Seminars in Immunology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Seminars in Immunology

journal homepage: www.elsevier.com/locate/ysmim

Review

NK cell-based immunotherapy for cancer

Fang Fang[a,b], Weihua Xiao[a,b,*], Zhigang Tian[a,b,*]

[a] Institute of Immunology and The CAS Key Laboratory of Innate Immunity and Chronic Disease, Innovation Center for Cell Signaling Network, School of Life Sciences and Medical Center, University Of Science and Technology of China, Hefei, Anhui 230027, China

[b] Hefei National Laboratory for Physical Sciences at Microscale, Engineering Technology Research Center of Biotechnology Drugs Anhui, University of Science and Technology of China, Hefei, Anhui 230027, China

ARTICLE INFO

Keywords:
Natural killer cell
Immunotherapy
Cancer

ABSTRACT

Natural killer (NK) cells can induce an antigen-independent immune response against malignant cells. A growing number of scientific reports and clinical studies have shown promising anti-tumor effects when using NK cell-based immunotherapy. Currently, various approaches are being used to enhance the number and function of NK cells. One approach uses cytokines to selectively boost both the number as well as the efficacy of anti-tumor functions of NK cells. Another emerging approach focuses on checkpoint inhibitors targeting the NK cell receptor. Furthermore, bi-specific and tri-specific engagers have been developed to enhance the specific immune response by cross-linking specific tumor antigens to effector cells. In addition, NK cell adoptive transfer therapies have shown promising prospects. Among the various sources of adoptive transfer NK cells, allogeneic haploidentical NK cells that have undergone short- or long-term activation or expansion have also demonstrated effective anti-tumor effects with a low rate of rejection and side effects. CAR-NKs, derived from a new type of genetic modification, show enhanced NK cell cytotoxicity, specificity, and targeting. These NK cell-based therapies have exhibited promising results in clinical trials with malignant tumors. In this review, the current progress on NK cell-based therapeutic approaches, NK cell manufacturing techniques and tumor therapy outcomes are discussed.

1. Introduction

Natural killer (NK) cells were first characterized in 1975 as a class of cells that differed from T cells and B cells. NK cells, which have the inherent ability to kill malignant cells without prior sensitization, play a major role in the immunosurveillance of malignant cells [1,2]. In addition, NK cells play an important role in maintaining immune homeostasis [3,4]. NK cell development occurs predominantly within the bone marrow and is divided into five stages. In the first stage, the cell is referred to as a common lymphoid progenitor (CLP). During this stage, CLPs are usually indicated by markers such as interleukin-7 receptor (IL-7Ra; CD127), c-kit (CD117), stem cells antigen-1 (Sca-1), and FMS-like tyrosine kinase-3 (Flt-3; CD135). During the second stage, the cell is most commonly referred to as a pre-NK precursor (pre-NKP). However, a recent study has demonstrated that this stage consists of a group of heterogeneous cells that includes NK precursors and innate lymphoid cell precursors. Next, the pre-NKP develops into a NKP, and the cells begin to express the IL-15 receptor complex (IL-15Rβ/γ), which is important for long-term NK cell development and survival. Also during the third stage, the cell begins to develop the symbolic NK cell marker CD56, which can be further subdivided into the two subsets $CD56^{bri}$ and $CD56^{dim}$. $CD56^{bri}$ NK cells, also known as immature NK cells, subsequently play a major role in the production of the cytokine interferon gamma (IFNγ), which is involved in immunomodulation. On the other hand, $CD56^{dim}$ NK cells, also known as mature NK cells, are the dominant cells in the peripheral blood and spleen and play a major role in cytotoxicity [5,6]. Alternatively, NK precursors that access the secondary lymphoid tissue can also develop into mature NK cells. After their development, NK cells circulate and become widely distributed throughout the body [6].

An increasing number of studies have confirmed the presence of a group of tissue-resident NK cells, such as in the liver and uterus [7–10]. In mice, liver-resident NK cells are characterized by $CD49a^{+}DX5^{-}$ expression [11], while $DX5^{-}$ NK cells or $CD49a^{+}$ NK cells reside in the uterus and the decidua, respectively [12]. However, how these tissue-resident NK cells develop remains controversial [11,13–17]. Additionally, while the tissue-resident NK cells in various tissues have similar phenotypes and functions, their function in tumor immunology requires further study.

NK cell activation is tightly regulated to avoid potentially dangerous

* Corresponding authors at: School of Life Sciences and Medical Center, University of Science and Technology of China, 443 Huangshan Road, Hefei, Anhui 230027, China.
E-mail addresses: xiaow@ustc.edu.cn (W. Xiao), tzg@ustc.edu.cn (Z. Tian).

http://dx.doi.org/10.1016/j.smim.2017.07.009
Received 9 June 2017; Accepted 24 July 2017

effects on the host. Various receptors are expressed on NK cells, and the activation of NK cells is dependent on the integration of signals from activating and inhibitory receptors [3,18,19]. NK cells can directly or indirectly receive signals from other immune cells and malignant cells during the immune response. Dendritic cells (DCs) play a role in regulating NK cell proliferation and function by secreting IL-12, type I IFN, *trans*-presenting IL-15 and secretory exosomes [20,21]. M1 polarized macrophage secrete cytokines such as IL-12, IL-18, IL-1β, IFN-β. Upon TLR stimulation, a subset of unpolarized and M2 macrophage can release soluble IL-18. These ultimately contribute to NK cell activation and cytotoxicity [22,23]. Monocytes regulate NK proliferation and function by secreting the factors IL-2, IL-12, IL-18 and IL-21 [24,25]. In addition, $CD4^+$ T cells can secrete IL-2, which is vital for NK cell survival and proliferation. Alternatively, regulatory T (Treg) cells can suppress NK cell proliferation and activity by secreting transforming growth factor beta (TGF-β) [26,27]. Various cell–cell interactions also transmit signals to NK cells [24,25]. Common activating receptors on NK cells include C-type lectin receptors (CD94/NKG2C, NKG2D, NKG2E/H and NKG2F), natural cytotoxicity receptors (NKp30, NKp44 and NKp46), killer cell C-type lectin-like receptor (NKp65, NKp80), Fc receptor FcγR (which mediates antibody-dependent cell cytotoxicity), SLAM family receptors (2B4, SLAM6 and SLAM7, which play a key role in the recognition of hematopoietic cells), killer cell immunoglobulin-like receptors (KIR) (KIR-2DS and KIR-3DS), DNAM-1 and CD137 (41BB), which is an important costimulatory receptor [18,28–30]. Inhibitory receptors include KIR-2DL, KIR-3DL and C-type lectin receptors CD94/NKG2A/B, which recognize HLA class I or class I-like molecules. Furthermore, both HLA class I and class I-like molecules play a key role in maintaining the self-tolerance of NK cells [18,29]. A series of immune checkpoints, including programmed death-1 (PD-1), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), T cell immunoglobulin and mucin domain containing-3 (TIM-3) and T cell immunoreceptor with Ig and ITIM domains (TIGIT), transmit inhibitory signals [18,29,31–33].

Activated NK cells can secrete cytokines and subsequently become cytotoxic or effector NK cells. As previously reported, human CMV (HCMV) infection can induce some activated NK cells to differentiate into long-lived memory cells against HCMV infection [34,35]. The in vitro incubation of NK cells with combinatorial inflammatory cytokines can also induce the production of long-term memory-like NK cells that are not antigen-specific [36].

In contrast to T cells or other adaptive immune cells, NK cells can rapidly release inflammatory cytokines and function as killer cells without pre-immunization. NK cells can also acquire immunological memory under certain conditions, which has previously been considered a function of adaptive immune cells. When NK cells are used in adoptive transfer immunotherapy, they are derived from a variety of sources, such as autologous, allogeneic, and peripheral blood mononuclear cells, stem cells, NK cell lines, and genetically modified NK cells [5,25,37]. Therefore, immunotherapy targeting NK cells has become attractive. In this review, we summarize the strategies for tumor immune therapy using NK cells, the manufacture of NK cells and the clinical outcomes of NK cell-based immunotherapy.

2. Tumor recognition and cytotoxicity of NK cells

Normally, almost all healthy cells express HLA class I molecules, which pair with inhibitory receptors KIRs or CD94/NKG2A/B on NK cells, to inhibit the killing of NK cells to themselves. However, tumor cells undergo surface marker changes. In one hand, certain types of tumor cells lost the expression of HLA class I molecules. On the other hand, stress can lead to the upregulation of damage-associated proteins. Therefore, NK cells recognize tumor cells through two models. In the first model, the loss of HLA class I molecules on tumor cells results also in the loss of inhibitory signals delivered through KIRs or CD94/NKG2A/B, causing the activation of NK cells. This model is called "missing-self" recognition [38]. Second, the upregulated damage-associated proteins can pair with activating receptors on NK cells, to trigger the cytotoxicity of NK cells, in a model known as "stress-induced recognition" [39,40]. Several damage-associated proteins have been found in malignant cells, such as the MHC class I chain-related protein A/B (MICA/MICB), UL16-binding proteins (ULBPs) paired with NKG2D [41], and B7-H6 and HLA-B-associated transcript 3 (BAT3) paired with NKp30. The activating receptor DNAM-1 can recognize CD155 and CD112. NKp44 can recognize an isoform of a mixed-lineage leukemia protein [28,29,42]. Additionally, tumor cells express some proteins which can pair with the inhibitory receptors on NK cells, to transmit the inhibitory signals to evade the immunosurveillance from NK cells. Inhibitory receptors PD-1 and TIM-3 in NK cells can bind to the PD-1 ligand and Gal-9 on tumor cells [43–47]. NK cell inhibitory receptor TIGIT which has higher affinity for CD155 and CD112, can compete with DNAM-1 to counterbalance the activation signal. CD96 subsequently displays an intermediate affinity for CD155, between its affinity for TIGIT and DNAM-1 [33]. The ligands for the activating receptors and inhibitory receptors expressed on the tumor cells simultaneously signal to the NK cells. NK cell activation is subsequently determined by the results of signal integration.

Activated NK cells can kill tumor cells through direct or indirect model [48]. Direct cytotoxicity is based on the formation of an immunological synapse between the tumor cell and the NK cell. In one mechanism, activated NK cells release granules, like secretory lysosomes that contain perforin and granzymes, toward the target cell. Perforin induces the perforation of the target cell membrane. Granzymes, which are a family of serine proteases, then induce caspase-dependent or −independent apoptosis. CD226, CD107a, perforin and granzymes play key roles in this process [49]. Antibody-dependent cell-mediated cytotoxicity (ADCC) is also an important class in this model. In humans, ADCC is usually mediated by IgG. In this model, the Fc portion and Fab portions of the antibody separately binds to the CD16 receptor on NK cell and the tumor antigen, forming a bridge between the two. After the cross-linking of CD16, the activated NK cell can kill the tumor cell through a granule-dependent mechanism [3,18,50]. Another direct killing model involves death receptor-dependent apoptosis. This model relies on the Fas ligand (FasL) and tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), which are expressed on the NK cells, as well as Fas and TRAIL receptors on the target cell. This interaction can trigger intracellular caspase activation. However, this pathway requires more NK-target cell conjugation and displays slower kinetics than granzyme-mediated pathways [29,42,51].

NK cells can also function via an indirect model as regulatory cells that modify the innate and adaptive immune response to gain cytotoxic functions [29]. Activated NK cells can secrete several factors, including the cytokine IFNγ, TNFα, IL-10, chemokine CC-chemokine ligand 3 (CCL3), CCL4, CCL5, granulocyte-macrophage colony-stimulating factor (GM-CSF) and adenosine [5,18,42,52]. Secretion of IFNγ can promote the activation of macrophages and DCs; induce the differentiation of $CD4^+$ T cells into Th1 cells; and induce the differentiation of $CD8^+$ T cell into cytotoxic T lymphocytes (CTL) [29]. Release of TNFα can subsequently promote B cell proliferation and the differentiation of monocytes and macrophages. Moreover, TNFα can directly induce tumor cell necrosis. Tumor cell releasers can be presented by antigen presenting cells, followed by the promotion of the adaptive immune response [3,29,42]. Secretion of chemokine CCL3/4/5 can recruit inflammatory cells, including monocytes, macrophages, eosinophils, and T cells, among others, to inflammatory sites [25].

Recent studies have found NK cells can acquire memory or memory-like properties under some conditions. In the mouse liver, a group of NK cells identified as $NK1.1^+DX5^-CD49a^+$ were found to have a memory response to several viral antigens [7]. In response to a cytomegalovirus infection, a group of NK cells with high levels of CD94-NKG2C provided an enhanced function in response to repeated viral exposure in humans [53]. Cytokine combinations can induce memory-like NK cells [36]. NK

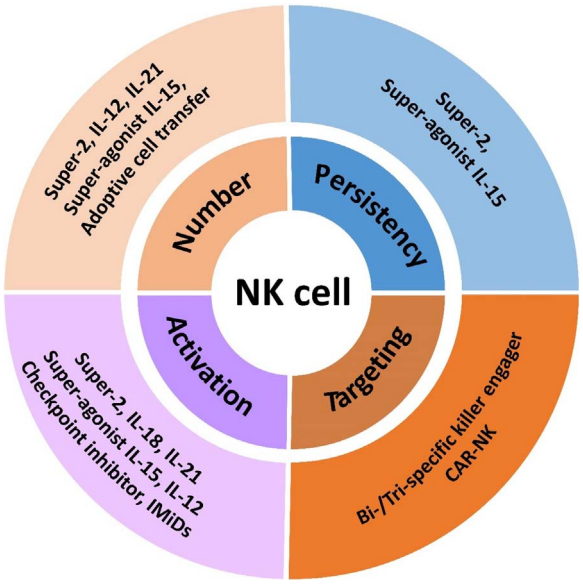

Fig. 1. Factors influencing the effect of NK cell immunotherapy. The outcome of NK cell immunotherapy could be improved from the following aspects, prolonging NK cell persistency by cytokine, or super-agonist; increasing NK cell number by cytokines or adoptive cell transfer; augmenting NK cell activation by cytokine, checkpoint inhibitor or immunomodulatory derivatives (IMiDs), and elevate NK cell tumor targeting by Bi-/Tri-specific killer engagers and CAR-NK.

cells with memory or memory-like properties can survive for a long time and have enhanced functions against target cells or re-stimulation, representing an emerging model for NK cell cytotoxicity.

3. Approaches using NK cell for cancer immunotherapy

Due to the key role that NK cells play in first-line immune response, as well as their rapid effects and absence of pre-immunization, an increasing number of studies have focused on NK cell-based cancer immunotherapy. The primary approaches used for NK cell-based cancer therapy involve cytokines, antibodies, and the adoptive transfer of ex vivo NK cells through improving the persistency, activation, numbers or tumor cell targeting of NK cells (Fig. 1).

3.1. Cytokines

Several cytokines have been confirmed to promote NK cell proliferation and/or boost NK cell function, most commonly through two means. First, the direct transfer of cytokines in vivo can improve the autologous NK cell numbers and functions. Second, the in vitro incubation of NK cells with single or combinatorial cytokines is usually a preliminary step in adoptive transfer for expansion or/and activation.

3.1.1. IL-2

IL-2 is one of the most popular cytokines used for boosting NK cells as well as the first cytokine approved for use in patients [54]. Initially, IL-2 was used to produce lymphokine-activated killer (LAK) cells (Table 1). LAK cells were transferred into patients together with IL-2, but only a limited clinical outcome was achieved. One explanation for this result is that IL-2 activates not only NK cells but also almost all T cells, including Treg cells, which express the high affinity receptor CD25 that can ultimately compete with NK cells for IL-2. Subsequently, Treg cells have been reported to suppress many immune cells, including NK cells. Furthermore, a high dose of IL-2 can cause severe side effects, including capillary leakage and organ injury due to activation of the vascular endothelium [25,54,55]. Therefore, several modifications based on IL-2 have been developed. A mutant form of IL-2 called "super-2" has an increased affinity to IL-2Rβ and can function independently of IL-2Rα (CD25). This superkine displayed an enhanced capacity to promote NK cell proliferation and also reverse the anergic state of NK cells in vivo. Superkine IL-2 can also promote the expansion of cytotoxic T cells but not Treg cells, resulting in fewer side effects when compared with wild type IL-2. Although the functional identification of super-2 was only performed in a mouse model, it shows potential in human models too [56]. Fusion proteins that combine IL-2 and NK cell-activating receptor ligands are another promising tool. The NKG2D ligand orthopoxvirus major histocompatibility complex class I-like protein (OMCP) has limited exposure in humans and has an equal or greater affinity than all other known NKG2D ligands. OMCP has previously been fused to the N-terminus of the mutant form of IL-2, R38A/F42K, which has a decreased affinity to IL-2Rα. This fusion protein selectively activates NKG2D-bearing cells and weakens the activation of IL-2Rα-bearing cells. Thus, this protein shows an enhanced capacity to selectively promote the expansion and activation of NK cells in vivo without expanding and activating Treg cells and also has fewer side effects [57]. In addition to being a therapeutic cytokine, IL-2 is used after NK cell adoptive transfer to support the survival and function of NK cells in vivo [58–63].

