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REVIEW

Technical advances in NK cell-based cellular immunotherapy

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ABSTRACT Natural killer (NK) cells represent a promising future for tumor immunotherapy because of their unique biological functions and characteristics. This review focuses on technical advances in NK cell-based cellular immunotherapy and summarizes the developments of recent years in cell sources, genetic modification, manufacturing systems, clinical programs, and outcomes. Future prospects and challenges in NK cell immunotherapy are also discussed, including off-the-shelf NK cell exploitation, automatic and closed manufacturing systems, cryopreservation, and therapies involving regulatory checkpoints.

KEYWORDS Natural killer cell; immunotherapy; adoptive cell transfer; genetic modification

Introduction

Natural killer (NK) cell-based immunotherapies are an attractive approach for treating malignancies because of their characteristic recognition and killing mechanisms[1-3]. Despite increasing interest in NK cell immunotherapy for cancer, some challenges have emerged. Owing to the low frequency of NK cells in peripheral blood (PB; 10%–15%) and cord blood (15%–20%)[4,5], expansion is necessary to obtain them in clinical quantities. Feeder cell-based NK cell expansion systems can be used to obtain highly purified and quantitative products[6]; however, the use of a feeder cell line may result in unpredictable risks. Although feeder-free NK cell expansion systems are relatively simple, the expansion fold is limited[7]. Moreover, the transduction efficiency of NK cells varies, ranging from 25% to 50% in NK cell lines[8], compared with less than 20% in primary NK cells and 6%–96% in *ex vivo* expanded NK cells[9]. The quantity of NK cells in a manufactured batch is not sufficient for multiple-dose adoptive transfer; thus, it is appropriate to cryopreserve NK cells for clinical use. However, NK cells are more sensitive than T cells to the freeze and thaw process, and the effects of cryopreservation on their cytotoxicity remain controversial[10,11]. Nevertheless, recent technical advances, including successful generation of stem-cell-derived NK cells, the development of an IL-15 super-agonist that can efficiently activate NK cells without side effects, and impressive clinical results from combination therapy and immune checkpoint inhibitors, have provided great encouragement and an indication of future trends in the development of NK cell immunotherapy.

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Development of NK cell manufacturing

Sources of NK cells

Allogeneic or autologous PB cells have traditionally been the major sources of NK cells for immunotherapy. However, this cell population is donor dependent and heterogeneous, and the efficiency of expansion systems varies. Recently, $CD34^+$ stem cells from sources such as cord blood and induced pluripotent stem cells (iPSCs) have been used to generate NK cells[12,13]. Various protocols involving xenogeneic stromal feeder cell lines[14] or a spin-embryoid body have been used to induce iPSC differentiation[12,15], producing more than 1,000-fold expansion of NK cells with purity of ≥ 90%. More importantly, by screening single iPSC clones, this approach

provides a genetically defined, homogeneous NK cell population that can be genetically modified and expanded on a large scale to produce multiple doses. Therefore, stem-cell-derived NK cells represent a possible means of achieving "off-the-shelf"production, genetic modification, and defined and stable supplementation for NK cell generation.

Optimization of CARs for NK cells

Chimeric antigen receptor (CAR) autologous T cells have shown promising clinical outcomes against hematopoietic malignancies. NK cells have been explored as candidates for CAR engineering, enabling them to be directed to specific targets[16]. In recent years, several researchers have focused on the optimization of CAR constructs, including the extracellular antigen recognition domain and intracellular costimulatory signaling domain. Previously, CARs were designed to recognize tumor cells using the extracellular part; more recently, the targeting of CARs has focused on suppressor cells in the tumor microenvironment. NK cells engineered with a CAR that recognizes myeloid-derived suppressor cells (MDSCs) with overexpression of molecular NKG2D ligands can efficiently kill intra-tumoral MDSCs. This is a viable way to relieve immunosuppression and support other forms of immunotherapy[17]. Currently, most intracellular signaling domains of CARs are CD3-ζ chains incorporated with costimulatory signaling domains such as CD28. However, CD28 is not naturally expressed in NK cells, so the function of the CD28 signaling domain in NK cells is not clearly defined. Therefore, CAR constructs in NK cells suited to costimulatory signaling domains are needed. Kaufman's group reported that CAR constructs in NK cells typically expressing costimulatory signaling domains NKG2D-2B4ζ showed greater capacity to induce NK cell cytotoxicity against targets. Notably, T cells engineered with T-CAR showed better activity than those engineered with NK-CAR[12]. Optimization of CAR intracellular costimulatory signaling domains is needed, in order to find CARs suited to NK cells and T cells, respectively.

