Chapter

# Natural Killer Cells for Cancer Immunotherapy: Opportunities and Challenges

*Leisheng Zhang, Xiaoming Feng, Zhihai Han and Zhongchao Han*

## Abstract

Natural killer (NK) cells are advantaged immune cells and play a pivotal role in both innate and adaptive immune responses. To date, autogenous and allogenic NK cells have been generated from a variety of origins, including perinatal blood (e.g., umbilical cord blood and placental blood), peripheral blood, and even stem cells (hematopoietic stem cells and pluripotent stem cells). NK cells function mainly *via* antibody-dependent cell-mediated cytotoxicity (ADCC), direct cytolytic effect, and paracrine effects (e.g., IFN-γ, GM-CSF, granzyme, and perforin). Distinguishing from the adaptive immunizing cells (e.g., T and B lymphocytes), NK cells, and chimeric antigen receptor-transduced NK (CAR-NK), cell-based cytotherapy is adequate to fulfill the biofunction of eliminating pathogenic infection, combating hematological malignancies and metastatic solid tumors, and delaying aging. In this chapter, we mainly focus on the state-of-the-art renewal of NK cell-based cytotherapy for cancer immunosurveillance and immunotherapy from the view of high-efficient *in vitro* preparation (e.g., candidate cell sources and *ex vivo* cultivation) and preclinical and clinical investigation. Furthermore, we also figure out the promising prospects and the concomitant challenges of NK cell-based remedies for cancer management in future, which will collectively benefit the development of NK cell-based cancer immunotherapy in future.

**Keywords:** natural killer cells, cancer immunotherapy, CAR-NK cells, preclinical and clinical investigation

## 1. Introduction

Cancer, including hematological malignancies and metastatic solid tumors, is one of the leading causes of death worldwide with increasing incidence and mortality, which has thus become a major public health issue and heavy burden to patients and their families [1, 2]. For example, a total of over 4,568,000 cases were newly diagnosed cancer in China, and over 3,002,000 cancer deaths occurred in 2020 [3]. Of them, lung cancer is the most frequent type, followed by colorectal cancer and gastric

cancer, whereas lung cancer, hepatocellular carcinoma, and stomach cancers are the top three most deadly subtypes of cancers in the general population.

For decades, depicting the cancer pattern has also been acknowledged to provide basic know-hows for developing novel strategies for cancer management [4, 5]. To date, a variety of modifiable risk factors for cancer have been identified to fuel the shift of cancer transition, such as oncogenes, air pollution, smoking, alcohol consumption, unhealthful dietary habits, obesity, physical inactivity, infectious agents, diabetes, improved livelihood, and even rapid economic development [6–8]. For example, cancer-related fatigue is one of the most distressing and prevalent symptoms among patients, which usually results in great research challenges [9].

For decades, effective implementation strategies have been developed to optimize the advancements in the fields of tumor survivorship, diagnosis, treatment, and end-of-life care [10]. For instance, a series of treatments have been developed to conquer cancers with high uncontrolled cell division and heterogeneity, including surgery (e.g., laparoscopic rectal surgery and robotic surgery) [11, 12], photothermal therapy (PTT) [13–15], radiotherapy [16], chemotherapy [17], oncolytic virotherapy [18], RNA vaccine [19, 20], hormone therapy [21], peptide-based neoantigen vaccine [22, 23], gene therapy [24–26], immunotherapy (e.g., immune cells and checkpoint inhibitors) [2], and even nanomaterial-mediated nanotheranostics (e.g., organic nanomaterials, inorganic nanomaterials, and organic–inorganic hybrid nanomaterials). Nevertheless, the aforementioned strategies have also revealed inherent shortcomings (e.g., severe toxicity, off-target effects, graft-versus-host disease, and drug delivery barriers), which collectively hinder the further improvement in cancer administration [27, 28].