3.1.2. IL-15

IL-15 is a special gamma chain cytokine which can function through *cis*-presentation or *trans*-presentation. The IL-15 receptor complex consists of IL-15Rα/β/γ. IL-15 can bind to the trimeric receptor complex expressed by a single cell through the *cis*-presentation model to fulfill its function. In response to LPS or Gram-negative bacteria, IL-15 *cis*-presentation is essential for NK cells activation [64]. IL-15 also can be *trans*-present to IL-15Rβ/γ-expressing cells by IL-15Rα-expressing cells due to the high affinity of IL-15Rα for IL-15. IL-15 has been reported to play an important role in the development, homeostasis and cytotoxicity of NK cells [54]. In vivo, IL-15 can be *trans*-presented to NK cells through several cell types, including monocytes, macrophages and DCs [54]. In contrast to IL-2, IL-15 does not promote Treg cell expansion [65]. In a mouse model, IL-15 therapy has been shown to elevate circulating NK cells and effector memory $CD8^+$ T cells by more than ten-fold without significant capillary leakage. The first clinical study investigating human single-chain recombinant IL-15 revealed that a bolus intravenous infusion induced the redistribution and expansion of circulating NK cells and CD8 effector memory T cells and resulted in a four- to eight-fold increase in cell numbers; however, intense cytokine secretion was also detected. The main cause of this side effect was IL-2 and IL-15, which share a common β chain and γc chain in their receptors [66,67]. Thus, several fusion proteins have been developed to improve the efficiency and half-life of IL-15. The fusion protein dsNKG2D-IL-15 has an enhanced NK cell-targeting ability and exhibits an enhanced tumor growth-suppressing capacity against human gastric cancer [68]. The chimeric protein fusion of IL-15 with IL-15Rα, called heterodimeric IL-15, shows an improved ability to promote NK cells and repress tumors [69]. Furthermore, a substitution of asparagine to aspartic acid at amino acid 72 in IL-15 can significantly prolong the half-life of the heterodimeric IL-15N72D:IL-15RαSu/Fc, termed super-agonist ALT-803, to nearly 25 h. In vivo, ALT-803 displays a nearly 25-fold increase in biological activity compared to the activity of IL-15. ALT-803 also shows an enhanced capacity to promote NK cell cytotoxicity and improve tumor repression in ovarian cancer and myeloid leukemia. Recently, the super-agonist has entered clinical trials as an immunomodulatory agent for cancer therapy [70]. Additionally, several clinical trials are currently underway to verify the effects of rIL-15 alone or as an adjuvant of NK cell adoptive transfer [71,72]. Thus, emerging prospects for IL-15 in tumor therapy are anticipated.

3.1.3. IL-12

IL-12 is a heterodimeric proinflammatory cytokine that consists of the p35 and p40 subunits. IL-12 was initially termed "natural killer cell stimulatory factor". IL-12 can stimulate the NK cell production of

Table 1
Manufacture of immunotherapeutic cellular products.

Cell type	Activating Inducers	Culture time	Component	Expansion fold	Ref.
LAK	IL-2	~7 days	$CD3^+CD56^-$ ~60%, $CD3^-CD56^+$ ~20%	< 10-fold ($CD3^-CD56^+$ group)	[183,184]
CIK	IFNγ, anti-CD3 Ab, IL-2	3–4 weeks	Dominant cell $CD3^+CD8^+TCR\alpha/\beta^+$, (~50% $CD3^+CD56^+$)	Max ~6000-fold ($CD3^+CD56^+$ group)	[183,185]
Feeder-co-NK	Genetic modified cells (K562, EBV-LCL) or primary cell (PBMC)	~3 weeks	~80% NK cells (initial cells PBMC)	Max ~40,000-fold ($CD3^-CD56^+$ group)	[186,187]
CI-NK	IL-2, OK432, anti-CD16 Ab	~3 weeks	~80% NK cells (initial cells PBMC)	Max ~5700-fold ($CD3^-CD56^+$ group)	[123]
CIML-NK	IL-12, IL-15, IL-18	12–18 h	Initial component	–	[36]

LAK, lymphokine-activated killer; CIK, cytokine-induced killer cell; Feeder-co-NK, feeder cell cocultured natural killer; CI-NK, cytokine-induced natural killer; CIML-NK, cytokine-induced memory-like natural killer.

cytokines, particularly IFN-γ, and enhance cytotoxicity by upregulating granule-associated protein. IL-12 also promotes $CD4^+$ T cell polarization into Th1 cells and enhances the cytolytic ability of $CD8^+$ T cells [54,73]. Indeed, initial clinical studies using a high dose of IL-12 showed immunomodulatory effects on NK cells and other lymphocytes; however, these studies were eventually suspended due to the occurrence of toxicity-related death in a phase II trial assessing metastatic RCC and melanoma. The side effects were later concluded to be largely due to the repeated dose regimen [54,74–76]. Recent studies have focused on the optimization of the dosage and modifications of IL-12. A phase I trial demonstrated that a subcutaneous injection of rhIL-12 at low-dose had significantly reduced side effects compared with a high-dose IL-12 treatment. Moreover, rhIL-12 can also alter the number of NK cells and other lymphocytes [77]. Furthermore, a modified novel cytokine, NHS-IL12, which consists of two molecules of IL-12 fused to a tumor necrosis-targeting human IgG1, has a prolonged half-life and reduced side effects. NHS-IL12 has also been shown to activate splenic NK and tumor-infiltrating NK cells in a tumor-bearing mouse model [78].

3.1.4. Other cytokines

Other cytokines, including IL-18 and IL-21, have been shown to promote NK cell functions in preclinical studies [54,79]. IL-21 has been confirmed to sustain the survival, promote the maturation, and lead to the apoptosis of NK cells. In a mouse tumor-bearing model, a high dose of IL-21 was shown to suppress tumor growth through NK and CD8T cell-mediated killing. To date, some clinical trials have demonstrated that rIL-21 only shows a modest response rate in tumor therapy and is accompanied by certain side effects. However, rIL-21 continues to show potential in combination other compounds, such as checkpoint inhibitor therapy [54]. IL-18 can also sustain the survival of NK cells through c-apoptosis inhibitor 2 (cIAP2) and TNF receptor-associated factor 1 (TRAF1) pathways [79,80]. Additionally, recent studies highlighted the costimulatory effects of IL-18 through the Fc receptor and the synergy between common γ chain family cytokines for driving CD25 expression and enhancing NK cell functions [81,82]. Due to the toxicity caused by high-dose or high-frequency cytokine therapy, low-dose cytokine combination therapy has become a research focus. Treatments with 100 ng IL-12 plus 100 ng IL-18 every other day in a tumor-bearing mouse model exerted effects that are similar to those following a 20 μg superkine IL-2 treatment [56]. An increasing number of in vitro studies have shown that cytokine combination treatment can promote NK cell proliferation, cytotoxicity and memory, which is better than any single cytokine treatment [54,81].

3.2. Antibody

There are three types of antibody treatments based on NK cells. First, ADCC relies on antibody targeting of the tumor-associated antigen. These monoclonal antibodies function partially by activating NK cell cytotoxicity through interactions with CD16. Second, antibodies can be used to target NK cell receptors, particularly immune checkpoints. These antibodies can block checkpoint proteins and release the brakes on cells. Third, bi- or tri-specific engagers can both increase and redirect NK cell cytotoxicity to tumor cells [25,42,52].

3.2.1. ADCC and cytokine combination

Activating receptor CD16a on NK cells provides NK cell recognition of tumor cells coated with IgG antibody. NK cells and other cells, including macrophages, that express CD16 and γδT cells contribute to the effects of ADCC in vivo [52]. Several tumor-specific antibodies have been developed, such as anti-CD20 for the treatment of B cell lymphoma, anti-Her2 for the treatment of Her2-overexpressing invasive breast cancer treatment, and anti-CD38 for the treatment of multiple myeloma (MM) and chronic lymphocytic leukemia (CLL) [55,83,84]. Alternatively, the modification of antibodies, including humanization and Fc portion modification, enhances their affinity to NK cells. An Fc-optimized CD133 antibody containing the S239D/I332E substitution shows an enhanced affinity to NK cells and an enhanced capacity to promote NK cell degranulation without relevant toxicity to hematopoietic progenitors in a human acute myeloid leukemia (AML) xenograft model [85]. BI836858, which is a fully humanized anti-CD33 antibody, shows increased binding to CD16 and decelerated internalization kinetics compared with the original CD33 antibody [86]. The effects of monoclonal antibody therapy can be synergistically enhanced in combinations with other treatments, particularly NK-stimulating cytokines. Cytokines such as IL-18, IL-2, IL-12, IL-15, and IL-21 have all been shown to augment NK cell-mediated ADCC [54]. In addition, immunocytokines, which involve cytokines linked to the Fc portion of an antibody, are a prominent example. The anti-CD20-IL2 immunocytokine has been demonstrated to effectively treat B cell lymphoma in vitro [87]. These engineered antibodies potently promote NK cell cytotoxicity to a greater extent than the original antibody and therefore could represent the next generation of therapy.

3.2.2. NK cell receptor as checkpoint

NKG2D is a well-studied NK cell-activating receptor. NKG2D is expressed in NK cells, activated T cells, and macrophages. NKG2D forms a homodimer and can recognize stress-induced ligands, such as MHC class I-like molecules. Tumor cells usually express NKG2D ligands, such as ULBPs, MICA, and MICB in humans and REA-1 and MULT1 in mice [88]. However, tumor cells can also develop an immune-evasive mechanism in which the NKG2D ligand is shed from the cell surface in a metalloproteinase-dependent manner. NKG2D activation triggers cytokine production and NK cell cytotoxicity. However, resting NK cells cannot be activated solely in response to NKG2D/NKG2D ligand ligation, often requiring other costimulatory signals. Thus, therapeutic strategies targeting NKG2D are often combined with other strategies that were previously mentioned in the section on cytokines and ADCC [89].

CD137 is a costimulatory factor that is expressed in activated T cells, NK cells and other lymphocytes. In vitro, CD137 antibody

treatment can induce IFN-γ production and the proliferation of NK cells [18]. When used alone to treat AML, CD137 causes an opposite effect with reduced cytotoxicity [90,91]. However, when used as an adjuvant therapy, promising results have been obtained. Trastuzumab, which is a humanized antibody against HER2, can induce the expression of CD137 in NK cells. The use of a combination of an agonistic antibody against CD137 and a HER2 antibody for the treatment of HER2-positive breast cancer shows enhanced cytotoxicity both in vitro and in vivo compared with the HER2 antibody treatment alone [92]. Additionally, the combination of a CD137 antibody and rituximab or trastuzumab shows an enhanced efficacy [93]. Agonists have already been developed for the TNFR family members OX40, GITR, and CD27, such as MOXR0916, MK-4166, and varlilumab [90,94–96], and although they are designed to promote T cell functions, their potential augmentation of NK cell functions remains to be addressed.

Due to immune homeostasis, feedback from an activating signal usually triggers an inhibitory signal or promotes cell death. Tumor cells can therefore also upregulate ligands of NK cell inhibitory receptors to evade immunosurveillance. Thus, the strategy of blocking an inhibitory signal is becoming a trend. Numerous drug and antibody studies have examined the ability to interrupt immune checkpoints to reverse the incapacity of NK cells.

Inhibitory KIR is an important class of inhibitory receptors that recognize MHC class I molecules. Tumor cells expressing MHC class I or class I-like molecules reduce the cytotoxicity of NK cells [18]. KIR blocking antibodies, such as IPH2101 and IPH2102, have been confirmed to promote NK cell-mediated lysis in vitro and in a xenograft mouse model of AML, relapsed/refractory MM and smoldering MM [97,98]. IPH2101 bound to KIR2D receptors was the first approved antibody to be used in clinical trials. A phase I trial with smoldering MM patients demonstrated its safety and tolerability [99]. However, a phase II clinical trial in smoldering MM patients showed a minimal response. The infusion of the antibody resulted in a significant depletion and reduced functionality of KIR2D-expressing NK cells [100]. The researchers subsequently provided a possible explanation of the mechanism underlying the results. The immune system of the patients was presumed to be intact, which is inconsistent with most previous studies conducted in patients who had received previous therapies that affect monocytes or neutrophils. The patients in this clinical trial received a high dose (1 mg/kg) of IPH2101 for a long period, resulting in the loss of KIR2D protein through monocyte- or neutrophil-mediated trogocytosis. The loss of KIR2D resulted in a decreased NK cell function due to the absence of NK cell education to reach functional maturation. Thus, the researchers hypothesized that this effect could be reversed by reducing the dosage or engineering the Fc portion to eliminate trogocytosis [101]. The results also revealed an optimistic response to the combination of IPH2101 and the immunomodulatory agent lenalidomide in a phase I trial of relapsed/refractory MM, in which 5 of 11 patients achieved very good or partial responses [102]. These clinical trials highlight that interruption of the immune checkpoint may trigger a back-fire event, and thus, careful optimization of this therapeutic strategy is needed.

NKG2A is expressed in NK and $CD8^+$ T cells, forming a heterodimer with CD94 and binding to HLA-E. The heterodimer contains two immunoreceptor Tyr-based inhibitory motifs (ITIMs) in the cytoplasmic domain that can transmit inhibitory signals and reduce NK cell function. HLA-E, the ligand of NKG2A, is often upregulated in malignant cells and used for immune evasion [18]. A humanized antibody against NKG2A humZ270 or IPH2201 demonstrated augmented NK cell cytotoxicity against human primary leukemia or Epstein-Barr virus cell lines in a mouse model. Clinical investigations are currently underway to test its safety and efficiency as a single agent or in combination with other drugs [103,104].

PD-1 is typically expressed on effector T cells, B cells and myeloid cells. PD-1 has two ligands, PD-L1 and PD-L2. PD-L1 is constitutively expressed in most hematopoietic cells; certain parenchymal cells, such as vascular endothelial cells; and various tumor cells, such as melanoma, breast cancer, ovarian and hematopoietic malignancy cells. PD-L2 is only expressed in macrophages and DCs [18,43]. A recent research found PD-1 is highly expressed in an NK cell subset in the peripheral blood in one-fourth of subjects among donors who were positive for human cytomegalovirus. NK cells from tumor patients, including Kaposi sarcoma, multiple myeloma, and ovarian carcinoma, have been reported to show an upregulated expression of PD-1 [43]. This subset of NK cells displays an exhausted function with reduced proliferation, degranulation and cytokine production, but these features can be reversed by a PD-1 antibody [32,105,106]. Several PD-1 antibodies have been developed, such as pidilizumab, lambrolizumab and nivolumab. Although these antibodies play a role in tumor suppression, mainly by targeting T cells, their potential to enhance endogenous NK cell function remains attractive [32,44,107].

TIM-3 is usually expressed in Th1 cells, Tc1 cells, macrophages, DCs and nearly all subtypes of NK cells. TIM-3 is further upregulated in NK cells from patients with tumors such as metastatic melanoma, lung adenocarcinoma and gastric cancer. TIM-3 can pair with galectin-9, high mobility group protein B1 (HMGB1), and ceacam-1 [32,45,108,109]. TIM-3 expression is considered a marker of T cell exhaustion. However, its function in NK cells remains controversial [32,109,110]. The human NK cell line NK92, which overexpresses TIM-3, shows a significant increase in IFN-γ production when paired with Gal-9 [45]. PBMC-derived NK cells show decreased cytolytic activity when treated with a TIM-3 antibody. Additionally, TIM-3 can act as a marker of NK cell exhaustion in advanced melanoma. The blockage of Tim-3 can reverse the function of NK cells to eliminate melanoma [108,111]. Thus, further studies are needed to fully understand the role of TIM-3 in the cell biology of NK cells.

Several checkpoint proteins are also expressed by NK cells, such as TIGIT and CD96, both of which contain ITIM motifs in the cytoplasmic domain. These proteins can recognize CD155 and CD112, which are equivalent to the activating receptor CD226 in NK cells. However, TIGIT and CD96 have a higher affinity for CD155 than for CD226 [33]. In vitro studies have revealed that the blockage of TIGIT can increase the cytolytic potential of NK cells. Additionally, CD96 knockout mice exhibit improved NK cell IFN-γ production and tumor control [112]. Moreover, related studies continue to constantly identify new checkpoints. Cytokine-inducible SH2-containing protein (CIS) has been shown to function as a negative regulator of IL-15 signaling in NK cells. The deletion of the CIS gene can reduce the threshold of NK cells to IL-15 and cause enhanced proliferation, survival and tumor cell cytotoxicity. Thus, CIS may be an intracellular checkpoint in NK cells [113].

3.2.3. Bi- or tri-specific killer engagers

Bi-specific or tri-specific killer engagers can enhance NK cell tumor targeting while boosting the cytotoxicity of NK cells. This technology relies on specific tumor antigens and specific markers of the effector cell. Engineered antibody is an innovative immunoglobulin with an engineered Fab or Fc portion. In these antibodies, two or three Fab fragments are fused against tumor-associated antigen or effector cell receptors. An Fc-optimized NKG2D-IgG1 displays highly enhanced ADCC [114]. The engineered antibody serves as a specific cross-link between the tumor cell and the effector cell [25,42,115]. Moreover, bi-specific or tri-specific killer engagers that are constructed by joining two or three single-chain Fv against the tumor-associated antigen and effector cell receptors have been developed. The CD16 in NK cells is a powerful candidate for mediating the cross-linking through bi- or tri-specific engagers to the tumor. A fully humanized bi-specific killer engager (BiKE) CD16 × CD33 has been confirmed to augment NK cell function to effectively eliminate AML in vitro. Furthermore, treatment with NK cells from patients with myelodysplastic syndromes can reverse the incapability and significantly increase the degranulation and IFN-γ and TNFα cytokine production [116,117]. BiKE CD16 × CD19 has been confirmed to directly trigger NK cell activation against CD19-

Table 2
Activation and expansion of NK cells.