Currently, the NK cell line, PB-NK, and stem-cell-derived NK cells can all be engineered with CARs. However, the efficiency of CAR gene transfer is lower in PB-NK, ranging from 10% to 60%, compared with the NK cell line and stem-cell-derived NK cells, which have efficiencies of up to 90%[18]. Moreover, which type of CAR-NK cell provides the greatest benefit is still the subject of research. The latest report shows that CAR-NK-92 cells have stronger cytotoxic activities compared with CAR-engineered PB-derived NK cells from healthy donors *in vivo*[19]. However, NK cell lines must be irradiated before infusion, resulting in reduced cytotoxicity and migration. Overall, CAR-NK strategies require further study.

The extracellular part of CARs is usually a single-chain variable fragment, designed to rely on the antibody. This extracellular part enables the cells to recognize specific antigens only expressed on the target cell surface. However, this recognition model represents a bottleneck in CAR-engineering of cells. Recently, the T cell receptor (TCR), which can recognize antigenic peptides, has been used to establish a TCR-NK cell. The transgenic NK-92 cell line expressing the CD3 signaling complex acquired a T-cell-like profile and showed pMHC-specific cytotoxic function[20]. TCR-NK cells can recognize all cellular proteins, enabling them to break through the bottleneck and expanding the range of potential applications.

Expansion systems for NK cells

Stem-cell-derived NK cells generation systems usually have two stages: the hematopoietic progenitor differentiation stage and the NK cell differentiation/expansion stage. Most early studies used two murine stromal cell lines, S17 or M210 in stage 1 and EL08-1D2 or AFT024 in stage 2[21]. However, the heterogeneity of these cell lines limited the clinical use of NK cells derived from such systems. A feeder-free system for differentiation and expansion of NK cells was established recently. This led to the development of a spin-dependent hematopoietic progenitor enrichment system that did not require sorting and could produce about 10 times more progenitor cells compared with a feeder-dependent sorting system. These two systems yielded a 1–2-log expansion of stem-cell-derived $CD45^+CD56^+$ NK cells in about 4 weeks[15].

Most of the protocols for PB-derived NK cell expansion fall into two categories: feeder-cell systems and feeder-free systems. Several type of cells, including EBV-transformed lymphoblastoids and genetically modified HEK293 or K562 cell lines, are used as feeder cells[22,23]. Genetically modified K562 is used most often. Expression of membrane-bound IL-21 in K562 feeder cells has significantly improved the expansion efficiency to 40,000-fold and the NK cell purity to 95% for 2–3-week cultures[24]. However, there are safety concerns regarding the clinical application of cancer cell-derived feeder cells, although the feeder cells are lethally irradiated before use. Recently, irradiated autologous PBMCs combined with anti-CD16 antibodies have been used as feeder cells, resulting in a greater than 5,000-fold expansion rate[23]. Furthermore, collected cell lysis particles from feeder cells have been used instead of whole cells for NK cell

expansion in vitro and in vivo; this may become an effective alternative to feeder-cell systems[25]. On the other hand, feeder-free systems based on cytokines and antibodies have been developed to expand NK cells, but their expansion efficiencies range from hundreds- to thousands-fold, and the purity of the resulting NK cells ranges from 30% to 90%. Thus, the major focus for feeder-free NK cell expansion protocols is improving their reliability and efficiency. An IL-15 super-agonist complex, ALT-803, has shown NK cell-specific proliferation and cytotoxicity stimulate activities than its nature form IL-15[26]. The development of these new artificial stimulators could improve feeder-free NK cell expansion systems and facilitate the production of NK cells of clinical grade in appropriate quantities.

Subsets of NK cells exert different cytotoxicities[27]. $CD56^{bright}$ NK cells harbor superior antitumor function compared with $CD56^{dim}$ cells[28,29] in general, although a subset defined as $CD56^{dim}FE\text{-}1H10^{-}$ exhibited higher cytotoxicity than their $CD56^{dim}FE\text{-}1H10^{+}$ counterparts[30]. In addition, NK cell subsets with memory or memory-like profiles exhibited robust responses to tumor cells[31-33]. These NK cell subsets could be exploited to improve NK cell-based immunotherapy, although strategies for their expansion require further study.