State-of-the-art renewal has highlighted the feasibility of cellular immunotherapy as promising remedy for cancer administration on the basis of the rapid progress of cryobiology and oncobiology [29–33]. For instance, pioneering and talented investigators in the field have conducted systematic and detailed studies from bench to bedside to conquer hematologic tumors and solid tumors, including the non-gene-edited natural killer (NK) cells and the CAR-transduced counterparts (e.g., CAR-T, CAR-NK cells). Distinguishing from the adaptive T cells, NK cells are adequate to efficaciously fulfill the requirement of combating the aforementioned metastatic solid tumors and hematological malignancies *via* antibody-dependent cell-mediated cytotoxicity (ADCC), direct cytolytic effect, and paracrine effects dispense with antigen presentation [2, 28, 33, 34].

Therewith, the overview of this literature aimed to summarize the current knowledge and scope of the evidence on NK cell-based immunotherapy for cancer patients from the view of cell source (e.g., peripheral blood, perinatal blood, and cell lines), *ex vivo* amplification and activation (e.g., coculture and monolayer culture), mode of action (e.g., ADCC and cytolytic effect), together with preclinical and clinical practice of NK cell-based cancer immunotherapy. Meanwhile, we also indicate the promising prospects and the concomitant challenges of NK cell-based cancer immunotherapy in regenerative medicine.

## 2. Natural killer (NK) cells

NK cells are pivotal part of the innate immune defense against cancer and infection, and in particular, in combating certain bacterial and viral pathogens [35]. In the

1970s, Kiessling and their colleagues firstly identified NK cells from a mouse with advantaged characteristics of cytolytic effector dispense with preliminary antigen presentation [36, 37]. Incipiently, NK cells were considered critical components to play an important role in host innate immunity and the first line of defense, and in particular, in the antiviral infection [38, 39]. To date, NK cells have been recognized as the third type of lymphocyte with vital roles in both innate and adaptive immune responses to malignancies and viral infection, which also function in delaying aging and benefiting hematopoietic reconstitution [28, 40].

As a heterogeneous lymphocyte subpopulation, NK cells can be divided into the cytotoxic $CD3^{-}CD56^{dim}CD16^{high}$ subset and the IFN-γ-producing $CD3^{-}CD56^{bright}CD16^{+}$ counterpart [28, 41]. Of note, state-of-the-art updates have also put forward the existence of long-lived memory-like NK cells with reinforced responsiveness against cancer and the CD16-dependent functional capability [42, 43].

### 2.1 Cell sources for NK cell generation

To date, NK cells have been generated from various origins, including umbilical cord blood (UCB) [44], placental blood [44], peripheral blood (PB)-derived mononuclear cells (PBMCs) [43, 45], NK cell lines (e.g., NK-92, YT) [28], and even derived from $CD34^{+}$ hematopoietic stem cells (HSCs) [28], human embryonic stem cells (hESCs), and induced pluripotent stem cells (iPSCs) [34, 46]. Interestingly, the content and cytotoxic activity of NK cells in perinatal and peripheral blood vary a lot. For example, NK cells take a proportion of PB cells ranging from 5–20% [47, 48], whereas the proportions of NK cells in UCB and placental blood were less than 5% [49] and 2% [50], respectively. Meanwhile, over 60% of NK cells in UCB and PB reveal the $CD3^{-}$ $CD56^{+}$ $CD16^{+}$ subset, whereas the rest belongs to the $CD3^{-}$ $CD56^{+}$ $CD16^{-}$ subset. Conversely, only 40% of NK cells in placental blood are $CD3^{-}$ $CD56^{+}$ $CD16^{+}$ cells compared with the rest $CD3^{-}CD56^{+}CD16^{-}$ subset with a percentage of 60% instead [50]. Additionally, Dogra and the colleagues found that immune responses of NK cells in diverse tissue sites were different, which indicated the tissue localization in the regulation of the NK cell development as well as the biofunctions involved in antiviral and tumor immunosurveillance [51].