Starting material	Medium	Manufacturing process	Culture time	Expansion fold/acquired cell number	Purity	Cytotoxicity	Ref.
CB-MNCs	1640 + 10% FBS + 10 IU/mL rIL-2	Irradiated K562mbIL15-41BBL coculture with CB-MNCs at 1:1 ratio.	7 days	~35-fold 1.3×10^9 NK cells from 3×10^7 CB-MNCs	~72%	Ramos (40%) SUDHL-6 (43.8) K562 (80%)	[188]
CB-MNCs	45% 1640 + 45% Click's media +10% human AB serum+ 100IU/mL IL-2	Cells were cultured with K562mbIL21 (also express 41BBL) at ratio 1:2, day7 $CD3^+$ cells were depleted, remaining cells restimulated with feeder cells in the same condition.	14 days	~1848-fold from fresh CB ~2389-fold from cryopreserved CB	> 95%	ARP-1 (17% 10:1) K562 (55% 10:1)	[189]
$CD34^+$ UCB cells	GBGM +2% human serum	Medium containing 10 pg/mL GM-CSF+ 250 pg/mL G-CSF + 50 pg/mL IL6 + 20 ng/mL IL7 + SCF + IL15 + 1000U/mL IL2 + 200 pg/mL IL12	3–4 weeks	–	> 80%	The UCB-NK could prominent inhibit growth of BM-residing K562 in mouse model.	[190]
hESC iPSC	1640 + 10–15% FBS	Use serum-free medium and spin-EB system to generate hESC or iPSC. Spin-EB were directly transferred to NK initiating conditions containing IL3, IL7, IL15, SCF, FLT3L and without exogenous stromal cells.	28–35 days	–	> 90%	K562 (50% 10:1) MA148 (15% 10:1) A1847 (35% 10:1)	[135,136]
$CD34^+$ UCB cells	GBGM +10% human serum	Medium containing 10 pg/mL GM-CSF+ 250 pg/mL G-CSF + 50 pg/mL IL6 + 25 ng/mL IL7, SCF, TPO, Flt-3L + 20 μg/mL heparin were used from D1-D14, TPO was replaced by 20 ng/mL IL15 from D9-D14. D15 add 2% HS 20 ng/mL IL7, SCF, IL15 and 1000U/mL IL2 to the basal medium as NK differentiation medium.	42 days		> 90%	Hela (30%) SiHa (55%) CaSki (47%)	[166]
$CD34^+$ UCB cells	GBGM +10% human serum	Medium containing GM-CSF+ G-CSF+ IL6 + IL7, SCF, TPO, Flt-3L + heparin were used from D1-D14, TPO was replaced by IL15 from D9-D14. D15 add IL2 to the basal medium as NK differentiation medium.	42 days	~2100-fold 1.6-3.7 × 10^9 total cells	> 90%	K562 (~60%)	[191]
UCB	SCGM + 5% human AB serum	IL15, IL2, OKT3, with or without dalteparin sodium and tacrolimus	21 days	> 1000-fold 40×10^6 NK cells from 1×10^6 unmanipulated CB cells	> 70%	K562 (80% 10:1) Primary AML (55% 10:1)	[192]
$CD3^-CD56^+$ cells from PBMC	1640 + 10% human AB serum	Pretreat with IL-12 + IL-18 + IL-15	16 ± 2 h	–	–	K562 (70%) Killing last for months	[58]
PBMC	NKGM-1 + 10% donor plasma + 500 U/mL IL-2	Cells were stimulated in the flask precoated with 0.1 μg/mL anti-CD3 and 20 μg/mL anti-CD52	14 days Mean 5.7×10^9	1537-fold (range, 695–4337)	60.4% (range, 27.1%–87.2%)	K562 (90%) PC-3 (95%)	[193]
PBMC	TexMACS Research Medium + 5% human AB serum + 500 U/mL IL-2	IL-21 was added to the medium at the start 7 days. Coculture with irradiated EBV-LCL at 1:20 ratio. And repeate stimulation on day 13,26,39.	6 weeks	10^{11}-fold		SK-MEL-28 (40%, 10:1) UKRV-MEL-02 (50%, 10:1) K562 (55%. 10:1) Daudi (50% 10:1)	[124]
PBMC	GT-T507α + 1% autologous plasma + IL-2 + OK-432	Cells were cocultured with RN-T (PBMC stimulated with OKT3 and FN-CH296) cells for 7 days, then GT-T510 + 1% plasma + IL-2 used for the expansion.	~21 days	4720-fold (range 1137–14116)	90.96% (range 65.94–99.45)	–	[63]
PBMC	SCGM + 10% FBS + 50 U/mL IL-2	Cells were stimulated with PM-particles (plasma membrane particles derived from K562mbIL15-41BBL)	17 days	1265-fold (range 280–4426)	> 70%	K562 (90% 5:1) KG1 (80% 5:1) HL60 (80% 5:1)	[194]
PBMC	X-VIVO15 + 10% human AB serum + 100IU/mL IL-2	Cells were co-incubated with 4-1BBL-IL-21-beads, and repeated stimulation weekly.	3 weeks	~140-fold	> 95%	K562 (90% 1:1)	[125]
PBMC	1640 + 10% FBS + 50 IU/ml IL-2	PBMCs were cocultured with irradiated K562mbIL-21 (express 441BBL) at ratio 1:2, restimulated with aAPC every 7days	21 days	Mean 47,967-fold	–	721.21 (90% 10:1)	[186]

(continued on next page)

Table 2 *(continued)*

Starting material	Medium	Manufacturing process	Culture time	Expansion fold/acquired cell number	Purity	Cytotoxicity	Ref.
PBMC	SCGM + 10% FBS + 10U/mL IL-2	PBMCs were cocultured with irradiated K562mbIL-15-41BBL at ratio 1:10	8–10 days	200-fold (range 93–442)	70 ± 11%	K562 (60% 5:1) Raji (60% 5:1) U266 (75% 5:1)	[127]
PBMC		PBMCs were cocultured with irradiated K562mbIL-15-41BBL	10–14 days	447-fold (range 20–10430)	~88%	K562 (80% 10:1) U266 (85% 10:1)	[195]
PBMC	OpTmizer CTS T cell expansion SFM + 10% human plasma	Cells were cultured in a flask immobilize with anti-CD16 mAb, and medium containing 700IU/mL IL-2, 0.01 KE/mL OK432 for 3 days, subsequent culture without anti-CD16 mAb and OK432	21 days	Range 637–5712 fold	78.9 ± 11.6%	K562 (80% 6:1) C1AK (35% 12:1) UB2MT (23% 12:1)	[123]
PBMC $CD3^+$ depletion	1640 + 10% FCS	Cells were weekly stimulated with CD137L/aAPC (expressing 41BBL, IL-15Rα, MICA/B) + rIL-15 at ratio 1:10	21 days	~1000-fold		Daudi (60% 10:1) K562 (95% 10:1)	[196]
PBMC	1640+ 10% FBS + 50 IU/ml IL-2	Cells were cocultured with irradiated Clone9.K562-CCR7(also express mbIL-21, 41BBL) at ratio 1:1	21 days	~21,000-fold	~99%	Enhanced homing to lymph nodes.	[187]
$CD56^+$ MACS from PBMC	α-MEM + 20% FBS	Cells were cultured in the medium containing 10^{-5}M HC and 20 ng/mL IL-15	~21 days	Mean 23-fold (range 3.2–131.3)	97.9% (range 82.7–99.6)	K562 (23% 1:1)	[178]
PBMC	SCGM + 5% human serum + 500U/mL IL2	Added OKT3(10 ng/mL) to the culture for first 5 days.	~21 days	Range 530–1100-fold	Mean 38%	K562 (70% 10:1)	[126]

CB, cord blood; MNC, mononuclear cell; UCB, umbilical cord blood; GBGM, glycostem basal growth medium; hESC, human embryonic stem cell; iPSC, induced pluripotent stem cell; EB, embryonic body; SCGM, stem cell growth medium; NKGM, NK cell growth medium; EBV-LCL, Epstein-Barr virus transformed lymphoblastoid cell line.

expressing B cells [115]. BiKE CD16 × CD133 has been confirmed to enhance NK cell cytotoxicity against the CD133-expressing cell line and even the NK-resistant cell line when combined with a drug that increases the expression of CD133 in tumor cells [118]. CD16 is often shed by activated NK cells via a metalloprotease called ADAM17. Consequently, the combination of a bi-specific antibody with the ADAM17 inhibitor improves therapeutic efficacy [117]. Additionally, there have been improvements in the format and design of the engagers. AFM13, which is a tetravalent bi-specific killer engager against CD30 and CD16A, contains two sites for both CD30 and CD16A without the Fc domain. AFM13 also has a longer half-life than other small bi-specific antibodies, and its safety and tolerability have been demonstrated in a phase I/II trial for the treatment of Hodgkin lymphoma, achieving an overall disease control rate of approximately 60–70%. A phase II trial is currently ongoing to optimize the dosage schedule [119–121]. In addition to redirecting the NK cell cytotoxicity, improvements also include facilitating NK cell survival and proliferation. A modified IL-15 cross-linker has been incorporated into the bi-specific killer engager to create a tri-specific killer engager, which has been confirmed to possess the capacity to enhance NK cell cytotoxicity with improved survival and proliferation in vitro [72,122].

3.3. Adoptive transfer of NK cells

3.3.1. Peripheral blood-derived NK cells

The usage of allogeneic or autologous PBMC-derived NK cells to treat tumor patients has already been evaluated in phase I or phase II clinical trials with high risk or chemotherapy refractory patients. Due to differences in the strategies used for the separation, expansion and activation of NK cells, the outcome of NK cell functions can vary (Tables 1 and 2) [37,42]. PBMC-derived NK cells are advantageous due to the ease of collection as well as the fact that they can be derived from either autologous or allogeneic sources. Additionally, NK cells derived from PBMCs can be obtained by separating or non-separating from ex vivo expanded cells, and activation is induced by cytokine or feeder cells [58,123–126]. Methods used to separate NK cells from PBMCs usually incorporate immunomagnetic beads by depleting $CD3^+$ cells, followed by enriching the $CD56^+$ cells. For clinical use, large numbers of cells are usually required, resulting in the development of apheresis technology and the CliniMACS device, which is fast and can yield more than 10^8 NK cells in each batch [127]. The enrichment of NK cells by depleting only $CD3^+$ cells from whole blood can result in a mean purity of 20% among cells including B cells and monocytes. Alternatively, selecting $CD56^+$ cells from peripheral blood can provide a purity of 95%, consisting of a small portion of $CD56^+CD3^+$ NKT cells. The usage of both $CD3^+$ cell depletion and $CD56^+$ cell enrichment yields up to 99% purity [128,129]. Because NK cells only account for approximately 10% of PBMCs, most of the purified NK cells must be expanded ex vivo to attain the requirements for clinical use. NK cell expansion and activation require signals from other immune cells, such as monocytes, and despite the higher purity obtained by amplifying the purified NK cells, non-separating ex vivo expansion and activation also provide good results [126]. The non-separating strategy usually utilizes cytokines, feeder cells, or their combination, to simultaneously induce the expansion and activation of NK cells. Deng et al. have established an approach in which PBMCs are cultured in a medium containing 10% human plasma, IL-2, OK432, and an anti-CD16 monoclonal antibody. By day 14, the purity of the NK cells in the culture is approximately 76.9%, and the expansion fold ranges from hundreds to thousands [123]. Feeder cells are also effective for promoting the production of ex vivo NK cells. PBMCs cultured with irradiated autologous stimulated T cells combined with IL-2 and OK432 can generate a NK cell purity of approximately 90% in approximately 20 days [63]. The major limitation of using PBMC-derived NK cells in therapy is difficulty in yielding an adequate cell number. Thus, ex vivo expansion and activation strategies remain a major focus. The production of peripheral blood-

derived NK cells is described in detail in Section 4.

3.3.2. Stem cell-derived NK cells

Due to the pluripotency of stem cells, they are also an essential source for the production of NK cells. Currently, there are multiple sources of stem cells, including umbilical cord blood (UCB), embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). UCB is an emerging source of NK cells. The advantages of using UCB include widely distributed storage banks and the ability to enrich hematopoietic stem cells and progenitor cells [130]. However, UCB-derived NK cells are slightly different from PBMC-derived NK cells. Most notably, a key difference, also a limitation associated with the use of UCB-derived NK cells in tumor therapy is UCB-derived NK cells express few inhibitory KIRs, a feature that is known to be associated with NK education and cytotoxicity. UCB-derived NK cells also lack CD57, an activation marker [130]. Thus, resting UCB-derived NK cells have poor function compared to PBMC-derived NK cells, which can be partially overcome by ex vivo expansion and activation using stimulators [73,131]. $CD34^+$ cells are usually separated from UCB using positive selection via magnetic beads. $CD34^+$ cells are then expanded in a bioreactor supplemented with stimulators. Current methods can generate large-scale clinical-grade NK cells, providing a $>$ 2,000-fold expansion and $>$ 90% purity [132]. Some UCB-derived NK cells have been tested in preclinical or clinical trials against AML and myelodysplastic syndromes [133].

Generation of NK cells from ESCs and iPSCs generally require two steps. First, $CD34^+$ hematopoietic precursors must be generated. The $CD34^+$ cells are then sorted and differentiated into NK cells with cytokines and feeder cells (usually murine stromal cells). The Kaufman research group established a feeder-free, sorting-free approach to generate NK cells from human ESCs. These authors used a spin-embryoid body (EB) system with BMP4 and VEGF to derive hematopoietic progenitors. After 11 days of culture, the spin-EBs were transferred to the NK cell culture containing the cytokines IL-3, IL-15, IL-7, SCF and Flt3L for 28 days. This feeder-free system can generate NK cells that have no difference from those derived from the murine embryonic liver cell line EL08-1D2 as feeder cells [134,135]. Meanwhile, this group established a clinical-scale derivation of NK cells from ESCs and iPSCs without cell sorting and in the absence of feeder cells. Denman et al. established an aAPC line expressing membrane-bound IL-21 which can induce the expansion of PBMC-derived NK cells and maintain the length of telomeres. Thus, Kaufman's research group used this cell line to further expand the hESC- or iPSC-derived NK cells, which resulted in a 2–3 log expansion that persisted for more than 2 months. Moreover, the functions of these NK cells derived from the different hESC and iPSC cell lines have been confirmed in vitro [135,136].

3.3.3. NK cell line

Compared to the ex vivo expansion of PBMC-derived NK cells and stem cell-derived NK cells, NK cell lines are easier to expand. Because of this advantage, NK cell lines are also a powerful tool for genetic modifications. Several cytotoxic cell lines, such as KHYG-1, NK-92, NKL, NKG and YT, have been established from malignant NK cell clones [37,42]. NK-92 shows consistent and highly cytotoxic effects against tumor cells in preclinical research studies and is the only FDA-approved cell line for use in clinical trials, although it has demonstrated minimal efficacy in clinical trials [137,138]. Other cell lines, such as NKG and NKL, are highly similar to NK-92. These cell lines have been tested in vitro and show cytotoxicity against several tumor cells but have never been tested in patients [25,37]. The limitations to using NK cell lines clinically are the in vivo persistence and the lack of CD16 expression in most cell lines. On the other hand, the absence of CD16 can be improved by transgene expression [139]. Although the in vivo persistence can be shortened by irradiation before infusion, this procedure can also results in reduced function, impaired migration and disrupted expansion after infusion [25,140].

3.3.4. Genetically modified NK cells

Genetic modification is a powerful tool used to increase the efficiency of NK cells in immunotherapy. NK cell lines, PBMC-derived NK cells and stem cell-derived NK cells can all serve as target cells for genetic modifications [42,141]. However, due to the resistance of NK cells to retroviral infection, the use of this strategy is limited by gene delivery efficiency [142]. Sutlu T et al. have established a method to elevate the transfection efficiency by targeting the intracellular antiviral defense mechanism. The transfection efficiency can be boosted, on average, by 3.8-fold using an inhibitor that targets the TBK1/IKKe complex [143]. Electroporation is another method used to deliver the gene into cells with a non-viral advantage. An improved technology using mRNA instead of cDNA can also increase the efficiency [144]. Linhong Li et al. used a non-viral approach to engineer NK cells and achieved an efficiency of approximately 80–90% and a viability of approximately 85% [145]. The authors also established a flow electroporation-based system for the large volume electroporation of cells with various molecules [146]. The modification of NK cells is varied, which can aim to improve the persistence, cytotoxicity and homing of NK cells. The introduction of CD16a into the NK cell line NK-92 can increase the ADCC effect [147]. Genetic modifications of NK cells to produce cytokines, such as IL-15 and IL-2, can improve their survival, persistence, proliferation and function in vivo [148–150]. Recent studies have focused on the introduction of tumor-associated antigens into NK cells to direct these antigens to specific target cells. Due to the successful application of the chimeric antigen receptor (CAR) in T cells, it is currently being applied to NK cells [42,141]. The first-generation CAR lacks a stimulatory domain and only has a CD3ζ chain as the signaling domain. Subsequent studies have resulted in the addition of one or two costimulatory domains to boost NK cell cytotoxicity [42,132,151]. In the second- and third-generation CARs, the NK cell-specific signaling endodomain has been designed to form a CAR intracellular domain to enhance cytotoxicity, such as 4-1BB plus CD3ζ and DAP-12 plus CD3ζ and 2B4 CD3ζ [152]. Currently, several tumor antigen-binding domains have been designed as CAR extracellular domains and tested in vitro or in vivo. A detailed description of these CARs is presented in Section 4.