Clinical programs for NK cell-based cellular immunotherapy in cancer

NK cell adoptive transfer monotherapy

Owing to the pan-specific tumor cell recognition model of NK cells[34], adoptive transfer has been widely used in tumor immunotherapy clinical research. There are several clinical schemes for NK cell adoptive transfer. Some of the reported studies employed a single NK cell infusion scheme, with $1 - 10 \times 10^7$ NK cells/kg[35-37], which was sufficient to evaluate safety and NK cell retention *in vivo*. However, considering that NK cells survive for about 2 weeks *in vivo*[38], a single infusion may not be sufficient to maximize the effect. Other studies employed an infusion scheme of $1 - 4 \times 10^7$ NK cells/kg three times weekly[39]; this may guarantee the safety of each return, while increasing the dose of NK cells for total infusion. In addition, an infusion scheme of $1 - 4 \times 10^7$ NK cells/kg in 2 – 4 infusions once per week was employed[40]. This infusion scheme may achieve the persistence and quantity of NK cells *in vivo* required for long-term therapeutic effect. However, this infusion scheme requires a weekly NK cells supply; considering the challenges of NK cells cryopreservation, the feasibility of this scheme is uncertain. Both autologous and allogenic NK cells can be used in adoptive transfer cell therapy. However, the impaired development and function of patient-derived autologous NK cells and cell lines limit the clinical applications[41,42]. Therefore, allogeneic NK cells have been employed in the majority of clinical trials of NK cell-based adoptive cell transfer[43]. In addition to allogeneic PB-NKs, stem-cell-derived and iPSC-derived NK cells have been developed, and their tumor suppression effects have been confirmed in preclinical models[13,44]. Clinical trials of stem-cell-derived and iPSC-derived NK cells against tumors are also ongoing (www.clinicaltrials.gov). Therefore, the optimization of the NK cells infusion scheme requires further study.

NK cell monotherapy has different clinical outcomes for different indications. Early clinical trials showed better clinical outcomes for allogeneic NK cell immunotherapy against hematopoietic malignancies, with complete and partial response rates of about 50%, compared with about 20% for NK cell immunotherapy against solid tumors[38,45]. In recent years, the clinical scheme for NK cells immunotherapy has been optimized, and clinical outcomes have gradually improved (**Table 1**).

Although NK cell therapy has shown a promising tumor suppressive effect, the clinical outcomes of NK cell monotherapy are still limited. Hence, studies using NK cell therapy in combination with other therapies to maximize effectiveness have become mainstream.

Combinational therapy with NK cell adoptive transfer

In previous studies, NK cell therapy was usually combined with conventional cancer treatments such as chemotherapy or radiotherapy. Patients treated with 2 Gy whole-body irradiation followed by an infusion of 2×10^7 NK cells/kg 1 day later had significantly prolonged overall survival[46]. Patients who received chemotherapy were subsequently infused with $1 - 3 \times 10^7$ NK cells/kg three times a week, leading to progression-free survival of about 4 months[47]. For hematological malignancies, NK cell therapy was usually combined with hematopoietic stem cell transplantation (SCT); transferring 1×10^7 NK cells/kg 1 – 3 months after allo-SCT transplantation enhanced hematopoietic stem cell engraftment[48,49]. However, several factors including dosage, intensity, and sequential order affect the outcomes of NK cells therapy. Therefore, increasing attention has focused on NK cell combination therapies in recent years (**Table 1**). Combined with chemotherapy, infusion of an increased dosage of 5×10^7 NK cells/kg prolonged the average disease-

Table 1 Clinical outcomes of NK cell immunotherapy (2017–2019)

