey J, Hernandez J, Cartron G, et al. Expanded NK cells from umbilical cord blood and adult peripheral blood combined with daratumumab are effective against tumor cells from multiple myeloma patients. OncoImmunology 2020;10:1853314.
83. Woll PS, Grzywacz B, Tian X, Marcus RK, Knorr DA, Verneris MR, et al. Human embryonic stem cells differentiate into a homogeneous population of natural killer cells with potent in vivo antitumor activity. Blood 2009;113:6094–101.
84. Gong Y, Klein Wolterink RGJ, Wang J, Bos GMJ, Germeraad WTV. Chimeric antigen receptor natural killer (CAR-NK) cell design and engineering for cancer therapy. J Hematol Oncol 2021;14:73.
85. Chmielewski M, Abken H. TRUCKs: the fourth generation of CARs. Expet Opin Biol Ther 2015;15:1145–54.
86. Caruso S, Quintarelli C, Angelis BD, Bufalo FD, Ciccone R, Donsante S, et al. CAR.CD123-NK cells have an Equally effective but safer off-tumor/on-target profile as compared to CARCD123-T cells for the treatment of acute myeloid Leukaemia. Blood 2022;140: 7369–70.
87. Kaulfuss M, Mietz J, Fabri A, Vom Berg J, Münz C, Chijioke O. The NK cell checkpoint NKG2A maintains expansion capacity of human NK cells. Sci Rep 2023;13:10555.
88. Bexte T, Alzubi J, Reindl LM, Wendel P, Schubert R, Salzmann-Manrique E, et al. CRISPR-Cas9 based gene editing of the immune checkpoint NKG2A enhances NK cell mediated cytotoxicity against multiple myeloma. OncoImmunology 2022;11: 2081415.
89. Albinger N, Bexte T, Buchinger L, Wendel P, Al-Ajami A, Gessner A, et al. CRISPR/Cas9 gene editing of immune checkpoint receptor NKG2A improves the efficacy of primary CD33-CAR-NK cells against AML. Blood 2022;140:4558–9.
90. Ma R, Lu T, Li Z, Teng KY, Mansour AG, Yu M, et al. An oncolytic virus expressing IL15/IL15Rα combined with off-the-shelf EGFR-CAR NK cells targets glioblastoma. Cancer Res 2021;81:3635–48.
91. Teng KY, Mansour AG, Zhu Z, Li Z, Tian L, Ma S, et al. Off-the-Shelf prostate stem cell antigen-directed chimeric antigen receptor natural killer cell therapy to treat pancreatic cancer. Gastroenterology 2022; 162:1319–33.
92. Wang J, Toregrosa-Allen S, Elzey BD, Utturkar S, Lanman NA, Bernal-Crespo V, et al. Multispecific targeting of glioblastoma with tumor microenvironment-responsive multifunctional engineered NK cells. Proc Natl Acad Sci U S A 2021;118:e2107507118.
93. Ueda T, Kumagai A, Iriguchi S, Yasui Y, Miyasaka T, Nakagoshi K, et al. Non-clinical efficacy, safety and stable clinical cell processing of induced pluripotent stem cell-derived anti-glypican-3 chimeric antigen receptor-expressing natural killer/innate lymphoid cells. Cancer Sci 2020;111:1478–90.
94. Da Y, Liu Y, Hu Y, Liu W, Ma J, Lu N, et al. STING agonist cGAMP enhances anti-tumor activity of CAR-NK cells against pancreatic cancer. OncoImmunology 2022;11:2054105.
95. Xia W, Chen J, Hou W, Chen J, Xiong Y, Li H, et al. Engineering a HER2-CAR-NK cell secreting soluble programmed cell death protein with superior antitumor efficacy. Int J Mol Sci 2023;24:6843.
96. Xia J, Minamino S, Kuwabara K. CAR-expressing NK cells for cancer therapy: a new hope. Biosci Trends 2020;14:354–9.
97. Gingras I, Gebhart G, de Azambuja E, Piccart-Gebhart M. HER2-positive breast cancer is lost in translation: time for patient-centered research. Nat Rev Clin Oncol 2017;14:669–81.
98. Liu E, Marin D, Banerjee P, Macapinlac HA, Thompson P, Basar R, et al. Use of CAR-transduced natural killer cells in CD19-positive lymphoid tumors. N Engl J Med 2020;382:545–53.