4. Manufacture of NK cells

4.1. Ex vivo expansion and functional enhancement of NK cells

In the early stage of adoptive cell transfer research, cytokines, such as IL-2 and IL-15 were applied to pretreated NK cells before transfer. However, two main reasons led to poor outcomes through this method. First, IL-2 or IL-15 can induce NK cell apoptosis upon contact with the sensitive target cell or vascular endothelium [153]. Second, methods using a single cytokine resulted in only a 10–20-fold expansion of NK cells. Subsequently, the use of feeder cells to amplify the NK cells was developed. Many types of original or genetically modified cells are used as feeder cells, such as PBMCs, Epstein-Barr virus-transformed lymphoblastoid cell lines, and genetically modified K562-expressing membrane-bound IL-15 or 41BB ligand. The use of feeder cells greatly increases the efficiency of NK cell expansion. The combination of feeder cells and cytokines can more effectively induce NK cell expansion and activation, ranging from 100- to 40,000-fold in approximately 2–3 weeks [154]. However, there are also problems associated with these techniques. The treated NK cells can become exhausted, indicating senescence of the expanded NK cells. Subsequently, overexpression of the telomerase reverse transcriptase gene (TERT) could reverse this phenomenon [155]. The use of feeder cells, which are lethally irradiated before use, to manufacture NK cells could also cause concerns regarding the risk of infusion. In response, a number of detection measures have been established, including monitoring the growth rate of feeder cells and detecting the presence of viable feeder cells. Therefore, ex vivo treatment strategies for NK cells have been an area of research focus in

recent years to optimize the feeder-free system or through the combination of cytokines and stimulators. As previously mentioned, Deng et al. established a feeder-free system to expand NK cells from PBMCs. The cells were cultured in a medium containing 700 IU/ml rhIL-2, 0.01 KE/ml OK432, and 10% heat-inactivated human plasma in an anti-CD16 monoclonal antibody-immobilized culture flask. Three days later, the cells were transferred to an anti-CD16 antibody-uncoated flask and cultured with IL-2 and 10% inactivated human plasma. This method resulted in the expansion of NK cells by thousands of fold and achieved a purity of approximately 70% [123]. More recently, Rizwan Romee et al. reported a method in which pretreated NK cells in a feeder-free cytokine combination system were activated within a short time. NK cell MACS from PBMCs pretreated with IL-12, IL-15 and IL-18 for 12–16 h can become memory-like NK cells with enhanced cytotoxicity in vivo [36]. A general summary of the 2010–2017 NK cells ex vivo manufacturing is presented in Table 2.

4.2. Ex vivo genetic manipulation of NK cells (CAR-NKs)

Recently, the ex vivo genetic manipulation of NK cells has focused on the expression of CARs, allowing them to recognize specific tumor-associated antigens with increased survival, proliferation and cytotoxicity. Recent studies examining the design of CARs are presented in Table 3.

5. Clinical utility and outcome of NK cells in cancer therapy

5.1. Clinical utility of NK cells

5.1.1. Autologous NK cells

Early studies reported that the adoptive transfer of purified $CD56^+$ autologous NK cells into patients with high-dose IL-2 led to a poor clinical outcome and severe side effects [156]. The severe toxicity, however, was due to the high dose of IL-2, and the transfer of ex vivo-treated autologous NK cells was found to be safe [63]. Unfortunately, clinical trials with autologous NK cells have also demonstrated a limited effect on tumor suppression [157,158]. Maia R. et al. showed that the adoptive transfer of approximately 4×10^{10} NK cells into metastatic melanoma or renal cell carcinoma patients resulted in high levels of NK cells in the circulation that persisted for one week or longer, but tumor regression was not observed. These NK cells expressed low levels of NKG2D and could lyse tumor cells in vitro when stimulated with IL-2. Sakamoto N et al. established a novel expansion method in which PBMCs were treated with OK432, IL-2 and FN-CH296-induced T cells, resulting in an NK purity of approximately 90% and an approximately 4720-fold (range 1372–14116) expansion. However, when these expanded NK cells were used in advanced digestive cancer patients, no clinical responses were observed [63]. The major reasons that contributed to these poor outcomes are the inhibition caused by the self-HLA molecules expressed by some tumor cells and the impaired development and function of patient NK cells [159–161]. Thus, strategies have been developed to overcome these limitations, such as an anti-KIR antibody [100,102]. Additionally, the combination of anti-tumor drugs with autologous NK cells is used to potentiate the effect of tumor therapy [61,162].

5.1.2. Allogeneic NK cells

Due to the limitations of autologous NK cells, allogeneic NK cells from selected donors have been investigated in adoptive transfer. Allogeneic NK cells obtained from healthy, related donors educated in a non-immunosuppressive environment are fully functional [163]. Existence of “Alloreactive” NK cells prove that NK cells could kill allogeneic cells [164]. However, a major risk of using allogeneic NK cells is the development of graft-versus-host disease (GVHD). Miller’s group was the first to perform a test of the infusion of allogeneic haploidentical NK cells in a non-transplantation setting in patients. These authors selected haploidentical related donors and obtained NK cells by $CD3^+$ cell depletion from peripheral blood. The NK cells were treated with a high-dose of IL-2 overnight and then transferred into patients who were treated with preparatory chemotherapy regimens, followed by IL-2 infusion. The allogeneic NK cells could persist and expand in vivo and demonstrated a clear association with the therapeutic response [165]. The use of haploidentical NK cells greatly improved the efficiency. Recently, Fehniger’s group established a new method to treat allogeneic haploidentical $CD3^-CD56^+$ MACSed NK cells from peripheral blood. The partial results of the phase I clinical trial showed that the cytokine combination of IL12, IL-15 and IL-18 to treat NK cells could provide a memory-like function and maintain cytotoxicity after approximately 1 week in vitro or in vivo. When used in relapsed/refractory AML patients who were pretreated with chemotherapy regimens, good tolerance was observed at the maximum cell dose of approximately 10^7/kg, yielding an overall response rate of 55%. Additionally, NK cells manufactured by this method can kill tumor cells regardless of the match status of KIR on NK cells and HLA on the tumor cells. Although it is only an in vitro test, and there is no statistical correlation between the match status of KIR-HLA and the clinical outcomes, this strategy remains attractive [36]. Allogeneic NK cells have been widely used in several tumor therapy clinical trials, including AML, CML, melanoma, breast cancer, ovarian cancer, neuroblastoma, and some types of solid tumors, such as renal cell carcinoma, colorectal cancer and hepatocellular cancer [128]. Some of these trials show exciting results with an enhanced response rate, complete remission or 1-year event-free survival [36,133,165–168].

5.2. Clinical outcomes of NK cell-based cancer therapy

5.2.1. Hematological malignancies

Miller J. S. et al. completed a study with 556 AML patients and showed that the absence of one or more KIR ligands could reduce the relapse incidence after unrelated HCT [169]. Further studies have shown that NK cells from HLA-mismatched donor play an important role in the graft-versus-leukemia (GVL) effect after T cell-depleted haplo-hematopoietic stem cell transplantation (HSCT) [164,170]. But NK cells from hematological malignancy patients, such as those with AML, have been shown to exhibit impaired cell maturation and functional activation [161,171]. After ex vivo activation, autologous NK cells from patients show an enhanced cytotoxicity to reduce the tumor burden [159,172]. However, as mentioned above, there are still limitations to the use of autologous NK cells. Because these studies demonstrate that allogeneic NK cells can exert anti-leukemia effects in patients without ligands for donor NK cell inhibitory KIRs, allogeneic NK cell adoptive transfer therapy is currently mainly applied therapeutically in patients with hematological malignancy in a transplantation or a non-transplantation setting [140,173]. Allogeneic haploidentical NK cells after ex vivo treatment have been used to treat various hematological malignancies, such as AML, CML, ALL, CLL, MM, myelodysplastic syndromes (MDS), and lymphoma. The outcomes are summarized in Table 4.

5.2.2. Melanoma

Melanoma is a highly malignant tumor that is characterized by an extremely high metastatic potential. Treatment using ex vivo-expanded and activated tumor-infiltrating T cells results in a nearly 50% response rate in patients [174]. However, approximately 60–80% of melanoma cells in situ show an absent or diminished expression of HLA class I molecules, which plays a key role in mediating the recognition of malignant cells by $CD8^+$ T cells [175]. Fregni et al. found that although melanoma cells can inhibit NK cell function by modulating the expression of activating receptors, the expression of NKp46 in NK cells from melanoma patients is positively correlated with the duration of stage IV [176]. Thus, NK cells are considered effective candidates for killing melanoma cells without the recognition of antigens presented by

Table 3
Chimeric antigen receptor (CARs) gene modification.

Target	Intracellular signaling molecules	NK cells	Indications	Outcome	Ref.
NKG2DL	DAP10, CD3δ	PB-NK	All	Gains in cytotoxicity against REH, MOLT4, CEM-C7, U-2OS, MG-36, HOS, DU145, PC-3, RH36	[197]
GD2	CD3δ	NK-92	Neuroblastoma Melanoma Breast carcinoma	Kelly (58% 5:1) LAN-1 (70% 6.3:1) SK-Mel23 (92% 10:1) SK-BR3 (40% 10:1)	[198]
GD2	2B4, CD3δ	PB-NK from patient or health donor	Neuroblastoma	CAR-patient NK cell overcome resistance of autologous leukemia cells Enhanced activation response to JF cell line.	[199]
CD19 CD19	CD3δ 41BB, CD3δ	NK-92 PB-NK	B-ALL, CLL B-ALL, CLL	High cytotoxicity than anti-CD19 antibody. OP-1 (> 80% 2:1) Raji (> 80% 2:1) Ramos (> 70% 2:1) 380 (> 70% 2:1) RS4;11 (> 70% 2:1)	[200–203] [145,204]
CD20	CD3δ	NK-92	B-ALL, CLL	High cytotoxicity than anti-CD20 antibody SUP-B15, TMD-5 (> 70% 2.5:1) Raji, DOHH-2 (> 60% 10:1) NK resistance was not overcome by CAR expression, e.g. NALM-6	[200,201,205]
CD20	41BB, CD3δ	PB-NK	B-ALL-CLL	Raji-2R (> 40% 10:1) Raji-4RH (> 35% 10:1)	[206]
SLAMF7	CD28, CD3δ	NK-92	MM	IM9 (~100% 20:1) L363 (80% 20:1) Increased lysis against primary myeloma cells compared to NK-92.	[207]
CD138	CD3δ	NK92	MM	RPMI8266 (56% 10:1) H929 (63% 10:1) U266 (91.5% 10:1) Increased lysis against primary myeloma cells compared to NK-92.	[208]
CEA	CD3δ	YT	Colon carcinoma	Target cells (85–95% 15:1)	[209]
GPA7	CD3δ	NK-92	Melanoma	Malme (70% 9:1) MEL-624 (65% 9:1) B16-AAD (50% 9:1) Efficiently lysis primary or metastasis cells (55%~75% 9:1)	[210]
EpCAM	CD28, CD3δ	NK-92, NKL	Breast carcinoma	K562 (77% 5:1) MDA-MB468 (61% 5:1) MDA-MB453 (64% 5:1)	[211]
HER-2	CD28, CD3δ	PB-NK	Breast, ovarian,	Higher levels of IFNγ (6400 pg/mL), enhanced degranulation against $HER2^+$ cells.	[212]
HER2	CD3δ or CD28, CD3δ or 41BB, CD3δ	NK-92	Breast cancer	More prominent cytotoxicity for cells carry 2nd generation CAR MDA-MB453 (55% 5:1)	[213]
EBNA3C	41BB, CD3δ	NK-92	EBV^+ T cell lymphoma	Enhanced cytotoxicity than EBNA clone 315 scFv-Fc-mediated.	[209]
EGFR	CD28, CD3δ	NK-92 PB-NK	Breast cancer Glioblastoma	MDA-MB-231 (> 50% 10:1) MCF-7 (> 35% 10:1) CAR-primary-NK show ~20% specific lysis against cell lines.	[214,215]

PB-NK, peripheral blood NK; MM, multiple myeloma; CEA, Carcinoembryonic antigen; GPA7, gp100/HLA/A2-specific antibody; EBNA, epstein-barr nuclear antigen.

HLA class I molecules. The recent NK cell-based trials are summarized in Table 4.

5.2.3. Other solid tumors

There are also trials using NK cells for the treatment of breast cancer, ovarian cancer, non-small cell lung cancer, colorectal cancer and glioblastoma. Twenty patients (14 ovarian cancer and 6 breast cancer) received haploidentical NK cells treatments, although the adoptive NK cells could be detected in the blood after 1 week. However, they did not show better expansion in vivo, and the outcome was poor [177]. Sixteen patients with non-small cell lung cancer (13 adenocarcinoma and 3 squamous cell carcinoma) received ex vivo-expanded and activated $CD56^+$ donor NK cells accompanied by immunodepletion treatment, and only 2 patients showed a partial response [178]. Promising evidence of NK cell treatment in patients with solid tumors remains sparse. Inadequate in vivo expansion, homing to tumor sites, or suppression of the tumor microenvironment contributes to the low effectiveness of NK cell adoptive therapy for solid tumors. Thus, studies are focusing on improving these issues. The related studies are summarized in Table 4.

6. Future perspectives

6.1. Combination of multiple strategies

Although the results of previous studies using various strategies to

Table 4
Clinical utility and outcome of NK cell-based cancer therapy.

Disease	Clinical trial	Source of NK	NK cells manufacture	Therapeutic regimen	Concomitant medication	Outcome	Ref.
Solid tumors/sarcomas (13) Leukemia/lymphoma (2)	I	NK-92	Ex vivo expanded in the presence of IL2, 10 Gy irradiated before infusion	1–10 × 10^9 cells/m^2, once repeated infusions in patients who had a presumed clinical benefit and did not have HLA antibodies against NK-92 cells, 48 h later	Pre-medicated with Methylprednisolone and anti-histamine	No infusion-related or long-term side effects 1/15 SD, 12/15 PD, 2/15 mixed response	[137]
Relapse or refractory MM (4)	I	Autologous	PBMC stimulated K562mbIL15-41BBL, mean 90% purity	7.5 × 10^6 NK cells/kg, once weekly, 2 infusions	Patients were treated with Lenalidomide only or Bor plus Bendamustine once weekly, 4 times	No toxicity exceeded grade 2 2/4 long-term response (> 1 year), 2/4 SD	[61]
Colorectal cancer (3) Esophageal cancer (3) Gastric cancer (2) Rectal cancer (2)	I	Autologous	PBMC stimulate with OK432, IL2 and FN-CH296 induced T cells, mean 90% purity	0.5–2 × 10^9/injection, weekly, 3 times	–	No toxicity exceeded grade 3 No clinical response	[63]
metastatic melanoma (7) renal cell carcinoma (1)	I/II	Autologous	CD3 depleted PBMC, activated with OKT3, IL2 and auto-PBMC, mean 90% purity	Mean 4.7 ± 2.1 × 10^{10} NK cells were infused, once	Flu × 5 doses Cy × 2 doses IL2 × 6 doses (high dose, every 8 h)	8/8 no clinical responses	[158]
Older AML patients with morphologic CR (10)	I	Partially matched donor	$CD34^+$ cells derived NK cells, Mean 75% purity	3–30 × 10^6/kg cells were infused, once	Cy and Flu (day −6, −3) Ace and Cle (day 0) Pegfilgrastim (day 8)	No GVHD and toxicity 6/10 relapse after mean duration of day 364, 4/10 disease free lasting	[133]
Relapse or refractory AML (9)	I	Haploidentical donor	$CD3^-CD56^+$ selected PB-NK cells, IL12, IL15, IL18 pretreate 12–16 h, > 90% purity.	0.5–10 × 10^6/kg, once	Cy (day −5, −4) Flu (day −6 to −2) IL2 × 7 doses (begin at day 0, every 48 h)	No toxic effects Response rate 55% 3/9 CR, 1/9 CRi	[36]
Relapse or persistent MDS (2) AML (6)	II	Haploidentical donor	$CD3^-CD56^+$ selected NK cells, > 90% purity.	Mean 10.6 × 10^6 cells/kg NK cells were infused after allo-HCT	Cy (day −3, −2) IL2 × 6 doses (begin at day −1, every 48 h)	No GVHD 2/8 (1 MDS, 1AML) CR Median duration of survival 12.9 months	[60]
Refractory AML (15)	II	Haploidentical donor	$CD3^+CD19^+$deplete PBMC, IL2 activate, mean 54% purity	Mean 2.6 ± 1.5 × 10^7/kg	Cy (day −5, −4) Flu (day −6 to −2) IL2DT (diphtheria toxin fusion protein) (day −1 or day −1 and 0)	No acute GVHD, no acute cytokine release symdrome 3/15 CR, 2/15 CRp, 3/15 CRi Median duration of remission 11.2 months	[182]
Relapsed MM (8) lack HLA-C I, II and/or Bw4	–	Haploidentical donor or autologous	PBMC activated with K562mbIL15-41BBL mean 78% purity	2–10 × 10^7/kg, once	Bor (day −9, −6, −2) Cy (day −7) Dex (day −6 to −3) Flu (day −6 to −2) (Bor only or combine with other three) IL2 × 13 doses, (begin at day 0, daily)	No GVHD and serious side effects 1 PR, 1 progression decrease, 5 PD	[62]

(continued on next page)