Indication	Source of NK cells	Therapeutic regimen	Outcome	Reference
Allogenic PB-NK	Hepatic carcinoma	20 × 10^6 NK cells/kg on day 1 to day 3, monthly, 1–6 infusions	3/16 PR 8/16 SD 5/16 PD PFS was 7.5 months (range, 2–12 months)	64
Allogenic PB-NK	Children with intermediate- or high-risk AML	Cyclophosphamide (day −7), fludarabine(days −6 through −2), and subcutaneous interleukin-2 (days −1, 1, 3, 5, 7, and 9), 3.6–62.2 × 10^6 NK cells/kg were infused on day 0, 4–5 infusions	8/21 relapse occurred between 186 and 629 days after NK cell infusion3/21 died of the disease	65
Allogenic PB-NK	Liver metastases of gastrointestinal carcinoma	3.1–12.1 × 10^6 NK cells/kg were infused on day 0, cetuximab intravenously first on day 7, weekly, 7 infusions	1/9 PR 2/9 SD 5/9 PD 1/9 Rd	66
Allogenic PB-NK	Children with recurrent/refractory neuroblastoma	hu14.18K322A, 40 mg/m2/dose, days 2–5, 7 infusions GM-CSF, and IL2 with chemotherapy: cyclophosphamide/topotecan (courses 1,2), irinotecan/temozolomide (courses 3,4), and ifosfamide/carboplatin/etoposide (courses 5,6) 4.7–59.5 x 10^6/kg NK cells were administered with courses 2, 4, and 6	4/13 CR 4/13 PR 5/13 SD Median time to progression was 274 days (range, 239–568 days)10 /13 patients survived 1 year	67
Allogenic PB-NK	High-risk neuroblastoma	NK cells at one of five dose levels ranging from 1 to 50 x 10^6 $CD3^-CD56^+$ cells/kg	10/35 CR or PR 8/35 PD 17/35 no response	68
Allogenic PB-NK	AML CML MDS	Melphalan (day -7), fludarabine (days -7, -6, -5, and -4). 200 cGy total body irradiation (day -3), 1 × 10^5 to 1 × 10^8/kg/dose NK cells (days -2, -7, and -28). Bone marrow graft was infused fresh on day 0	54% developed grade 1–2 aGVHD; a low incidence of viral complications was observed. 1/13 died of non-relapse mortality 1/13 relapsed 11/13 alive and in remission at last follow-up (median 14.7 months)	52
Autologous PB-NK	Gastric or colorectal cancer	IgG1 antibodies treated on weeks -3, 3, 6, 10. NK cells, 3 doses 0.5 × 10^9 cells/injection on week 0, 3 doses 1.0 × 10^9 cells/injection on week 3, 2.0 ×10^9 cells/injection on week 6	4/6 SD 2/6 PD	69
Autologous PB-NK	Locally advanced colon carcinoma	Pre-treatment with 5-fluorouracil or oxaliplatin, then patients were treated with chemotherapy combined with NK cells 2.4–4.0 × 10^9/injection	Median PFS 23.5 months Prevented recurrence Prolonged survival with acceptable adverse effects Poorly differentiated carcinomas	50
CB-NK	MM	Lenalidomide on days -8 to -2, melphalan on day-7, 5 × 10^6 to 1 × 10^8 CB-NK cells/kg on day -5 and auto-HCT on day 0	8/10 CR Median follow-up of 21 months, 4/10 progressed or relapsed, 2/10 died	51

AML: acute myeloid leukemia; CML: chronic myeloid leukemia; CR: complete response; MDS: myelodysplastic syndrome; MM: multiple myeloma; PD: disease progression; PFS: progression-free survival; PR: partial response; Rd: dissociated response; SD: disease stabilization.

free survival time to 23.5 months[50]. In addition, NK cell infusion 1 week before hematopoietic stem cell transplantation benefited the regeneration of hematopoietic stem cells[51]. Moreover, patients with hematopoietic malignancies treated with radiotherapy followed by hematopoietic stem cell transplantation and three courses of 1×10^8 NK cells/kg achieved 85% remission lasting for about 14.7 months[52]. Based on the regulation of other cells by NK cells[53,54], NK cell therapy can be combined with novel immunotherapies. Copik's group reported that adoptive transfer NK cells could induce the expression of PD-L1 on tumor cells; thus, NK cell infusion combined with anti-PD-L1 treatment could significantly improve the NK cells' anti-tumor efficacy, with prolonged NK cell persistence and retention[55]. However, this has only been shown in animal models, with no clinical trial outcomes reported as yet.

Recently, a case study showed that a combination of radiochemotherapy, NK cell adoptive transfer, and PD-1 inhibition could effectively induce long-term tumor control in a patient with stage IIIb non-small cell lung cancer[56]. However, NK cell combination therapies require further study and clinical verification.

Future prospects

Exploitation of off-the-shelf NK cells

Owing to the one-donor, one-patient strategy, the manufacture of clinical grade NK cells is a labor-intensive process. In addition, each batch of cell product may differ in yield, purity, and cell activity. In clinical applications, this requires a huge amount of work on product verification and a resulting lag-time to patient infusion. Although researchers have tried to introduce some criteria to predict or guarantee the success rate of cell production, it remains difficult to guarantee 100% cell infusion in less than 3–5 weeks. This may greatly limit the clinical applications of cell therapy. Therefore, there is a great need for an "off-the-shelf" product. Such a product should have sufficient cells for clinical use, and a clearly illustrated cell profile. Taking into account these quality and quantity criteria, monoclonal $CD34^+$ stem cells, including iPSC-derived NK cells, may be suitable candidates. $CD34^+$ stem cells are an unlimited NK cell source. NK cells derived from monoclonal $CD34^+$ stem cells are homogeneous and can be clearly genetically defined. Kaufman's group has established a manufacturing system for clinical grade iPSC-derived NK cells. Using genetic modification, they established a CAR-iPSC-NK cell line. A xenograft mouse model showed that CAR-iPSC-NK cell infusion could significantly inhibit tumor growth and prolong survival[12]. This manufacturing strategy for monoclonal $CD34^+$ stem-cell-derived NK cells may represent a feasible off-the-shelf cell product system, although this awaits confirmation through clinical research.