99. Dickinson M, Hamad N, Bryant CE, Kothari N, Ojeras P, Vohra A, et al. First in human data of NKX019, an allogeneic CAR NK for the treatment of relapsed/refractory (R/R) B-cell malignancies. Hematol Oncol 2023;41:526–7.
100. Cho C, Hansen K, Zhang M, Chan C, Trager J. Abstract 3183: combination of anti-EGFR antibody cetuximab with NKX101, an allogeneic NKG2D-L targeting NK cell therapy, enhances potency and in vitro cytotoxicity against solid tumors. Cancer Res 2023;83:3183.
101. Huang R, Wen Q, Wang X, Yan H, Ma Y, Mai-Hong W, et al. Off-the-Shelf CD33 CAR-NK cell therapy for relapse/refractory AML: first-in-human, phase I trial. Blood 2022;140:7450–1.
102. Dhakal B, Berdeja JG, Gregory T, Ly T, Bickers C, Zong X, et al. Interim phase I clinical data of FT576 as monotherapy and in combination with daratumumab in subjects with relapsed/refractory multiple myeloma. Blood 2022;140:4586–7.
103. Bachier C, Borthakur G, Hosing C, Blum W, Rotta M, Ojeras P, et al. A phase 1 study of NKX101, an allogeneic CAR natural killer (NK) cell therapy, in subjects with relapsed/refractory (R/R) acute myeloid leukemia (AML) or higher-risk myelodysplastic syndrome (MDS). Blood 2020;136:42–3.
104. Xiao L, Cen D, Gan H, Sun Y, Huang N, Xiong H, et al. Adoptive transfer of NKG2D CAR mRNA-engineered natural killer cells in colorectal cancer patients. Mol Ther 2019;27:1114–25.
105. Seery TE, Nangia CS, McKean HA, Sender LS, Bhar P, Reddy SK, et al. Promising survival and disease control in third-line or greater metastatic or locally advanced pancreatic cancer patients following

chemo-radiation and novel combination of aldoxorubicin, N-803 IL-15 superagonist, and PDL1- NK cell therapy. J Clin Oncol 2022;40:582.
106. Hermanson DL, Bendzick L, Pribyl L, McCullar V, Vogel RI, Miller JS, et al. Induced pluripotent stem cell-derived natural killer cells for treatment of ovarian cancer. Stem Cell 2016;34:93–101.
107. Cichocki F, Bjordahl R, Gaidarova S, Mahmood S, Abujarour R, Wang H, et al. iPSC-derived NK cells maintain high cytotoxicity and enhance in vivo tumor control in concert with T cells and anti-PD-1 therapy. Sci Transl Med 2020;12:eaaz5618.
108. Shankar K, Capitini CM, Saha K. Genome engineering of induced pluripotent stem cells to manufacture natural killer cell therapies. Stem Cell Res Ther 2020;11:234.
109. Meng F, Zhang S, Xie J, Zhou Y, Wu Q, Lu B, et al. Leveraging CD16 fusion receptors to remodel the immune response for enhancing anti-tumor immunotherapy in iPSC-derived NK cells. J Hematol Oncol 2023;16:62.
110. Woan KV, Kim H, Bjordahl R, Davis ZB, Gaidarova S, Goulding J, et al. Harnessing features of adaptive NK cells to generate iPSC-derived NK cells for enhanced immunotherapy. Cell Stem Cell 2021;28:2062–75 e5.
111. Zeng J, Tang SY, Toh LL, Wang S. Generation of “Off-the-Shelf” natural killer cells from peripheral blood cell-derived induced pluripotent stem cells. Stem Cell Rep 2017;9:1796–812.
112. Li Y, Hermanson DL, Moriarity BS, Kaufman DS. Human iPSC-derived natural killer cells engineered with chimeric antigen receptors enhance anti-tumor activity. Cell Stem Cell 2018;23:181–92 e5.
113. Wang Y, Wang L, Shao M, He X, Yue Y, Zhou Y, et al. Off-the-Shelf, multiplexed-engineered iPSC-derived CD33 CAR-NK cells for treatment of acute myeloid leukemia. Blood 2022;140:12685.
114. Bachanova V, Ghobadi A, Patel K, Park JH, Flinn IW, Shah P, et al. Safety and efficacy of FT596, a first-in-class, multi-antigen targeted, off-the-shelf, iPSC-derived CD19 CAR NK cell therapy in relapsed/refractory B-cell lymphoma. Blood 2021;138:823.