Table 4 *(continued)*

Disease	Clinical trial	Source of NK	NK cells manufacture	Therapeutic regimen	Concomitant medication	Outcome	Ref.
High risk AML(7)	I	Haploidentical donor	$CD56^+$ selected PBMC were stimulated with CTV-a leukemia cells lysate	1–10 × 10^6 NK cells/kg were infused, once	Flu (day −4 to −2) Total body irradiation 2 Gy (day −1)	No GVHD 3/7 CR, 1/7 PR, 3/7 PD Median overall survival was 400 days	[216]
AML active disease (5) molecular relapse (2) morphologic CR (6)	I	Haploidentical donor	$CD3^-CD56^+$ cells selected from PBMC, activated with PHA, IL2 and healthy donor mixed PBMC, median purity 93.5%	Mean 2.74 × 10^6 $CD3^-CD56^+$ cells/kg, once	Flu (day −7 to −3) Cy (day −2) IL2 × 6 doses (3 times weekly)	No NK cell–related toxicity, including GVHD 1/5 CR, 4/5 no benefit 2/2 CR 3/6 CR last for more than 18 months, 3/6 relapse	[167]
Childhood AML in CR (10)	II	Haploidentical donor	$CD3^-CD56^+$ cells selected from PBMC	Median 2.9 × 10^7 NK cells/kg were infused	Cy (day −7) Flu (day −6 to −2) IL–2 × 6 doses (begin at day −1, every 48 h)	Limited Nonhematologic toxicity, no GVHD 2-year event-free survival 100%	[168]
MM (5) HL (2) NHL (6)	I	Haploidentical donor	PBMC deplete $CD3^+$ cells, $CD3^-CD56^+$ cells > 20%	About 90 days after allo-SCT, cells infused 1–20 × 10^5 cells/m^2	pretreat with diphenhudramine	No side effects exceed grade 3, no GVHD or marrow suppression 6/13 Relapse 7/13 Remission	[217]
relapsed or refractory $CD20^+$ NHL (6)	Not stated	Haploidentical donor	CD3-depleted PBMC stimulated with IL2, mean 43% purity	Mean 2.1 ± 1.9 × 10^7 NK cells/kg were infused	Cy (day −6) Flu (day −6 to −2) Rituximab (days −8, −1, +6, +15) IL–2 × 6 doses (begin at day 0, every 48 h)	No GVHD No toxicity exceed grade 3 2/6 CR, 2/6 PR, 2/6 no response	[181]
Refractory solid tumor in pediatric patients after allo-SCT (7)	I/II	Haploidentical donor	$CD3^-CD56^+$ PB-NK stimulated by IL15, > 90% purity	Mean 11.3 × 10^6/kg, 30 days after haplo-SCT, once or twice	–	No toxic effects 3 PR, 1 SD	[218]
Lymphoma (2) Advanced solid tumor (15)	I	Random donor	$CD3^+$ depleted PBMC expanded with autologous PBMC, OKT3 and IL2, mean 98% purity	1 × 10^6–3 × 10^7/kg, once weekly, triple infusion	chemotherapies	33% dose-limiting toxicity 8/17 SD, 9/17 PD Median progression-free survival with SD 4 months	[59]
recurrent ovarian(14) breast cancer(6)	II	Haploidentical donor	CD3-depleted PBMC stimulated with IL2, NK cells median purity 33%	Mean 2.15 × 10^7 NK cells/kg were infused	Cy (day −5, −4) Flu (day −6 to −2) In 7 patients, 200cGy total body irradiation (day −1) IL–2 × 6 doses (begin at day 0, 3 times weekly)	4/20 PR (all ovarian) 12/20 SD (8 ovarian cancer and 4 breast cancer) 3/20 PD (1 ovarian cancer and 2 breast cancer)	[177]

(continued on next page)

Table 4 *(continued)*

Disease	Clinical trial	Source of NK	NK cells manufacture	Therapeutic regimen	Concomitant medication	Outcome	Ref.
Advanced NSCLC(15)	I	Haploidentical donor	$CD56^+$ selected PBMC stimulated with IL5 and hydrocortisone, mean 97% purity	Mean 4.15×10^6 NK cells/kg, 2–4 infusions	Pre-medicated with corticosteroids and/or H1 inhibitors	No side effects and GVHD 2/15 PR, 6/15 SD, 7/15 PD Median overall survival 15 months	[178]

SD, stable disease; PD, progressive disease; Cy, cyclophosphamide; Flu, fludarabine; Ace, acetaminophen; Cle, clemastine; CR, complete remission; CRi, CR with incomplete blood count recovery; CRp, CR without platelet recovery; PR, partial response; MDS, myelodysplastic syndrome; HCT, hematopoietic cell transplantation; Bor, bortezomib; Dex, dexamethasone; HL, Hodgkin's lymphoma; NHL, non-Hodgkin's lymphoma; SCT, stem cell transplantation; NSCLC, non-small cell lung cancer.

target NK cells for tumor therapy are promising, their long-term anti-tumor efficiency remains modest. To benefit patients, a combination of multiple strategies, including targeting NK cells, targeting tumor cells and targeting the immune environment, is a focus of future research. The combination of cytokines with antibodies targeting tumor-specific antigens has been confirmed to augment NK function to a greater extent than their use in isolation. IL-18 has been shown to augment NK cell production via IFN-γ stimulation through Fc receptors [79]. The combination of the overexpression of IL-15 and CAR in NK cells can significantly increase proliferation and tumor cell-killing activity [122]. In addition, checkpoint inhibitors, antibodies targeting NK cells that activate receptors or drugs potentiating NK cell cytotoxicity can combine with tumor-specific antibodies to significantly improve the treatment efficiency [83,92,179]. Furthermore, regular tumor treatment approaches, such as chemotherapy, radiotherapy and surgery, can be used in combination with NK cell-based immunotherapy. NK cell adoptive transfer in combination with chemotherapy or radiotherapy has been widely used in clinical trials. Chemotherapy or radiotherapy could help create space for NK cell expansion and integration [36,165]. Chemotherapy can also induce genotoxic stress, which promotes the tumor cell sensitivity to NK cells [180]. In addition, adoptively transferred NK cells can be used as an adjuvant therapy combined with regular therapy to elevate patient benefits. Dolstra et al. demonstrated that the adoptive transfer of $CD34^+$ cell-derived NK cells into elder AML patients who had reached morphologically complete remission during the previous treatment could prolong the disease-free duration for longer than 1 year [133]. This study demonstrated that a combination of HCT and chemotherapy with NK infusion could significantly delay relapse. Another study of childhood AML achieved 100% (10/10) disease free for two years, strongly indicating that NK cell immunotherapy as an adjuvant therapy may effectively control or delay the recurrence. Although the efficacy of the NK cell treatment alone in patients with solid tumors remains poor, the tumor burden and immune repression are largely reduced after regular therapy, such as chemotherapy, radiotherapy or surgery. Thus, the adoptive transfer of NK cells might exert its function to improve the therapeutic outcome. The combining scheme, treatment order and intensity are challenges for the combination therapies.

6.2. Homeostatic proliferation of NK cells in vivo

The differences in the NK cells ex vivo manufacture strategies and patient preparatory regimen result in differences in vivo persistence and proliferation. These factors are thought to affect clinical outcomes [34,55]. Miller J.S. et al. found that when patients received a preparatory regimen of Hi-Cy/Flu, the donor NK cells showed good expansion, and nearly 30% of the poor-prognosis AML patients achieved complete remission (CR), although the CR was not long-lasting. When total body irradiation was added to the initial protocol, a higher rate of NK cell expansion occurred, and an elevated leukemia clearance rate was observed compared with patients who did not undergo NK cell expansion in vivo [165]. Bachanova et al. found that four of six patients with advanced B cell non-Hodgkin lymphoma showed an objective clinical response, and two patients showed early donor cell persistence [181]. The depletion of Treg cells could also improve the in vivo NK cell expansion and was associated with higher frequencies of CR in AML patients [182]. Although the level of in vivo NK cell expansion has not been shown to be relevant to the clinical response and outcome, recent studies using short-term cytokine-pretreated NK cells in AML therapy have reported favorable results. The combination of IL-12, IL-15 and IL-18 for the pretreatment of NK cells for approximately 12 h could result in a memory-like NK cell phenotype. These NK cells showed a significant expansion and persistence in vivo, resulting in a nearly 55% response rate in relapse and refractory AML patients [36]. Thus, challenges remain for improving clinical outcomes with NK cell adoptive transfer therapy, which can be achieved by optimizing clinical strategies to strengthen the persistence and proliferation of NK cells after

infusion.

Conflicts of interest

None.

Acknowledgments

This work was supported by the National Natural Science Foundation of China [grant numbers 91429300, 1571440, 81671558, 31570893]; the Ministry of Science and Technology of China [2013CB944900, 2016YFC1303503] and Chinese Academy of Science [XDA12020344, XDA12020312].

References

[1] R.B. Herberman, M.E. Nunn, D.H. Lavrin, Natural cytotoxic reactivity of mouse lymphoid-cells against syngeneic and allogeneic tumors. 1. Distribution of reactivity and specificity, Int. J. Cancer 16 (2) (1975) 216–229.
[2] R. Kiessling, E. Klein, H. Wigzell, Natural killer cells in mouse. 1. Cytotoxic cells with specificity for mouse moloney leukemia-cells—specificity and distribution according to genotype, Eur. J. Immunol. 5 (2) (1975) 112–117.
[3] E. Vivier, E. Tomasello, M. Baratin, T. Walzer, S. Ugolini, Functions of natural killer cells, Nat. Immunol. 9 (5) (2008) 503–510.
[4] M.W. Robinson, C. Harmon, C. O'Farrelly, Liver immunology and its role in inflammation and homeostasis, Cell. Mol. Immunol. 13 (3) (2016) 267–276.
[5] T.L. Geiger, J.C. Sun, Development and maturation of natural killer cells, Curr. Opin. Immunol. 39 (2016) 82–89.
[6] A.G. Freud, J. Yu, M.A. Caligiuri, Human natural killer cell development in secondary lymphoid tissues, Semin. Immunol. 26 (2) (2014) 132–137.
[7] H. Peng, X. Jiang, Y. Chen, D.K. Sojka, H. Wei, X. Gao, R. Sun, W.M. Yokoyama, Z. Tian, Liver-resident NK cells confer adaptive immunity in skin-contact inflammation, J. Clin. Invest. 123 (4) (2013) 1444–1456.
[8] R. Sharma, A. Das, Organ-specific phenotypic and functional features of NK cells in humans, Immunol. Res. 58 (1) (2014) 125–131.
[9] H. Peng, E. Wisse, Z. Tian, Liver natural killer cells: subsets and roles in liver immunity, Cell. Mol. Immunol. 13 (3) (2016) 328–336.
[10] Z. Shuai, M.W.Y. Leung, X. He, W. Zhang, G. Yang, P.S.C. Leung, M.E. Gershwin, Adaptive immunity in the liver, Cell. Mol. Immunol. 13 (3) (2016) 354–368.
[11] H. Peng, Z. Tian, Tissue-resident natural killer cells in the livers, Sci. China Life Sci. 59 (12) (2016) 1218–1223.
[12] W.M. Yokoyama, D.K. Sojka, H. Peng, Z. Tian, Tissue-resident natural killer cells, Cold Spring Harb. Symp. Quant. Biol. 78 (2013) 149–156.
[13] D.K. Sojka, B. Plougastel-Douglas, L. Yang, M.A. Pak-Wittel, M.N. Artyomov, Y. Ivanova, C. Zhong, J.M. Chase, P.B. Rothman, J. Yu, J.K. Riley, J. Zhu, Z. Tian, W.M. Yokoyama, Tissue-resident natural killer (NK) cells are cell lineages distinct from thymic and conventional splenic NK cells, Elife 3 (2014) e01659.
[14] J.E. Melsen, G. Lugthart, A.C. Lankester, M.W. Schilham, Human Circulating, Human circulating and tissue-resident CD56(bright) natural killer cell populations, Front. Immunol. 7 (2016) 262.
[15] X. Zhou, J. Tang, H. Cao, H. Fan, B. Li, Human circulating and tissue resident regulatory T cells: novel therapeutic targets for human disease, Cell. Mol. Immunol. 12 (5) (2015) 543–552.
[16] B. Gao, Basic liver immunology, Cell. Mol. Immunol. 13 (3) (2016) 265–266.
[17] A.K. Horst, K. Neumann, L. Diehl, G. Tiegs, Modulation of liver tolerance by conventional and nonconventional antigen-presenting cells and regulatory immune cells, Cell. Mol. Immunol. 13 (3) (2016) 277–292.
[18] L. Martinet, M.J. Smyth, Balancing natural killer cell activation through paired receptors, Nat. Rev. Immunol. 15 (4) (2015) 243–254.
[19] C. Sun, H. Sun, C. Zhang, Z. Tian, NK cell receptor imbalance and NK cell dysfunction in HBV infection and hepatocellular carcinoma, Cell. Mol. Immunol. 12 (3) (2015) 292–302.
[20] A. Moretta, Natural killer cells and dendritic cells: rendezvous in abused tissues, Nat. Rev. Immunol. 2 (12) (2002) 957–964.
[21] M. Lucas, W. Schachterle, K. Oberle, P. Aichele, A. Diefenbach, Dendritic cells prime natural killer cells by trans-presenting interleukin 15, Immunity 26 (4) (2007) 503–517.
[22] I. Mattiola, M. Pesant, P.F. Tentorio, M. Molgora, E. Marcenaro, E. Lugli, M. Locati, D. Mavilio, Priming of human resting NK cells by autologous M1 macrophages via the engagement of IL-1 beta, IFN-beta, and IL-15 pathways, J. Immunol. 195 (6) (2015) 2818–2828.
[23] F. Bellora, R. Castriconi, A. Dondero, P. Carrega, A. Mantovani, G. Ferlazzo, A. Moretta, C. Bottino, Human NK cells and NK receptors, Immunol. Lett. 161 (2) (2014) 168–173.
[24] W.H. Hallett, W.J. Murphy, Positive and negative regulation of natural killer cells: therapeutic implications, Semin. Cancer Biol. 16 (5) (2006) 367–382.
[25] Z.B. Davis, M. Felices, M.R. Verneris, J.S. Miller, Natural killer cell adoptive transfer therapy exploiting the first line of defense against cancer, Cancer J. 21 (6) (2015) 486–491.
[26] Z. Li, D. Li, A. Tsun, B. Li, FOXP3(+) regulatory T cells and their functional regulation, Cell. Mol. Immunol. 12 (5) (2015) 558–565.
[27] S. Zhang, X. Ke, S. Zeng, M. Wu, J. Lou, L. Wu, P. Huang, L. Huang, F. Wang, S. Pan, Analysis of CD8(+) Treg cells in patients with ovarian cancer: a possible mechanism for immune impairment, Cell. Mol. Immunol. 12 (5) (2015) 580–591.
[28] P. Xiong, H.-W. Sang, M. Zhu, Critical roles of co-activation receptor DNAX accessory molecule-1 in natural killer cell immunity, Immunology 146 (3) (2015) 369–378.
[29] C. Chester, K. Fritsch, H.E. Kohrt, Natural killer cell immunomodulation: targeting activating inhibitory, and Co-stimulatory receptor signaling for cancer immunotherapy, Front. Immunol. 6 (2015) 601.
[30] J. Spreu, S. Kuttruff, V. Stejfova, K.M. Dennehy, B. Schittek, A. Steinle, Interaction of C-type lectin-like receptors NKp65 and KACL facilitates dedicated immune recognition of human keratinocytes, Proc. Natl. Acad. Sci. U. S. A. 107 (11) (2010) 5100–5105.
[31] V. Beziat, H.G. Hilton, P.J. Norman, J.A. Traherne, Deciphering the killer-cell immunoglobulin-like receptor system at super-resolution for natural killer and T-cell biology, Immunology 150 (3) (2017) 248–264.
[32] D. Romero, PD-1 says goodbye, TIM-3 says hello, Nat. Rev. Clin. Oncol. 13 (4) (2016) 202–203.
[33] W.C. Dougall, S. Kurtulus, M.J. Smyth, A.C. Anderson, TIGIT and CD96: new checkpoint receptor targets for cancer immunotherapy, Immunol. Rev. 276 (1) (2017) 112–120.
[34] A. Cerwenka, L.L. Lanier, Natural killer cell memory in infection, inflammation and cancer, Nat. Rev. Immunol. 16 (2) (2016) 112–123.
[35] S. Jonjic, CMV immunology, Cell. Mol. Immunol. 12 (2) (2015) 125–127.
[36] R. Romee, M. Rosario, M.M. Berrien-Elliott, J.A. Wagner, B.A. Jewell, T. Schappe, J.W. Leong, S. Abdel-Latif, S.E. Schneider, S. Willey, C.C. Neal, L. Yu, S.T. Oh, Y.-S. Lee, A. Mulder, F. Claas, M.A. Cooper, T.A. Fehniger, Cytokine-induced memory-like natural killer cells exhibit enhanced responses against myeloid leukemia, Sci. Transl. Med. 8 (357) (2016).
[37] C. Chabannon, B. Mfarrej, S. Guia, S. Ugolini, R. Devillier, D. Blaise, E. Vivier, B. Calmels, Manufacturing natural killer cells as medicinal products, Front. Immunol. 7 (2016) 504.
[38] H.G. Ljunggren, K. Karre, In search of the missing self—MHC molecules and NK cell recognition, Immunol. Today 11 (7) (1990) 237–244.
[39] C. Bottino, A. Dondero, F. Bellora, L. Morette, F. Locatelli, V. Pistoia, A. Moretta, R. Castriconi, Natural killer cells and neuroblastoma: tumor recognition, escape mechanisms, and possible novel immunotherapeutic approaches, Front. Immunol. 5 (2014).
[40] H.J. Close, H. Wurdak, S.C. Short, A.A. Melcher, L.F. Stead, E.B. Wilson, G.P. Cook, Natural killer cell recognition of glioblastoma, Immunology 143 (2014) 138–138.
[41] J. Ren, Y. Nie, M. Lv, S. Shen, R. Tang, Y. Xu, Y. Hou, S. Zhao, T. Wang, Estrogen upregulates MICA/B expression in human non-small cell lung cancer through the regulation of ADAM17, Cell. Mol. Immunol. 12 (6) (2015) 768–776.
[42] S. Carotta, Targeting NK cells for anticancer immunotherapy: clinical and preclinical approaches, Front. Immunol. 7 (2016) 152.
[43] L. Zitvogel, G. Kroemer, Targeting PD-1/PD-L1 interactions for cancer immunotherapy, Oncoimmunology 1 (8) (2012) 1223–1225.
[44] A. Beldi-Ferchiou, M. Lambert, S. Dogniaux, F. Vely, E. Vivier, D. Olive, S. Dupuy, F. Levasseur, D. Zucman, C. Lebbe, D. Sene, C. Hivroz, S. Caillat-Zucman, PD-1 mediates functional exhaustion of activated NK cells in patients with Kaposi sarcoma, Oncotarget 7 (45) (2016) 72961–72977.
[45] M.K. Gleason, T.R. Lenvik, V. McCullar, M. Felices, M.S. O'Brien, S.A. Cooley, M.R. Verneris, F. Cichocki, C.J. Holman, A. Panoskaltsis-Mortari, T. Niki, M. Hirashima, B.R. Blazar, J.S. Miller, Tim-3 is an inducible human natural killer cell receptor that enhances interferon gamma production in response to galectin-9, Blood 119 (13) (2012) 3064–3072.
[46] R. Rekik, N.B. Hmida, A. Ben Hmid, I. Zamali, N. Kammoun, M. Ben Ahmed, PD-1 induction through TCR activation is partially regulated by endogenous TGF-beta, Cell. Mol. Immunol. 12 (5) (2015) 648–649.
[47] Y.-H. Li, W.-H. Zhou, Y. Tao, S.-C. Wang, Y.-L. Jiang, D. Zhang, H.-L. Piao, Q. Fu, D.-J. Li, M.-R. Du, The galectin-9/Tim-3 pathway is involved in the regulation of NK cell function at the maternal-fetal interface in early pregnancy, Cell. Mol. Immunol. 13 (1) (2016) 73–81.
[48] C.M. Sungur, W.J. Murphy, Positive and negative regulation by NK cells in cancer, Crit. Rev. Oncog. 19 (1–2) (2014) 57–66.
[49] S. Carotta, Targeting NK cells for anticancer immunotherapy: Clinical and preclinical approaches, Front Immunol. 7 (2016) 152.
[50] N. Shimasaki, E. Coustan-Smith, T. Kamiya, D. Campana, Expanded and armed natural killer cells for cancer treatment, Cytotherapy 18 (11) (2016) 1422–1434.
[51] Y. Zhu, B. Huang, J. Shi, Fas ligand and lytic granule differentially control cytotoxic dynamics of natural killer cell against cancer target, Oncotarget 7 (30) (2016) 47163–47172.
[52] R.W. Childs, M. Carlsten, Therapeutic approaches to enhance natural killer cell cytotoxicity against cancer: the force awakens, Nat. Rev. Drug Discov. 14 (7) (2015) 487–498.
[53] T.A. Fehniger, M.A. Cooper, Harnessing NK cell memory for cancer immunotherapy, Trends Immunol. 37 (12) (2016) 877–888.
[54] T. Floros, A.A. Tarhini, Anticancer cytokines: clinical effects of interferon-alpha 2, interleukin (IL)-2, IL-15, IL-21, and IL-12, Semin. Oncol. 42 (4) (2015) 539–548.
[55] R.W. Childs, M. Carlsten, Therapeutic approaches to enhance natural killer cell cytotoxicity against cancer: the force awakens, Nat. Rev. Drug Discov. 14 (7) (2015) 487–498.
[56] A.M. Levin, D.L. Bates, A.M. Ring, C. Krieg, J.T. Lin, L. Su, I. Moraga, M.E. Raeber, G.R. Bowman, P. Novick, V.S. Pande, C.G. Fathman, O. Boyman, K.C. Garcia, Exploiting a natural conformational switch to engineer an interleukin-2 'superkine', Nature 484 (7395) (2012) 529–U159.