Manufacturing cell products

Currently, NK cells used for preclinical studies or clinical applications are usually cultured in T-flasks or bags. Handling these culture vessels is labor-intensive work and requires highly skilled experts. Using these culture vessels for every batch product leads to low levels of standardization. Thus, there is a great need to explore closed and automatic manufacturing systems, with the aim of achieving highly standardized cell manufacturing and reducing dependency on specialized cell manufacturing centers. Miltenyi Biotech has developed a fully automated, large-scale NK cell expansion system, in which centrifugation, magnetic cell separation, and cell cultivation are performed in a closed system. NK cells produced by this automated system have similar yield and cell function to manually produced NK cells[57]. Although this system was developed before 2015, its application is not universal. This is probably owing to the diversity of NK cell manufacturing processes with different initial cells, stimulators, and cell passaging principles. Currently, Boya Beijing, Novartis, and Kite Pharma are developing closed and automatic cell manufacturing systems. It is both challenging and necessary to develop an automated system suitable for diverse expansion systems.

NK cells cryopreservation

The condition of a patient, especially in cases of hematopoietic malignancy, may change at any time. It is difficult to guarantee that an NK cell product can be delivered to the patient at the appropriate time during the stable period of NK cell product quality, meaning that every newly started course of NK cell therapy needs the NK cells to be re-produced. Thus, there is a great need for NK cell cryopreservation technologies; these would increase the convenience of NK cell therapy in clinical applications, while reducing production costs and promoting the development of off-the-shelf products. However, current cryopreservation technology for NK cells has some limitations. The optimization of the cryoprotectant formula is ongoing, including the usage and proportion of autologous plasma, dimethyl sulfoxide (DMSO), and plasma substitution[58,59]. A recent study showed that NK cells cryopreserved using freezing media containing RPMI1640, albumin, dextran, and DMSO retained about 90% viability and had no reduction in cytotoxicity after thawing. Moreover, viability and cytotoxicity were not significantly influenced after thawing when the cells were incubated in the presence of IL-2[59]. This is a promising result for NK cell immunotherapy, although it requires verification.

Investigating the regulatory checkpoints of NK cell therapies to ensure safety and efficacy

Until now, regardless of whether autologous or allogeneic NK cell therapy was used, most clinical trials have shown better safety, less severe uncontrollable graft-versus-host disease (GVHD), and fewer severe adverse effects above grade 4. However, other cells in the final product used for NK cell

therapy represent a potential risk in clinical infusion; these include $CD4^+$ T cells and B cells. At present, there are no criteria for the composition of NK cell therapy products. It is necessary to establish standards to define the proportion of NK cells and other immune cell subsets to avoid potential risks. NK cells can pan-specifically recognize malignant cells and have a broad-spectrum cytotoxicity to various malignant cells. However, clinical trial outcomes show that NK cell therapy has different effects on different tumors. Activation of NK cell function is determined by the integration of the received activating and inhibitory signals, so the activation trigger of NK cells is partially affected by the number and abundance of activating and inhibitory ligands expressed on the surface of target cells. In addition, various factors including cytokines and immunosuppressive cells in the tumor microenvironment affect NK cell activation and the clinical outcomes of NK cell therapy[27,60-63]. Therefore, some regulatory checkpoints from the perspective of tumor cells and the tumor microenvironment will be beneficial to improve the outcomes of NK cell therapy. Establishing molecular and cell indices for NK cell therapy will also promote its clinical applications. The overall goal is to maximize the benefits of NK cell therapy while minimizing the risk.

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Conflict of interest statement

No potential conflicts of interest are disclosed.