During the past decades, NK cells were isolated and identified from PB to determine the specific characteristics associated with anti-infection and anticancer as well as pathogenesis (e.g., recurrent miscarriage and hematologic malignancies) [45]. PB-derived NK (PB-NK) cells have been recognized as cytotoxic innate lymphocytes, which are endowed with a unique capacity to kill a broad spectrum of virus-infected cells, cancers, and even chronic obstructive pulmonary disease (COPD) [52, 53]. Currently, PBMCs are considered the dominant ingredient for clinical- or GMP- grade NK cell manufacturing [48]. However, the stability and yield of NK cell production are far from adequate largely attributed to the limitations in blood donators and the present procedures [43, 54]. Distinguishing from those of PB-NK cells, perinatal blood-derived NK cells reveal advantaged properties such as vigorous cytolytic activity, preferable cellular vitality, and enhanced cytotoxicity [28]. Of note, the latest kinds of literature have further indicated the large-scale preparation of NK cells from human pluripotent stem cells (hPSCs), which are considered excellent candidates for "off-the-shelf" anticancer immunotherapy and NK cell development [55].

### 2.2 *Ex vivo* preparation of NK cells

Strategies for *ex vivo* expansion of NK cells have allowed preparing enough amounts of NK cells for clinical trials and new drug application (NDA). However, current donor cell-dependent strategies for *ex vivo* preparation of NK cells can only produce limited amount of "made-to-order" therapeutic cells for limited patients [46, 56]. For decades, a series of methods have been developed for large-scale *ex vivo* NK cell preparation, including cell sorting, feeder cell coculture (e.g., K562 cells), gene editing, low oxygen treatment, multiple cytokine cocktail stimulation, three-dimensional rotation, and bioreactors [28]. For instance, Mu *et al.* developed a simple, safe, and cost-effective strategy for *ex vivo* purification and expansion of NK cells from CB *via* zoledronate and streptococcus stimulation, and dispense of cell sorting or feeder cells/multiple cytokines within 21 days [57]. Alves *et al.* reported the *ex vivo* expansion of $CD56^+$ lymphocytes, including NK cells and NKT-like subset, from PBMCs in CellGro medium supplemented with IL-2 and fetal bovine serum (FBS), anti-CD3 (9–10 initial days) for 21 days [56]. Meanwhile, distinguishing from the resting subset, the expanded NK cells showed a higher expression level of activating receptors (e.g., CD16, NKp44, NKp46, NKG2D, NKp30, CD62L, and CD69) and a higher concentration of cytokine secretion (e.g., IFN-γ, TNF-α, and GM-CSF). Instead, Radice and the colleagues verified that PB-derived NK cells from inpatients with early-stage colorectal cancer showed enhanced *ex vivo* cytotoxicity against cancer cell lines (e.g., HT-29, K562, and human CRC Caco-2) after the stimulation of low-doses of sequential-kinetic-activated (SKA) interferon-γ (IFN-γ) *in vitro* [58].

Interestingly, we took advantage of the "3ILs"-based cell programming and induced high-efficient generation of NK cells from peripheral and perinatal blood within 14 days [43, 44]. Of note, we and other investigators in the field found that the expanded NK cells revealed elevated content and enhanced cytotoxicity against various tumor cell lines *in vitro* compared to the resident counterpart [44, 57]. Additionally, Klingemann and the colleagues tested the feasibility of a broadly applicable method for blood progenitor cell and NK cell preparation based on the well-established NK-92 cell line, which is derived from a patient with non-Hodgkin's lymphoma [59]. Collectively, NK cell expansion and activation require multiple cell signal cascades for proliferation, survival, and activation. Therewith, most of the current *ex vivo* expansion strategies are mainly focused on either using genetically modified allogeneic feeder cells or substituting the indicated factors by utilizing the autologous feeder cells [60].

### 2.3 Mode of action for NK cell-based cytotherapy

Aforesaid, autologous and allogeneic NK cells act as critical effector lymphocytes and mediate cancer immune clearance surveillance [61]. Generally, NK cells spontaneously function in abnormal cell elimination, pathogenic microorganism, and tumor immunosurveillance *via* ADCC, direct cytolytic effect, paracrine of cytokines, and receptor-ligand-associated natural cytotoxicity effects as well [48, 62, 63].