[57] R. Ghasemi, E. Lazear, X. Wang, S. Arefanian, A. Zheleznyak, B.M. Carreno, R. Higashikubo, A.E. Gelman, D. Kreisel, D.H. Fremont, A.S. Krupnick, Selective targeting of IL-2 to NKG2D bearing cells for improved immunotherapy, Nat. Commun. 7 (2016).
[58] R. Romee, M. Rosario, M.M. Berrien-Elliott, J.A. Wagner, B.A. Jewell, T. Schappe, J.W. Leong, S. Abdel-Latif, S.E. Schneider, S. Willey, C.C. Neal, L.Y. Yu, S.T. Oh, Y.S. Lee, A. Mulder, F. Claas, M.A. Cooper, T.A. Fehniger, Cytokine-induced memory-like natural killer cells exhibit enhanced responses against myeloid leukemia, Sci. Transl. Med. 8 (357) (2016).
[59] Y. Yang, O. Lim, T.M. Kim, Y.-O. Ahn, H. Choi, H. Chung, B. Min, J.H. Her, S.Y. Cho, B. Keam, S.-H. Lee, D.-W. Kim, Y.K. Hwang, D.S. Heo, 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. 4 (3) (2016) 215–224.
[60] B.C. Shaffer, J.B. Le Luduec, C. Forlenza, A.A. Jakubowski, M.A. Perales, J.W. Young, K.C. Hsu, 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. 22 (4) (2016) 705–709.
[61] A. Leivas, A. Perez-Martinez, M. Jesus Blanchard, E. Martin-Clavero, L. Fernandez, J. Jose Lahuerta, J. Martinez-Lopez, Novel treatment strategy with autologous activated and expanded natural killer cells plus anti-myeloma drugs for multiple myeloma, Oncoimmunology 5 (12) (2016).
[62] S. Szmania, N. Lapteva, T. Garg, A. Greenway, J. Lingo, B. Nair, K. Stone, E. Woods, J. Khan, J. Stivers, S. Panozzo, D. Campana, W.T. Bellamy, M. Robbins, J. Epstein, S. Yaccoby, S. Waheed, A. Gee, M. Cottler-Fox, C. Rooney, B. Barlogie, F. van Rhee, Ex vivo-expanded natural killer cells demonstrate robust proliferation in vivo in high-risk relapsed multiple myeloma patients, J. Immunother. 38 (1) (2015) 24–36.
[63] N. Sakamoto, T. Ishikawa, S. Kokura, T. Okayama, K. Oka, M. Ideno, F. Sakai, A. Kato, M. Tanabe, T. Enoki, J. Mineno, Y. Naito, Y. Itoh, T. Yoshikawa, Phase I clinical trial of autologous NK cell therapy using novel expansion method in patients with advanced digestive cancer, J. Transl. Med. 13 (2015).
[64] I. Zanoni, R. Spreafico, C. Bodio, M. Di Gioia, C. Cigni, A. Broggi, T. Gorletta, M. Caccia, G. Chirico, L. Sironi, M. Collini, M.P. Colombo, N. Garbi, F. Granucci, IL-15 cis presentation is required for optimal NK cell activation in lipopolysaccharide-mediated inflammatory conditions, Cell Rep. 4 (6) (2013) 1235–1249.
[65] A.-H. Pillet, J. Theze, T. Rose, Interleukin (IL)-2 and IL-15 have different effects on human natural killer lymphocytes, Hum. Immunol. 72 (11) (2011) 1013–1017.
[66] J.S. Miller, M.R. Verneris, J. Curtsinger, T.E. DeFor, D. McKenna, T.A. Waldmann, B.R. Blazar, D.J. Weisdorf, S. Cooley, A phase I study of intravenous NCI IL-15 to enhance adoptively transferred NK cells uncovers defects in CD16 triggered IFN gamma production and competition between donor NK and recipient T cells, Blood 126 (23) (2015).
[67] K.C. Conlon, E. Lugli, H.C. Welles, S.A. Rosenberg, A.T. Fojo, J.C. Morris, T.A. Fleisher, S.P. Dubois, L.P. Perera, D.M. Stewart, C.K. Goldman, B.R. Bryant, J.M. Decker, J. Chen, T.Y.A. Worthy, W.D. Figg, Sr., C.J. Peer, M.C. Sneller, H.C. Lane, J.L. Yovandich, S.P. Creekmore, M. Roederer, T.A. Waldmann, Redistribution, hyperproliferation, activation of natural killer cells and CD8 T cells, and cytokine production during first-in-human clinical trial of recombinant human interleukin-15 in patients with cancer, J. Clin. Oncol. 33 (1) (2015) (74-U123).
[68] Y. Chen, B. Chen, T. Yang, W. Xiao, L. Qian, Y. Ding, M. Ji, X. Ge, W. Gong, Human fused NKG2D-IL-15 protein controls xenografted human gastric cancer through the recruitment and activation of NK cells, Cell. Mol. Immunol. 14 (3) (2017) 293–307.
[69] M. Thaysen-Andersen, E. Chertova, C. Bergamaschi, E.S.X. Moh, O. Chertov, J. Roser, R. Sowder, J. Bear, J. Lifson, N.H. Packer, B.K. Felber, G.N. Pavlakis, Recombinant human heterodimeric IL-15 complex displays extensive and reproducible N- and O-linked glycosylation, Glycoconj. J. 33 (3) (2016) 417–433.
[70] M. Rosario, B. Liu, L. Kong, L.I. Collins, S.E. Schneider, X. Chen, K. Han, E.K. Jeng, P.R. Rhode, J.W. Leong, T. Schappe, B.A. Jewell, C.R. Keppel, K. Shah, B. Hess, R. Romee, D.R. Piwnica-Worms, A.F. Cashen, N.L. Bartlett, H.C. Wong, T.A. Fehniger, The IL-15-based ALT-803 complex enhances fc gamma RIIIa-triggered NK cell responses and in vivo clearance of B cell lymphomas, Clin. Cancer Res. 22 (3) (2016) 596–608.
[71] D. Upreti, M. Zhang, S.K.P. Kung, IL-15 activated NK cells overcame DC maturation defects induced by head and neck cancer microenvironment, Eur. J. Immunol. 46 (2016) 1244–1244.
[72] J.U. Schmohl, M. Felices, J.S. Miller, D.A. Vallera, Improvement in ADCC and NK cell activation of an anti-carcinoma bispecific antibody by genetic insertion of IL-15 as a cross-linker, Oncol. Res. Treatm. 39 (2016) 280–280.
[73] D. Lehmann, J. Spanholtz, C. Sturtzel, M. Tordoir, B. Schlechta, D. Groenewegen, E. Hofer, IL-12 directs further maturation of ex vivo differentiated NK cells with improved therapeutic potential, PLoS One 9 (1) (2014).
[74] C. Trudeau, M.M. Cotreau, L. Stonis, K.H. Dykstra, J.L. Oestreicher, A. Strahs, A.J. Dorner, V.H. Van Cleave, W.L. Trepicchio, U.S. Schwertschlag, A single administration of recombinant human interleukin-12 is associated with increased expression levels of interferon-gamma and signal transducer and activator of transcription in healthy subjects, J. Clin. Pharmacol. 45 (6) (2005) 649–658.
[75] J.A. Gollob, K.G. Veenstra, R.A. Parker, J.W. Mier, D.F. McDermott, D. Clancy, L. Tutin, H. Koon, M.B. Atkins, Phase I trial of concurrent twice-weekly recombinant human interleukin-12 plus low-dose IL-2 in patients with melanoma or renal cell carcinoma, J. Clin. Oncol. 21 (13) (2003) 2564–2573.
[76] J.A. Gollob, J.W. Mier, K. Veenstra, D.F. McDermott, D. Clancy, M. Clancy, M.B. Atkins, Phase I trial of twice-weekly intravenous interleukin 12 in patients with metastatic renal cell cancer or malignant melanoma: ability to maintain IFN-gamma induction is associated with clinical response, Clin. Cancer Res. 6 (5) (2000) 1678–1692.
[77] M.S. Gokhale, V. Vainstein, J. Tom, S. Thomas, C.E. Lawrence, Z. Gluzman-Poltorak, N. Siebers, L.A. Basile, Single low-dose rHuIL-12 safely triggers multi-lineage hematopoietic and immune-mediated effects, Exp. Hematol. Oncol. 3 (1) (2014) 11.
[78] J. Fallon, R. Tighe, G. Kradjian, W. Guzman, A. Bernhardt, B. Neuteboom, Y. Lan, H. Sabzevari, J. Schlom, J.W. Greiner, The immunocytokine NHS-IL12 as a potential cancer therapeutic, Oncotarget 5 (7) (2014) 1869–1884.
[79] S. Srivastava, D. Pelloso, H. Feng, L. Voiles, D. Lewis, Z. Haskova, M. Whitacre, S. Trulli, Y.-J. Chen, J. Toso, Z.L. Jonak, H.-C. Chang, M.J. Robertson, Effects of interleukin-18 on natural killer cells: costimulation of activation through Fc receptors for immunoglobulin, Cancer Immunol. Immunother. 62 (6) (2013) 1073–1082.
[80] D.L. Hodge, J.J. Subleski, D.A. Reynolds, M.D. Buschman, W.B. Schill, M.W. Burkett, A.M. Malyguine, H.A. Young, The proinflammatory cytokine interleukin-18 alters multiple signaling pathways to inhibit natural killer cell death, J. Interferon Cytokine Res. 26 (10) (2006) 706–718.
[81] C.M. Nielsen, A.-S. Wolf, M.R. Goodier, E.M. Riley, Synergy between common gamma chain family cytokines and IL-18 potentiates innate and adaptive pathways of NK cell activation, Front. Immunol. 7 (2016).
[82] S. Agaugue, E. Marcenaro, B. Ferranti, L. Moretta, A. Moretta, Human natural killer cells exposed to IL-2, IL-125 IL-18, or IL-4 differently modulate priming of naive T cells by monocyte-derived dendritic cells, Blood 112 (5) (2008) 1776–1783.
[83] C. Kellner, A. Guenther, A. Humpe, R. Repp, K. Klausz, S. Derer, T. Valerius, M. Ritgen, M. Brueggemann, J.G.J. van de Winkel, P.W.H.I. Parren, M. Kneba, M. Gramatzki, M. Peipp, Enhancing natural killer cell-mediated lysis of lymphoma cells by combining therapeutic antibodies with CD20-specific immunoligands engaging NKG2D or NKp30, Oncoimmunology 5 (1) (2016).
[84] J.S. Miller, Therapeutic applications: natural killer cells in the clinic, hematology, Am. Soc. Hematol. Educ. Progr. 2013 (2013) 247–253.
[85] S.P. Koerner, M.C. Andre, J.S. Leibold, P.C. Kousis, A. Kuebler, M. Pal, S.P. Haen, H.J. Buehring, L. Grosse-Hovest, G. Jung, H.R. Salih, An Fc-optimized CD133 antibody for induction of NK cell reactivity against myeloid leukemia, Leukemia 31 (2) (2017) 459–469.
[86] S. Vasu, S. He, C. Cheney, B. Gopalakrishnan, R. Mani, G. Lozanski, X. Mo, V. Groh, S.P. Whitman, R. Konopitzky, C. Koessl, D. Bucci, D.M. Lucas, J. Yu, M.A. Caligiuri, W. Blum, P.J. Adam, E. Borges, B. Rueter, K.-H. Heider, G. Marcucci, N. Muthusamy, Decitabine enhances anti-CD33 monoclonal antibody BI 836858-mediated natural killer ADCC against AML blasts, Blood 127 (23) (2016) 2879–2889.
[87] M.C. Ochoa, L. Minute, I. Rodriguez, S. Garasa, E. Perez-Ruiz, S. Inoges, I. Melero, P. Berraondo, Antibody-dependent cell cytotoxicity: immunotherapy strategies enhancing effector NK cells, Immunol. Cell Biol. 95 (4) (2017) 347–355.
[88] L.L. Lanier, NKG2D receptor and its ligands in host defense, Cancer Immunol. Res. 3 (6) (2015) 575–582.
[89] R. Pawlick, B. Gala-Lopez, A.R. Pepper, M. McCall, O. Ziff, A.M.J. Shapiro, The combination of anti-NKG2D and CTLA-4 Ig therapy prolongs islet allograft survival in a murine model, Am. J. Transplant. 14 (10) (2014) 2367–2374.
[90] M.F. Sanmamed, F. Pastor, A. Rodriguez, J. Luis Perez-Gracia, M.E. Rodriguez-Ruiz, M. Jure-Kunkel, I. Melero, Agonists of co-stimulation in cancer immunotherapy directed against CD137, 0 × 40, GITR, CD27, CD28, and ICOS, Semin. Oncol. 42 (4) (2015) 640–655.
[91] D.M. Pardoll, The blockade of immune checkpoints in cancer immunotherapy, Nat. Rev. Cancer 12 (4) (2012) 252–264.
[92] H.E. Kohrt, R. Houot, K. Weiskopf, M.J. Goldstein, F. Scheeren, D. Czerwinski, A.D. Colevas, W.K. Weng, M.F. Clarke, R.W. Carlson, F.E. Stockdale, J.A. Mollick, L. Chen, R. Levy, Stimulation of natural killer cells with a CD137-specific antibody enhances trastuzumab efficacy in xenotransplant models of breast cancer, J. Clin. Invest. 122 (3) (2012) 1066–1075.
[93] H. Kohrt, R. Houot, K. Weiskopf, M. Goldsteini, P. Lund, D. Czerwinski, J. Sunwoo, T. Tedder, L. Chen, R. Levy, Stimulation of natural killer cells with an anti-CD137 antibody enhances the efficacy trastuzumab, cetuximab, and rituximab in HER2-expressing breast cancer, EGFR plus head and neck cancer, and CD20 + lymphoma, Cancer Res. 72 (2012).
[94] S.-M. Wei, J.-X. Fei, F. Tao, H.-L. Pan, Q. Shen, L. Wang, Y.-J. Wu, L. Zhou, S.-X. Zhu, W.-B. Liao, H. Ji, Z.-L. Xin, Anti-CD27 antibody potentiates antitumor effect of dendritic cell-based vaccine in prostate cancer-bearing mice, Int. Surg. 100 (1) (2015) 155–163.
[95] R. Prell, W. Halpern, J. Beyer, J. Tarrant, S. Sukumaran, M. Huseni, R. Kaiser, D. Wilkins, S. Karanth, H. Chiu, J. Ruppel, C. Zhang, K. Lin, L. Damico-Beyer, J. Kim, H. Taylor, Nonclinical safety assessment of a humanized anti-OX40 agonist antibody, MOXR0916, Eur. J. Cancer 50 (2014) 136–136.
[96] J. Miska, A. Rashidi, A.L. Chang, M.E. Muroski, Y. Han, L. Zhang, M.S. Lesniak, Anti-GITR therapy promotes immunity against malignant glioma in a murine model, Cancer Immunol. Immunother. 65 (12) (2016) 1555–1567.
[97] F. Romagne, P. Andre, P. Spee, S. Zahn, N. Anfossi, L. Gauthier, M. Capanni, L. Ruggeri, D.M. Benson Jr., B.W. Blaser, M. Della Chiesa, A. Moretta, E. Vivier, M.A. Caligiuri, A. Velardi, N. Wagtmann, Preclinical characterization of 1–7F9, a novel human anti-KIR receptor therapeutic antibody that augments natural killer-mediated killing of tumor cells, Blood 114 (13) (2009) 2667–2677.
[98] N. Wagtmann, P. Andre, S. Zahn, P. Spee, N. Anfossi, L. Gauthier, B.W. Blaser, M.A. Caligiuri, M. Capanni, L. Ruggeri, A. Velardi, F. Romagne, Anti-KIR(1–7F9): a fully human monoclonal antibody (mAb) that blocks KIR2DL1, −2 and-3,