References

1. Ramos CA, Heslop HE, Brenner MK. CAR-T cell therapy for lymphoma. Annu Rev Med. 2016; 67: 165-83.
2. Handgretinger R, Lang P, André MC. Exploitation of natural killer cells for the treatment of acute leukemia. Blood. 2016; 127: 3341-9.
3. He YK, Tian ZG. NK cell education via nonclassical MHC and non-MHC ligands. Cell Mol Immunol. 2017; 14: 321-30.
4. Luevano M, Daryouzeh M, Alnabhan R, Querol S, Khakoo S, Madrigal A, et al. The unique profile of cord blood natural killer cells balances incomplete maturation and effective killing function upon activation. Hum Immunol. 2012; 73: 248-57.
5. Dalle JH, Menezes J, Wagner E, Blagdon M, Champagne J, Champagne MA, et al. Characterization of cord blood natural killer cells: implications for transplantation and neonatal infections. Pediatr Res. 2005; 57: 649-55.
6. Fujisaki H, Kakuda H, Shimasaki N, Imai C, Ma J, Lockey T, et al. Expansion of highly cytotoxic human natural killer cells for cancer cell therapy. Cancer Res. 2009; 69: 4010-7.
7. Gras Navarro A, Bjorklund AT, Chekenya M. Therapeutic potential and challenges of natural killer cells in treatment of solid tumors. Front Immunol. 2015; 6: 202.
8. Tassev DV, Cheng M, Cheung NK. Retargeting NK92 cells using an HLA-A2-restricted, EBNA3C-specific chimeric antigen receptor. Cancer Gene Ther. 2012; 19: 84-100.
9. Carlsten M, Childs RW. Genetic manipulation of NK cells for cancer immunotherapy: techniques and clinical implications. Front Immunol. 2015; 6: 266.
10. Streltsova MA, Erokhina SA, Kanevskiy LM, Grechikhina MV, Kobyzeva PA, Lee DA, et al. Recurrent stimulation of natural killer cell clones with k562 expressing membrane-bound interleukin-21 affects their phenotype, interferon-γ production, and lifespan. Int J Mol Sci. 2019; 20: 443.
11. Lapteva N, Szmania SM, Van Rhee F, Rooney CM. Clinical grade purification and expansion of natural killer cells. Crit Rev Oncog. 2014; 19: 121-32.
12. 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.
13. Zeng JM, 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.
14. Woll PS, Grzywacz B, Tian XH, 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.
15. Knorr DA, Ni ZY, Hermanson D, Hexum MK, Bendzick L, Cooper LJN, 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.
16. Mehta RS, Rezvani K. Chimeric antigen receptor expressing natural killer cells for the immunotherapy of cancer. Front Immunol. 2018; 9: 283.
17. Parihar R, Rivas C, Huynh M, Omer B, Lapteva N, Metelitsa LS, et al. NK cells expressing a chimeric activating receptor eliminate MDSCs and rescue impaired CAR-T cell activity against solid tumors. Cancer Immunol Res. 2019; 7: 363-75.
18. Hermanson DL, Kaufman DS. Utilizing chimeric antigen receptors to direct natural killer cell activity. Front Immunol. 2015; 6: 195.
19. Kloess S, Oberschmidt O, Dahlke J, Vu XK, Neudoerfl C, Kloos A,

et al. Preclinical assessment of suitable natural killer cell sources for chimeric antigen receptor natural killer-based "off-the-shelf " acute myeloid leukemia immunotherapies. Hum Gene Ther. 2019; 30: 381-401.