Generally, the mode of action for NK cell-based cytotherapy is mainly based on "NK cell education," which has also been recognized as functional maturation of NK cells. As recently reviewed by Zhang and the colleagues, this process usually involves a series of step-wise intermediate stages and obtains self-major histocompatibility complex class I (MHC-I) recognition [64]. Meanwhile, as mentioned above, the IFN-γ-producing $CD3^-CD56^{bright}$ NK cells were less mature when compared with the

cytotoxic $CD3^{-}CD56^{dim}$ subset [41]. Interestingly, new insights into the long-lived memory-like NK cells indicated the enhanced CD16-dependent functional capability as well as responsiveness, which thus hold advantages over the CD25- subset in NK cell-based interventions against cancer [42, 43].

## 3. Chimeric antigen receptor-transduced NK (CAR-NK) cells

In recent years, CAR-transduced NK (CAR-NK) cells are considered as novel therapeutic options to reduce the incidence of relapse and recurrent cancers. A number of preclinical and clinical investigations have indicated CAR-NK cells as "off-the-shelf" products for cancer immunotherapy attributed to CAR-dependent and NK cell receptor-dependent signaling pathways [34]. Differing from those of CAR-T cells, CAR-NK cells avoid the adverse effect during cancer administration such as immune cell-associated neurotoxicity syndrome (ICANS), acute cytokine release syndrome (CRS), and graft-versus-host disease (GVHD), which thus represent the therapeutic paradigm for boosting the immune system to reinforce anticancer responses and eventually eliminate malignancies [34].

To date, a considerable number of cancer-specific antigens and the concomitant costimulatory molecules as well as the well-established subsets (e.g., NKp44, NKp46, and NKG2D) have been involved in CAR-NK cell-based immunotherapy. For example, the existing CARs transduced to generate CAR-T cells were directly transferred to NK cells, including 4-1BB, CD19, CD20, CD22, CD276, BCMA, CD3ζ, and CD28 [28, 65–70]. Simultaneously, pioneering and talented investigators in the field have dedicated themselves to optimize the constructs of CARs to enhance the efficacy of anticancer effects [32, 71]. For example, suicide genes have been integrated into the fourth generation of CARs to remove the unanticipated toxicity [72]. Furthermore, due to the advances in genome-editing techniques, the applicability and feasibility of CAR-NK cells have vastly accelerated cancer immunotherapy. Despite with deficiency in CAR constructs delivery into NK cells, a plethora of groups have highlighted the possibility for high-efficient CAR transduction *via* Lentivirus [28, 73, 74], retrovirus [75, 76], and even nonviral-mediated transfection (e.g., lipofection and electroporation, PiggyBac, and the sleeping beauty (SB) subsets) [77, 78]. Collectively, recent developments in the clinical practice of genetically modified NK cells with chimeric antigen receptors (CARs) are promising and challenging for NK cell-based immunotherapy.

## 4. NK cell-based cancer immunotherapy

Cancer immunotherapy with diverse preclinical and clinical attempts has been attractive and received considerable attention [79]. As time passes by, the feasibility of using NK cells in the treatment of human disorders such as refractory lung diseases, pathogenic infection, hematopoietic reconstitution, hematological malignancies, and metastatic solid tumors has increased in recent years [80]. In particular, the antileukemic potential of NK cells has raised considerable interest among researchers and clinicians for hematological malignancy administration. Nowadays, immune-based remedies characterized by the infusion of *ex vivo* activated or freshly isolated NK cells have become a research hotspot in cancer immunotherapy [34, 81–83].

In the 1980s, the dysfunction of NK cells was reported with a higher incidence of cancers, including Chédiak–Higashi syndrome and X-linked lymphoproliferative syndrome, which was first indicated in cancer immunosurveillance [41, 84, 85]. Recently, Bryce *et al.* found that NK cells in patients with chronic lymphocytic leukemia (CLL) showed reduction in content and abnormality in gene expression pattern (e.g., GATA-1, GATA-2, PU.1, and HIF-1α) in bone marrow [86].