promoting natural killer (NK) cell-mediated lysis of tumor cells in vitro and in vivo, Blood 110 (11) (2007) (179A-179A).
[99] D.M. Benson, S. Padmanabhan, C.C. Hofmeistar, C.E. Bakan, R. Abonour, A. Suvannasankha, Y.A. Efebera, P.J. Squiban, M.A. Caligiuri, S. Farag, A phase I study of IPH2101, a novel anti-inhibitory monoclonal antibody, in patients with multiple myelonia, J. Clin. Oncol. 28 (15) (2010).
[100] N. Korde, M. Carlsten, M.-J. Lee, A. Minter, E. Tan, M. Kwok, E. Manasanch, M. Bhutani, N. Tageja, M. Roschewski, A. Zingone, R. Costello, M. Mulquin, D. Zuchlinski, I. Maric, K.R. Calvo, R. Braylan, P. Tembhare, C. Yuan, M. Stetler-Stevenson, J. Trepel, R. Childs, O. Landgren, A phase II trial of pan-KIR2D blockade with IPH2101 in smoldering multiple myeloma, Haematologica 99 (6) (2014) E81–E83.
[101] M. Felices, J.S. Miller, Targeting KIR blockade in multiple myeloma: trouble in checkpoint paradise? Clin. Cancer Res. 22 (21) (2016) 5161–5163.
[102] D.M. Benson Jr., A.D. Cohen, S. Jagannath, N.C. Munshi, G. Spitzer, C.C. Hofmeister, Y.A. Efebera, P. Andre, R. Zerbib, M.A. Caligiuri, A phase I trial of the anti-KIR antibody IPH2101 and lenalidomide in patients with relapsed/refractory multiple myeloma, Clin. Cancer Res. 21 (18) (2015) 4055–4061.
[103] L. Ruggeri, E. Urbani, P. Andre, A. Mancusi, A. Tosti, F. Topini, M. Blery, L. Animobono, F. Romagne, N. Wagtmann, A. Velardi, Effects of anti-NKG2A antibody administration on leukemia and normal hematopoietic cells, Haematologica 101 (5) (2016) 626–633.
[104] N. Nielsen, E.D. Galsgaard, D. Ahern, M. Andersen, P. Spee, M. Feldmann, F. Brennan, K. Soederstroem, Blocking the inhibitory CD94/NKG2A NK cell receptor with a novel anti-NKG2A MAB enhances the susceptibility of rheumatoid arthritis fibroblast-like synoviocytes (FLS) to NK cell-mediated cytotoxicity, Ann. Rheum. Dis. 71 (2012) 316–316.
[105] S. Pesce, M. Greppi, G. Tabellini, F. Rampinelli, S. Parolini, D. Olive, L. Moretta, A. Moretta, E. Marcenaro, Identification of a subset of human natural killer cells expressing high levels of programmed death 1: a phenotypic and functional characterization, J. Allergy Clin. Immunol. 139 (1) (2017) 335–346 (e3).
[106] M. Della Chiesa, S. Pesce, L. Muccio, S. Carlomagno, S. Sivori, A. Moretta, E. Marcenaro, Features of memory-like and PD-1(+) human nK cell subsets, Front. Immunol. 7 (2016).
[107] M. Giuliani, B. Janji, G. Berchem, Activation of NK cells and disruption of PD-axis: two different ways for lenalidomide to block myeloma progression, Oncotarget (2017) L1/PD–L1/P1.
[108] Z. Sun, J. Fourcade, O. Pagliano, J.M. Chauyin, C. Sander, J. Kirkwood, V. Kuchroo, H. Zarour, Tim-3 is expressed by a majority of NK cells at periphery but not at tumor site in patients with advanced melanoma, J. Immunol. 192 (2014).
[109] S.F. Ngiow, M.W.L. Teng, M.J. Smyth, Prospects for TIM3-targeted antitumor immunotherapy, Cancer Res. 71 (21) (2011) 6567–6571.
[110] Z.-Z. Yang, D.M. Grote, S.C. Ziesmer, T. Niki, M. Hirashima, A.J. Novak, T.E. Witzig, S.M. Ansell, IL-12 upregulates TIM-3 expression and induces T cell exhaustion in patients with follicular B cell non-Hodgkin lymphoma, J. Clin. Invest. 122 (4) (2012) 1271–1282.
[111] I.P. da Silva, A. Gallois, S. Jimenez-Baranda, S. Khan, A.C. Anderson, V.K. Kuchroo, I. Osman, N. Bhardwaj, Reversal of NK-cell exhaustion in advanced melanoma by Tim-3 blockade, Cancer Immunol. Res. 2 (5) (2014) 410–422.
[112] J. Bi, Q. Zhang, D. Liang, L. Xiong, H. Wei, R. Sun, Z. Tian, T-cell ig and ITIM domain regulates natural killer cell activation in murine acute viral hepatitis, Hepatology 59 (5) (2014) 1715–1725.
[113] R.B. Delconte, T.B. Kolesnik, L.F. Dagley, J. Rautela, W. Shi, E.M. Putz, K. Stannard, J.G. Zhang, C. Teh, M. Firth, T. Ushiki, C.E. Andoniou, M.A. Degli-Esposti, P.P. Sharp, C.E. Sanvitale, G. Infusini, N.P. Liau, E.M. Linossi, C.J. Burns, S. Carotta, D.H. Gray, C. Seillet, D.S. Hutchinson, G.T. Belz, A.I. Webb, W.S. Alexander, S.S. Li, A.N. Bullock, J.J. Babon, M.J. Smyth, S.E. Nicholson, N.D. Huntington, CIS is a potent checkpoint in NK cell-mediated tumor immunity, Nat. Immunol. 17 (7) (2016) 816–824.
[114] J. Steinbacher, K. Baltz-Ghahremanpour, B.J. Schmiedel, A. Steinle, G. Jung, A. Kuebler, M.C. Andre, L. Grosse-Hovest, H.R. Salih, An Fc-optimized NKG2D-immunoglobulin G fusion protein for induction of natural killer cell reactivity against leukemia, Int. J. Cancer 136 (5) (2015) 1073–1084.
[115] S. Chen, J. Li, Q. Li, Z. Wang, Bispecific antibodies in cancer immunotherapy, Hum. Vaccines Immunother. 12 (10) (2016) 2491–2500.
[116] M.K. Gleason, J.A. Ross, E.D. Warlick, T.C. Lund, M.R. Verneris, A. Wiernik, S. Spellman, M.D. Haagenson, A.J. Lenvik, M.R. Litzow, P.K. Epling-Burnette, B.R. Blazar, L.M. Weiner, D.J. Weisdorf, D.A. Vallera, J.S. Miller, CD16xCD33 bispecific killer cell engager (BiKE) activates NK cells against primary MDS and MDSC CD33(+) targets, Blood 123 (19) (2014) 3016–3026.
[117] A. Wiernik, B. Foley, B. Zhang, M.R. Verneris, E. Warlick, M.K. Gleason, J.A. Ross, X. Luo, D.J. Weisdorf, B. Walcheck, D.A. Vallera, J.S. Miller, Targeting natural killer cells to acute myeloid leukemia in vitro with a CD16 x 33 bispecific killer cell engager and ADAM17 inhibition, Clin. Cancer Res. 19 (14) (2013) 3844–3855.
[118] J.U. Schmohl, M.K. Gleason, P.R. Dougherty, J.S. Miller, D.A. Vallera, Heterodimeric bispecific single chain variable fragments (scFv) killer engagers (BiKEs) enhance NK-cell activity against CD133 + colorectal cancer cells, Targeted Oncol. 11 (3) (2016) 353–361.
[119] J.P. Marschner, M. Treder, H.E. Kohrt, M.S. Topp, A. Engert, AFM13, a novel bispecific (CD30xCD16A) tetravalent antibody (TandAb (R)) specifically engaging NK-cells to fight Hodgkin lymphoma (HL), Oncol. Res. Treat. 39 (2016) (28-28).
[120] J. Wu, J. Fu, M. Zhang, D. Liu, AFM13: a first-in-class tetravalent bispecific anti-CD30/CD16A antibody for NK cell-mediated immunotherapy, J. Hematol. Oncol. 8 (2015).
[121] A. Rothe, S. Sasse, M.S. Topp, D.A. Eichenauer, H. Hummel, K.S. Reiners, M. Dietlein, G. Kuhnert, J. Kessler, C. Buerkle, M. Ravic, S. Knackmuss, J.-P. Marschner, E.P. von Strandmann, P. Borchmann, A. Engert, A phase 1 study of the bispecific anti-CD30/CD16A antibody construct AFM13 in patients with relapsed or refractory Hodgkin lymphoma, Blood 125 (26) (2015) 4024–4031.
[122] J.S. Miller, M. Felice, R. McElmurry, V. McCullar, X. Zhou, J. Tolar, J.U. Schmohl, A. Panoskaltsis-Mortari, B. Zhang, E. Taras, M.R. Verneris, S. Cooley, D.J. Weisdorf, B.R. Blazar, D. Vallera, Trispecific killer engagers (TriKEs) that contain IL-15 to make NK cells antigen specific and to sustain their persistence and expansion, Blood 126 (23) (2015).
[123] X. Deng, H. Terunuma, M. Nieda, W. Xiao, A. Nicol, Synergistic cytotoxicity of ex vivo expanded natural killer cells in combination with monoclonal antibody drugs against cancer cells, Int. Immunopharmacol. 14 (4) (2012) 593–605.
[124] M. Granzin, A. Stojanovic, M. Miller, R. Childs, V. Huppert, A. Cerwenka, Highly efficient IL-21 and feeder cell-driven ex vivo expansion of human NK cells with therapeutic activity in a xenograft mouse model of melanoma, Oncoimmunology 5 (9) (2016).
[125] X. Li, C. He, C. Liu, J. Ma, P. Ma, H. Cui, H. Tao, B. Gao, Expansion of NK cells from PBMCs using immobilized 4-1BBL and interleukin-21, Int. J. Oncol. 47 (1) (2015) 335–342.
[126] T. Sutlu, B. Stellan, M. Gilljam, H.C. Quezada, H. Nahi, G. Gahrton, E. Alici, Clinical-grade, large-scale, feeder-free expansion of highly active human natural killer cells for adoptive immunotherapy using an automated bioreactor, Cytotherapy 12 (8) (2010) 1044–1055.
[127] N. Lapteva, A.G. Durett, J. Sun, L.A. Rollins, L.L. Huye, J. Fang, V. Dandekar, Z. Mei, K. Jackson, J. Vera, J. Ando, M.C. Ngo, E. Coustan-Smith, D. Campana, S. Szmania, T. Garg, A. Moreno-Bost, F. Vanrhee, A.P. Gee, C.M. Rooney, Large-scale ex vivo expansion and characterization of natural killer cells for clinical applications, Cytotherapy 14 (9) (2012) 1131–1143.
[128] M. Cheng, Y.Y. Chen, W.H. Xiao, R. Sun, Z.G. Tian, NK cell-based immunotherapy for malignant diseases, Cell. Mol. Immunol. 10 (3) (2013) 230–252.
[129] D.A. Knorr, V. Bachanova, M.R. Verneris, J.S. Miller, Clinical utility of natural killer cells in cancer therapy and transplantation, Semin. Immunol. 26 (2) (2014) 161–172.
[130] R.S. Mehta, E.J. Shpall, K. Rezvani, Cord blood as a source of natural killer cells, Front. Med. (Lausanne) 2 (2015) 93.
[131] D.X. Xing, A.G. Ramsay, J.G. Gribben, W.K. Decker, J.K. Burks, M. Munsell, S.F. Li, S.N. Robinson, H. Yang, D. Steiner, N. Shah, J.D. McMannis, R.E. Champlin, C. Hosing, P.A. Zweidler-Mckay, E.J. Shpall, C.M. Bollard, Cord blood natural killer cells exhibit impaired lytic immunological synapse formation that is reversed with IL-2 exvivo expansion, J. Immunother. 33 (7) (2010) 684–696.
[132] G. Pittari, P. Filippini, G. Gentilcore, J.C. Grivel, S. Rutella, Revving up natural killer cells and cytokine-induced killer cells against hematological malignancies, Front. Immunol. 6 (2015) 230.
[133] H. Dolstra, M.W.H. Roeven, J. Spanholtz, B.N. Hangalapura, M. Tordoir, F. Maas, M. Leenders, F. Bohme, N. Kok, C. Trilsbeek, J. Paardekooper, A.B. van der Waart, P.E. Westerweel, T.J.F. Snijders, J.J. Cornelissen, G.M.J. Bos, H.F.M. Pruijt, A.O. De Graaf, B. van der Reijden, J.H. Jansen, A. van der Meer, G. Huls, J. Cany, F. Preijers, N.M.A. Blijlevens, N.M. Schaap, Successful transfer of umbilical cord blood C+ hematopoietic stem and progenitor-derived NK cells in older acute myeloid leukemia patients, Clin. Cancer Res. (2017) D34.
[134] A.M. Bock, D. Knorr, D.S. Kaufman, Expansion, and in vivo monitoring of human NK cells from human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs), Jove-J. Vis. Exp. (74) (2013).
[135] D.A. Knorr, Z. Ni, D. Hermanson, M.K. Hexum, L. Bendzick, L.J.N. Cooper, D.A. Lee, D.S. Kaufman, Clinical-scale derivation of natural killer cells from human pluripotent stem cells for cancer therapy, Stem Cells Transl. Med. 2 (4) (2013) 274–283.
[136] D.L. Hermanson, L. Bendzick, L. Pribyl, V. McCullar, R.I. Vogel, J.S. Miller, M.A. Geller, D.S. Kaufman, Induced pluripotent stem cell-derived natural killer cells for treatment of ovarian cancer, Stem Cells 34 (1) (2016) 93–101.
[137] T. Tonn, D. Schwabe, H.G. Klingemann, S. Becker, R. Esser, U. Koehl, M. Suttorp, E. Seifried, O.G. Ottimann, G. Bug, Treatment of patients with advanced cancer with the natural killer cell line NK-92, Cytotherapy 15 (12) (2013) 1563–1570.
[138] G. Suck, M. Odendahl, P. Nowakowska, C. Seidl, W.S. Wels, H.G. Klingemann, T. Tonn, NK-92: an 'off-the-shelf therapeutic' for adoptive natural killer cell-based cancer immunotherapy, Cancer Immunol. Immunother. 65 (4) (2016) 485–492.
[139] C. Jochems, J.W. Hodge, M. Fantini, R. Fujii, Y.M. Morillon II, J.W. Greiner, M.R. Padget, S.R. Tritsch, K.Y. Tsang, K.S. Campbell, H. Klingemann, L. Boissel, S. Rabizadeh, P. Soon-Shiong, J. Schlom, An NK cell line (haNK) expressing high levels of granzyme and engineered to express the high affinity CD16 allele, Oncotarget 7 (52) (2016) 86359–86373.
[140] J.O.J. Davies, K. Stringaris, A.J. Barrett, K. Rezvani, Opportunities and limitations of natural killer cells as adoptive therapy for malignant disease, Cytotherapy 16 (11) (2014) 1453–1466.
[141] P. Oberoi, W.S. Wels, Arming NK cells with enhanced antitumor activity: CARs and beyond, Oncoimmunology 2 (8) (2013) e25220.
[142] J.D. Brandstadter, Y. Yang, Natural killer cell responses to viral infection, J. Innate Immun. 3 (3) (2011) 274–279.
[143] T. Sutlu, S. Nystrom, M. Gilljam, B. Stellan, S.E. Applequist, E. Alici, Inhibition of intracellular antiviral defense mechanisms augments lentiviral transduction of human natural killer cells: implications for gene therapy, Hum. Gene Ther. 23 (10) (2012) 1090–1100.
[144] V.F.I. Van Tendeloo, P. Ponsaerts, F. Lardon, G. Nijs, M. Lenjou, C. Van Broeckhoven, D.R. Van Bockstaele, Z.N. Berneman, Highly efficient gene delivery by mRNA electroporation in human hematopoietic cells: superiority to lipofection and passive pulsing of mRNA and to electroporation of plasmid cDNA for tumor