20. Mensali N, Dillard P, Hebeisen M, Lorenz S, Theodossiou T, Myhre MR, et al. NK cells specifically TCR-dressed to kill cancer cells. EBioMedicine. 2019; 40: 106-17.
21. Zhu H, Lai YS, Li Y, Blum RH, Kaufman DS. Concise review: human pluripotent stem cells to produce cell-based cancer immunotherapy. Stem Cells. 2018; 36: 134-45.
22. Baggio L, Laureano ÁM, Silla LMDR, Lee DA. Natural killer cell adoptive immunotherapy: coming of age. Clin Immunol. 2017; 177: 3-11.
23. Lee HR, Son CH, Koh EK, Bae JH, Kang CD, Yang K, et al. Expansion of cytotoxic natural killer cells using irradiated autologous peripheral blood mononuclear cells and anti-CD16 antibody. Sci Rep. 2017; 7: 11075.
24. Denman CJ, Senyukov VV, Somanchi SS, Phatarpekar PV, Kopp LM, Johnson JL, et al. Membrane-bound IL-21 promotes sustained *ex vivo* proliferation of human natural killer cells. PLoS One. 2012; 7: e30264.
25. Oyer JL, Pandey V, Igarashi RY, Somanchi SS, Zakari A, Solh M, et al. Natural killer cells stimulated with PM21 particles expand and biodistribute *in vivo*: clinical implications for cancer treatment. Cytotherapy. 2016; 18: 653-63.
26. Felices M, Chu S, Kodal B, Bendzick L, Ryan C, Lenvik AJ, et al. IL-15 super-agonist (ALT-803) enhances natural killer (NK) cell function against ovarian cancer. Gynecol Oncol. 2017; 145: 453-61.
27. Andreotti JP, Paiva AE, Prazeres PHDM, Guerra DAP, Silva WN, Vaz RS, et al. The role of natural killer cells in the uterine microenvironment during pregnancy. Cell Mol Immunol. 2018; 15: 941-3.
28. Poznanski SM, Nham T, Chew MV, Lee AJ, Hammill JA, Fan IY, et al. Expanded $CD56^{superbright}CD16^{+}$ NK cells from ovarian cancer patients are cytotoxic against autologous tumor in a patient-derived xenograft murine model. Cancer Immunol Res. 2018; 6: 1174-85.
29. Poznanski SM, Ashkar AA. Shining light on the significance of NK cell CD56 brightness. Cell Mol Immunol. 2018; 15: 1071-3.
30. Khummuang S, Chuensirikulchai K, Pata S, Laopajon W, Chruewkamlow N, Mahasongkram K, et al. Characterization and functional analysis of novel circulating NK cell sub-populations. Int Immunol. 2019; 31: 515-30.
31. 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.
32. Wu LSH, Wang JY. Warm up, cool down, and tearing apart in NK cell memory. Cell Mol Immunol. 2018; 15: 1095-7.
33. Wang XW, Peng H, Tian ZG. Innate lymphoid cell memory. Cell Mol Immunol. 2019; 16: 423-9.
34. Sivori S, Vacca P, Del Zotto G, Munari E, Mingari MC, Moretta L. Human NK cells: surface receptors, inhibitory checkpoints, and translational applications. Cell Mol Immunol. 2019; 16: 430-41.
35. 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.
36. 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.
37. Rubnitz JE, Inaba H, Ribeiro RC, Pounds S, Rooney B, Bell T, et al. NKAML: a pilot study to determine the safety and feasibility of haploidentical natural killer cell transplantation in childhood acute myeloid leukemia. J Clin Oncol. 2010; 28: 955-9.
38. Fang F, Xiao WH, Tian ZG. Challenges of NK cell-based immunotherapy in the new era. Front Med. 2018; 12: 440-50.
39. Sakamoto N, Ishikawa T, Kokura S, Okayama T, Oka K, Ideno M, et al. Phase I clinical trial of autologous NK cell therapy using novel expansion method in patients with advanced digestive cancer. J Transl Med. 2015; 13: 277.
40. Leivas A, Perez-Martinez A, Blanchard MJ, Martín-Clavero E, Fernández L, Lahuerta JJ, et al. Novel treatment strategy with autologous activated and expanded natural killer cells plus anti-myeloma drugs for multiple myeloma. OncoImmunology. 2016; 5: e1250051.
41. Rezvani K, Rouce RH. The application of natural killer cell immunotherapy for the treatment of cancer. Front Immunol. 2015; 6: 578.
42. Knorr DA, Bachanova V, Verneris MR, Miller JS. Clinical utility of natural killer cells in cancer therapy and transplantation. Semin Immunol. 2014; 26: 161-72.
43. Veluchamy JP, Kok N, Van Der Vliet HJ, Verheul HMW, De Gruijl TD, Spanholtz J. The rise of allogeneic natural killer cells as a platform for cancer immunotherapy: recent innovations and future developments. Front Immunol. 2017; 8: 631.
44. Wang KJ, Han Y, Cho WC, Zhu H. The rise of human stem cell-derived natural killer cells for cancer immunotherapy. Expert Opin Biol Ther. 2019; 19: 141-8.
45. Habif G, Crinier A, André P, Vivier E, Narni-Mancinelli E. Targeting natural killer cells in solid tumors. Cell Mol Immunol. 2019; 16: 415-22.
46. Geller MA, Cooley S, Judson PL, Ghebre R, Carson LF, Argenta PA, et al. A phase II study of allogeneic natural killer cell therapy to treat patients with recurrent ovarian and breast cancer. Cytotherapy. 2011; 13: 98-107.
47. Yang Y, Lim O, Kim TM, Ahn YO, Choi H, Chung H, et al. Phase I study of random healthy donor-derived allogeneic natural killer cell therapy in patients with malignant lymphoma or advanced solid tumors. Cancer Immunol Res. 2016; 4: 215-24.
48. Shaffer BC, Le Luduec JB, Forlenza C, Jakubowski AA, Perales MA, Young JW, et al. Phase II study of haploidentical natural killer cell infusion for treatment of relapsed or persistent myeloid malignancies following allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2016; 22: 705-9.
49. Pérez-Martínez A, Fernández L, Valentín J, Martínez-Romera I,