To date, nearly 900 NK cell-based clinical trials, including the observational and interventional subtypes, were registered upon multiple tumors according to the Clinicaltrials.gov website. Generally, most of the trials were involved in hematologic malignancy administration, including acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myelogenous leukemia (CML), and myelodysplastic syndromes (MDS). Meanwhile, clinical pieces of evidence have also indicated the therapeutic effect of NK cell-based cytotherapy upon various solid tumors such as gastric carcinoma, colorectal cancer, liver cancer, and squamous cell lung cancer [87–89]. However, the definite NK cell-based immunotherapy upon metastatic solid tumors has not been verified, which was largely attributed to the dysregulation of NK cell function and immune subversion by various factors (e.g., IDO, PGE2, and TGF-β) in the tumor microenvironment [48].

## 5. Discussion and conclusions

Nowadays, with the rapid progress of available remedies for cancer treatments, patients are adequate to live with the consequences of cancer. NK cells have been regarded as the first line of innate immune cells, which are involved in providing the surveillance and elimination of the stressed, infected, damaged, and malignant cells [90, 91]. NK cell-based cancer immunotherapy has largely benefited the treatments of patients for longer living and better outcomes, which also supplies new preferences for cancer administration and helps improve decisions about optimal treatment. Of note, recent kinds of literature have suggested the promising aspect of achieving considerable amount of NK cells for adoptive immunotherapy after a period of *ex vivo* expansion with multifaceted stimulation. Nevertheless, the improvement of *ex vivo* expansion procedures for the clinical application of NK cells generated under good manufacturing practice conditions is of prime importance in future.

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## Conflict of interest

The authors declare no conflict of interest.

## Notes/thanks/other declarations

Not applicable.

## Abbreviation

| | |
|---|---|
| ADCC | Antibody-dependent cell-mediated cytotoxicity |
| CAR-NK | Chimeric antigen receptor-transduced NK |
| NK | Natural killer |
| PTT | Photothermal therapy |
| CAR-T | CAR-transduced T cells |
| UCB | Umbilical cord blood |
| PB | Peripheral blood |
| PBMCs | Peripheral blood-derived mononuclear cells |
| PB-NK | Peripheral blood-derived NK |
| COPD | Chronic obstructive pulmonary disease |
| NDA | New drug application |
| FBS | Fetal bovine serum |
| HSCs | Hematopoietic stem cells |
| CRS | Cytokine release syndrome |
| GVHD | Graft-versus-host disease |
| ICANS | Immune cell-associated neurotoxicity syndrome |
| GMP | Good manufacturing practices |
| CML | Chronic myelogenous leukemia |
| hPSCs | Human pluripotent stem cells |
| MDS | Myelodysplastic syndromes |
| ALL | Acute lymphoblastic leukemia |
| hESCs | Human embryonic stem cells |
| hiPSCs | Human induced PSCs |
| AML | Acute myeloid leukemia |
| CLL | Chronic lymphocytic leukemia |
| SKA | Sequential-kinetic-activated |
| IFN-γ | Interferon-γ |

## Author details

Leisheng Zhang[1,2,3,4,5*], Xiaoming Feng[6], Zhihai Han[4] and Zhongchao Han[3,4]

1 Key Laboratory of Molecular Diagnostics and Precision Medicine for Surgical Oncology in Gansu Province and NHC Key Laboratory of Diagnosis and Therapy of Gastrointestinal Tumor, Gansu Provincial Hospital, China

2 Key Laboratory of Radiation Technology and Biophysics, Hefei Institutes of Physical Science, Chinese Academy of Sciences, China

3 Institute of Health-Biotech, Health-Biotech (Tianjin) Stem Cell Research Institute Co., Ltd., China

4 Jiangxi Research Center of Stem Cell Engineering, Jiangxi Health-Biotech Stem Cell Technology Co., Ltd., China

5 Center for Cellular Therapies, The First Affiliated Hospital of Shandong First Medical University, China

6 State Key Laboratory of Experimental Hematology, National Clinical Research Center for Blood Diseases, Haihe Laboratory of Cell Ecosystem, Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin, China

*Address all correspondence to: leisheng_zhang@163.com

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