Corral MD, Ramírez M, et al. A phase I/II trial of interleukin-15-stimulated natural killer cell infusion after haplo-identical stem cell transplantation for pediatric refractory solid tumors. Cytotherapy. 2015; 17: 1594-603.
50. Li LY, Li W, Wang C, Yan X, Wang YZ, Niu C, et al. Adoptive transfer of natural killer cells in combination with chemotherapy improves outcomes of patients with locally advanced colon carcinoma. Cytotherapy. 2018; 20: 134-48.
51. Shah N, Li L, McCarty J, Kaur I, Yvon E, Shaim H, et al. Phase I study of cord blood-derived natural killer cells combined with autologous stem cell transplantation in multiple myeloma. Br J Haematol. 2017; 177: 457-66.
52. Ciurea SO, Schafer JR, Bassett R, Denman CJ, Cao K, Willis D, et al. Phase 1 clinical trial using mbIL21 *ex-vivo* expanded donor-derived NK cells after haploidentical transplantation. Blood. 2017; 130: 1857-68.
53. Liu Y, Zheng J, Liu YP, Wen LY, Huang L, Xiang Z, et al. Uncompromised NK cell activation is essential for virus-specific CTL activity during acute influenza virus infection. Cell Mol Immunol. 2018; 15: 827-37.
54. Barry KC, Hsu J, Broz ML, Cueto FJ, Binnewies M, Combes AJ, et al. A natural killer-dendritic cell axis defines checkpoint therapy-responsive tumor microenvironments. Nat Med. 2018; 24: 1178-91.
55. Oyer JL, Gitto SB, Altomare DA, Copik AJ. PD-L1 blockade enhances anti-tumor efficacy of NK cells. OncoImmunology. 2018; 7: e1509819.
56. Kokowski K, Stangl S, Seier S, Hildebrandt M, Vaupel P, Multhoff G. Radiochemotherapy combined with NK cell transfer followed by second-line PD-1 inhibition in a patient with NSCLC stage IIIb inducing long-term tumor control: a case study. Strahlenther Onkol. 2019; 195: 352-61.
57. Granzin M, Soltenborn S, Müller S, Kollet J, Berg M, Cerwenka A, et al. Fully automated expansion and activation of clinical-grade natural killer cells for adoptive immunotherapy. Cytotherapy. 2015; 17: 621-32.
58. Lee DA. Regulatory considerations for NK cells used in human immunotherapy applications. In: Somanchi SS. Natural Killer Cells: Methods and Protocols. New York, NY: Humana Press; 2016; 347-61.
59. Min B, Choi H, Her JH, Jung MY, Kim HJ, Jung MY, et al. Optimization of large-scale expansion and cryopreservation of human natural killer cells for anti-tumor therapy. Immune Netw. 2018; 18: e31.
60. Huang Q, Huang M, Meng FT, Sun R. Activated pancreatic stellate cells inhibit NK cell function in the human pancreatic cancer microenvironment. Cell Mol Immunol. 2018; 16: 87-9.
61. Zhang SP, Wu M, Wang F. Immune regulation by $CD8^+$ treg cells: novel possibilities for anticancer immunotherapy. Cell Mol Immunol. 2018; 15: 805-7.
62. Trotta AM, Pacelli R, Scala S. Predictive immune biomarkers: an unattainable chimera? Cell Mol Immunol. 2018; 15: 740-2.
63. Zou WP. Mechanistic insights into cancer immunity and immunotherapy. Cell Mol Immunol. 2018; 15: 419-20.
64. Qin ZL, Chen JB, Zeng JY, Niu LZ, Xie SL, Wang XH, et al. Effect of NK cell immunotherapy on immune function in patients with hepatic carcinoma: a preliminary clinical study. Cancer Biol Ther. 2017; 18: 323-30.
65. Nguyen R, Wu HY, Pounds S, Inaba H, Ribeiro RC, Cullins D, et al. A phase II clinical trial of adoptive transfer of haploidentical natural killer cells for consolidation therapy of pediatric acute myeloid leukemia. J ImmunoTher Cancer. 2019; 7: 81.
66. Adotevi O, Godet Y, Galaine J, Lakkis Z, Idirene I, Certoux JM, et al. In situ delivery of allogeneic natural killer cell (NK) combined with cetuximab in liver metastases of gastrointestinal carcinoma: a phase I clinical trial. OncoImmunology. 2018; 7: e1424673.
67. Federico SM, McCarville MB, Shulkin BL, Sondel PM, Hank JA, Hutson P, et al. A pilot trial of humanized anti-GD2 monoclonal antibody (hu14.18K322A) with chemotherapy and natural killer cells in children with recurrent/refractory neuroblastoma. Clin Cancer Res. 2017; 23: 6441-9.
68. Modak S, Le Luduec JB, Cheung IY, Goldman DA, Ostrovnaya I, Doubrovina E, et al. Adoptive immunotherapy with haploidentical natural killer cells and anti-GD2 monoclonal antibody m3F8 for resistant neuroblastoma: results of a phase I study. OncoImmunology. 2018; 7: e1461305.
69. Ishikawa T, Okayama T, Sakamoto N, Ideno M, Oka K, Enoki T, et al. Phase I clinical trial of adoptive transfer of expanded natural killer cells in combination with igG1 antibody in patients with gastric or colorectal cancer. Int J Cancer. 2018; 142: 2599-609.

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