antigen loading of dendritic cells, Blood 98 (1) (2001) 49–56.
[145] L. Li, L.N. Liu, S. Feller, C. Allen, R. Shivakumar, J. Fratantoni, L.A. Wolfraim, H. Fujisaki, D. Campana, N. Chopas, S. Dzekunov, M. Peshwa, Expression of chimeric antigen receptors in natural killer cells with a regulatory-compliant non-viral method, Cancer Gene Ther. 17 (3) (2010) 147–154.
[146] L. Li, C. Allen, R. Shivakumar, M.V. Peshwa, Large volume flow electroporation of mRNA: clinical scale process, Methods Mol. Biol. (Clifton N.J.) 969 (2013) 127–138.
[147] B. Clemenceau, R. Vivien, C. Pellat, M. Foss, G. Thibault, H. Vie, The human natural killer cytotoxic cell line NK-92, once armed with a murine CD16 receptor, represents a convenient cellular tool for the screening of mouse mAbs according to their ADCC potential, Mabs 5 (4) (2013) 587–594.
[148] C. Sahm, K. Schoenfeld, W.S. Wels, Expression of IL-15 in NK cells results in rapid enrichment and selective cytotoxicity of gene-modified effectors that carry a tumor-specific antigen receptor, Cancer Immunol. Immunother. 61 (9) (2012) 1451–1461.
[149] S. Nagashima, R. Mailliard, Y. Kashii, T.E. Reichert, R.B. Herberman, P. Robbins, T.L. Whiteside, Stable transduction of the interleukin-2 gene into human natural killer cell lines and their phenotypic and functional characterization in vitro and in vivo, Blood 91 (10) (1998) 3850–3861.
[150] W. Jiang, J. Zhang, Z. Tian, Functional characterization of interleukin-15 gene transduction into the human natural killer cell line NKL, Cytotherapy 10 (3) (2008) 265–274.
[151] H. Klingemann, Are natural killer cells superior CAR drivers? Oncoimmunology 3 (2014) e28147.
[152] K. Rahman, S.S. Iyer, Costimulatory molecules as vaccine adjuvants: to 4-1BB or not to 4-1BB? Cell. Mol. Immunol. 12 (4) (2015) 508–509.
[153] L. Rodella, L. Zamai, R. Rezzani, M. Artico, G. Peri, M. Falconi, A. Facchini, G. Pelusi, M. Vitale, Interleukin 2 and interleukin 15 differentially predispose natural killer cells to apoptosis mediated by endothelial and tumour cells, Br. J. Haematol. 115 (2) (2001) 442–450.
[154] S.A. Koepsell, J.S. Miller, D.H. McKenna, Natural killer cells: a review of manufacturing and clinical utility, Transfusion (Paris) 53 (2) (2013) 404–410.
[155] H. Fujisaki, H. Kakuda, C. Imai, C.G. Mullighan, D. Campana, Replicative potential of human natural killer cells, Br. J. Haematol. 145 (5) (2009) 606–613.
[156] S.A. Rosenberg, M.T. Lotze, L.M. Muul, S. Leitman, A.E. Chang, S.E. Ettinghausen, Y.L. Matory, J.M. Skibber, E. Shiloni, J.T. Vetto, C.A. Seipp, C. Simpson, C.M. Reichert, Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer, N. Engl. J. Med. 313 (23) (1985) 1485–1492.
[157] E. Ishikawa, K. Tsuboi, K. Saijo, H. Harada, S. Takano, T. Nose, T. Ohno, Autologous natural killer cell therapy for human recurrent malignant glioma, Anticancer Res. 24 (3B) (2004) 1861–1871.
[158] M.R. Parkhurst, J.P. Riley, M.E. Dudley, S.A. Rosenberg, 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. 17 (19) (2011) 6287–6297.
[159] M. Morvan, G. David, V. Sebille, A. Perrin, K. Gagne, C. Willem, N. Kerdudou, L. Denis, B. Clemenceau, G. Follea, J.-D. Bignon, C. Retiere, Autologous and allogeneic HLA KIR ligand environments and activating KIR control KIR NK-cell functions, Eur. J. Immunol. 38 (12) (2008) 3474–3486.
[160] P. Carrega, G. Pezzino, P. Queirolo, I. Bonaccorsi, M. Falco, G. Vita, D. Pende, A. Misefari, A. Moretta, M.C. Mingari, L. Moretta, G. Ferlazzo, Susceptibility of human melanoma cells to autologous natural killer (NK) cell killing: HLA-related effector mechanisms and role of unlicensed NK cells, PLoS One 4 (12) (2009).
[161] B. Mundy-Bosse, M. Kathleen, C. Mao, E. Ahmed, L. Chen, S.D. Scoville, A.G. Freud, J. Yu, M.A. Caligiuri, Acute myeloid leukemia alters natural killer cell maturation and functional activation, Blood 124 (21) (2014).
[162] A. Lundqvist, M. Berg, A. Smith, R.W. Childs, Bortezomib treatment to potentiate the anti-tumor immunity of ex-vivo expanded adoptively infused autologous natural killer cells, J. Cancer 2 (2011) 383–385.
[163] T. Krneta, A. Gillgrass, M. Chew, A.A. Ashkar, The breast tumor microenvironment alters the phenotype and function of natural killer cells, Cell. Mol. Immunol. 13 (5) (2016) 628–639.
[164] L. Moretta, F. Locatelli, D. Pende, E. Marcenaro, M.C. Mingari, A. Moretta, Killer Ig-like receptor-mediated control of natural killer cell alloreactivity in haploidentical hematopoietic stem cell transplantation, Blood 117 (3) (2011) 764–771.
[165] J.S. Miller, Y. Soignier, A. Panoskaltsis-Mortari, S.A. McNearney, G.H. Yun, S.K. Fautsch, D. McKenna, C. Le, T.E. Defor, L.J. Burns, P.J. Orchard, B.R. Blazar, J.E. Wagner, A. Slungaard, D.J. Weisdorf, I.J. Okazaki, P.B. McGlave, Successful adoptive transfer and in vivo expansion of human haploidentical NK cells in patients with cancer, Blood 105 (8) (2005) 3051–3057.
[166] J.P. Veluchamy, A.M. Heeren, J. Spanholtz, J.D.H. van Eendenburg, D.A.M. Heideman, G.G. Kenter, H.M. Verheul, H.J. van der Vliet, E.S. Jordanova, T.D. de Gruijl, High-efficiency lysis of cervical cancer by allogeneic NK cells derived from umbilical cord progenitors is independent of HLA status, Cancer Immunol. Immunother. 66 (1) (2017) 51–61.
[167] A. Curti, L. Ruggeri, A. D'Addio, A. Bontadini, E. Dan, M.R. Motta, S. Trabanelli, V. Giudice, E. Urbani, G. Martinelli, S. Paolini, F. Fruet, A. Isidori, S. Parisi, G. Bandini, M. Baccarani, A. Velardi, R.M. Lemoli, Successful transfer of alloreactive haploidentical KIR ligand-mismatched natural killer cells after infusion in elderly high risk acute myeloid leukemia patients, Blood 118 (12) (2011) 3273–3279.
[168] J.E. Rubnitz, H. Inaba, R.C. Ribeiro, S. Pounds, B. Rooney, T. Bell, C.H. Pui, W. Leung, NKAML: a pilot study to determine the safety and feasibility of haploidentical natural killer cell transplantation in childhood acute myeloid leukemia, J. Clin. Oncol. 28 (6) (2010) 955–959.
[169] J.S. Miller, S. Cooley, P. Parham, S.S. Farag, M.R. Verneris, K.L. McQueen, L.A. Guethlein, E.A. Trachtenberg, M. Haagenson, M.M. Horowitz, J.P. Klein, D.J. Weisdorf, Missing KIR ligands are associated with less relapse and increased graft-versus-host disease (GVHD) following unrelated donor allogeneic HCT, Blood 109 (11) (2007) 5058–5061.
[170] M. Alvarez, W. Murphy, Combination of IL-2 and anti-TGF-beta increases natural killer cell reconstitution after hematopoietic stem cell transplantation, J. Immunol. 186 (2011).
[171] R.T. Costello, S. Sivori, E. Marcenaro, M. Lafage-Pochitaloff, M.J. Mozziconacci, D. Reviron, J.A. Gastaut, D. Pende, D. Olive, A. Moretta, Defective expression and function of natural killer cell-triggering receptors in patients with acute myeloid leukemia, Blood 99 (10) (2002) 3661–3667.
[172] E. Alici, T. Sutlu, B. Boejrkstrand, M. Gilljam, B. Stellan, H. Nahi, H.C. Quezada, G. Gahrton, H.-G. Ljunggren, M.S. Dilber, Autologous antitumor activity by NK cells expanded from myeloma patients using GMP-compliant components, Blood 111 (6) (2008) 3155–3162.
[173] G. Suck, Y.C. Linn, T. Tonn, Natural killer cells for therapy of leukemia, Transfus. Med. Hemother. 43 (2) (2016) 89–95.
[174] M.I.G. Raaijmakers, S. Rozati, S.M. Goldinger, D.S. Widmer, R. Dummer, M.P. Levesque, Melanoma immunotherapy: historical precedents, recent successes and future prospects, Immunotherapy-Uk 5 (2) (2013) 169–182.
[175] T. Kageshita, Z.G. Wang, L. Calorini, A. Yoshii, T. Kimura, T. Ono, S. Gattonicelli, S. Ferrone, Selective loss of human-leukocyte class-I allospecificities and staining of melanoma-cells by monoclonal-antibodies recognizing monomorphic determinants of class-I human-leukocyte antigens, Cancer Res. 53 (14) (1993) 3349–3354.
[176] G. Pietra, C. Manzini, S. Rivara, M. Vitale, C. Cantoni, A. Petretto, M. Balsamo, R. Conte, R. Benelli, S. Minghelli, N. Solari, M. Gualco, P. Queirolo, L. Moretta, M.C. Mingari, Melanoma cells inhibit natural killer cell function by modulating the expression of activating receptors and cytolytic activity, Cancer Res. 72 (6) (2012) 1407–1415.
[177] M.A. Geller, S. Cooley, P.L. Judson, R. Ghebre, L.F. Carson, P.A. Argenta, A.L. Jonson, A. Panoskaltsis-Mortari, J. Curtsinger, D. McKenna, K. Dusenbery, R. Bliss, L.S. Downs, J.S. Miller, A phase II study of allogeneic natural killer cell therapy to treat patients with recurrent ovarian and breast cancer, Cytotherapy 13 (1) (2011) 98–107.
[178] E.G. Iliopoulou, P. Kountourakis, M.V. Karamouzis, D. Doufexis, A. Ardavanis, C.N. Baxevanis, G. Rigatos, M. Papamichail, S.A. Perez, A phase I trial of adoptive transfer of allogeneic natural killer cells in patients with advanced non-small cell lung cancer, Cancer Immunol. Immunother. 59 (12) (2010) 1781–1789.
[179] A.M. James, A.D. Cohen, K.S. Campbell, Combination immune therapies to enhance anti-tumor responses by NK cells, Front. Immunol. 4 (2013).
[180] J.H. Fine, P. Chen, A. Mesci, D.S.J. Allan, S. Gasser, D.H. Raulet, J.R. Carlyle, Chemotherapy-induced genotoxic stress promotes sensitivity to natural killer cell cytotoxicity by enabling missing-self recognition, Cancer Res. 70 (18) (2010) 7102–7113.
[181] V. Bachanova, L.J. Burns, D.H. McKenna, J. Curtsinger, A. Panoskaltsis-Mortari, B.R. Lindgren, S. Cooley, D. Weisdorf, J.S. Miller, Allogeneic natural killer cells for refractory lymphoma, Cancer Immunol. Immunother. 59 (11) (2010) 1739–1744.
[182] V. Bachanova, S. Cooley, T.E. Defor, M.R. Verneris, B. Zhang, D.H. McKenna, J. Curtsinger, A. Panoskaltsis-Mortari, D. Lewis, K. Hippen, P. McGlave, D.J. Weisdorf, B.R. Blazar, J.S. Miller, Clearance of acute myeloid leukemia by haploidentical natural killer cells is improved using IL-2 diphtheria toxin fusion protein, Blood 123 (25) (2014) 3855–3863.
[183] I.G.H. Schmidtwolf, P. Lefterova, V. Johnston, D. Huhn, K.G. Blume, R.S. Negrin, Propagation of large numbers of T-Cells with natural-killer-cell markers, Br. J. Haematol. 87 (3) (1994) 453–458.
[184] J.H. Phillips, L.L. Lanier, Dissection of the lymphokine-activated killer phenomenon—relative contribution of peripheral-blood natural-killer-cells and lymphocytes-T to cytolysis, J. Exp. Med. 164 (3) (1986) 814–825.
[185] G.D. SchmidtWolf, R.S. Negrin, I.G.H. SchmidtWolf, Activated T cells and cytokine-induced CD3(+)CD56(+) killer cells, Ann. Hematol. 74 (2) (1997) 51–56.
[186] C.J. Denman, V.V. Senyukov, S.S. Somanchi, P.V. Phatarpekar, L.M. Kopp, J.L. Johnson, H. Singh, L. Hurton, S.N. Maiti, M.H. Huls, R.E. Champlin, L.J. Cooper, D.A. Lee, Membrane-bound IL-21 promotes sustained ex vivo proliferation of human natural killer cells, PLoS One 7 (1) (2012) e30264.
[187] S.S. Somanchi, A. Somanchi, L.J. Cooper, D.A. Lee, Engineering lymph node homing of ex vivo-expanded human natural killer cells via trogocytosis of the chemokine receptor CCR7, Blood 119 (22) (2012) 5164–5172.
[188] J. Ayello, J. Hochberg, A. Flower, Y. Chu, L.V. Baxi, W. Quish, C. van de Ven, M.S. Cairo, Genetically re-engineered K562 cells significantly expand and functionally activate cord blood natural killer cells: potential for adoptive cellular immunotherapy, Exp. Hematol. 46 (2017) 38–47.
[189] N. Shah, B. Martin-Antonio, H. Yang, S. Ku, D.A. Lee, L.J. Cooper, W.K. Decker, S. Li, S.N. Robinson, T. Sekine, S. Parmar, J. Gribben, M. Wang, K. Rezvani, E. Yvon, A. Najjar, J. Burks, I. Kaur, R.E. Champlin, C.M. Bollard, E.J. Shpall, Antigen presenting cell-mediated expansion of human umbilical cord blood yields log-scale expansion of natural killer cells with anti-myeloma activity, PLoS One 8 (10) (2013) e76781.
[190] J. Cany, A.B. van der Waart, M. Tordoir, G.M. Franssen, B.N. Hangalapura, J. de Vries, O. Boerman, N. Schaap, R. van der Voort, J. Spanholtz, H. Dolstra, Natural killer cells generated from cord blood hematopoietic progenitor cells efficiently target bone marrow-residing human leukemia cells in NOD/SCID/IL2Rg(null) mice, PLoS One 8 (6) (2013).
[191] J. Spanholtz, F. Preijers, M. Tordoir, C. Trilsbeek, J. Paardekooper, T. de Witte, N. Schaap, H. Dolstra, Clinical-grade generation of active NK cells from cord blood

hematopoietic progenitor cells for immunotherapy using a closed-system culture process, PLoS One 6 (6) (2011) e20740.
[192] J. Tanaka, J. Sugita, S. Shiratori, A. Shigematu, S. Asanuma, K. Fujimoto, M. Nishio, T. Kondo, M. Imamura, Expansion of NK cells from cord blood with antileukemic activity using GMP-compliant substances without feeder cells, Leukemia 26 (5) (2012) 1149–1152.
[193] J.-i. Masuyama, T. Murakami, S. Iwamoto, S. Fujita, Ex vivo expansion of natural killer cells from human peripheral blood mononuclear cells co-stimulated with anti-CD3 and anti-CD52 monoclonal antibodies, Cytotherapy 18 (1) (2016) 80–90.
[194] J.L. Oyer, R.Y. Igarashi, A.R. Kulikowski, D.A. Colosimo, M.M. Solh, A. Zakari, Y.A. Khaled, D.A. Altomare, A.J. Copik, Generation of highly cytotoxic natural killer cells for treatment of acute myelogenous leukemia using a feeder-free, particle-based approach, Biol. Blood Marrow Transplant. 21 (4) (2015) 632–639.
[195] T.K. Garg, S.M. Szmania, J.A. Khan, A. Hoering, P.A. Malbrough, A. Moreno-Bost, A.D. Greenway, J.D. Lingo, X. Li, S. Yaccoby, L.J. Suva, B. Storrie, G. Tricot, D. Campana, J.D. Shaughnessy Jr., B.P. Nair, W.T. Bellamy, J. Epstein, B. Barlogie, F. van Rhee, Highly activated and expanded natural killer cells for multiple myeloma immunotherapy, Haematologica 97 (9) (2012) 1348–1356.
[196] H. Zhang, Y. Cui, N. Voong, M. Sabatino, D.F. Stroncek, S. Morisot, C.I. Civin, A.S. Wayne, B.L. Levine, C.L. Mackall, Activating signals dominate inhibitory signals in CD137L/IL-15 activated natural killer cells, J. Immunother. 34 (2) (2011) 187–195.
[197] Y.H. Chang, J. Connolly, N. Shimasaki, K. Mimura, K. Kono, D. Campana, A chimeric receptor with NKG2D specificity enhances natural killer cell activation and killing of tumor cells, Cancer Res. 73 (6) (2013) 1777–1786.
[198] R. Esser, T. Muller, D. Stefes, S. Kloess, D. Seidel, S.D. Gillies, C. Aperlo-Iffland, J.S. Huston, C. Uherek, K. Schonfeld, T. Tonn, N. Huebener, H.N. Lode, U. Koehl, W.S. Wels, NK cells engineered to express a GD2 −specific antigen receptor display built-in ADCC-like activity against tumour cells of neuroectodermal origin, J. Cell. Mol. Med. 16 (3) (2012) 569–581.
[199] B. Altvater, S. Landmeier, S. Pscherer, J. Temme, K. Schweer, S. Kailayangiri, D. Campana, H. Juergens, M. Pule, C. Rossig, 2B4 (CD244) signaling by recombinant antigen-specific chimeric receptors costimulates natural killer cell activation to leukemia and neuroblastoma cells, Clin. Cancer Res. 15 (15) (2009) 4857–4866.
[200] L. Boissel, M. Betancur-Boissel, W. Lu, D.S. Krause, R.A. Van Etten, W.S. Wels, H. Klingemann, Retargeting NK-92 cells by means of CD19- and CD20-specific chimeric antigen receptors compares favorably with antibody-dependent cellular cytotoxicity, Oncoimmunology 2 (10) (2013) e26527.
[201] L. Boissel, M. Betancur, W. Lu, W.S. Wels, T. Marino, R.A. Van Etten, H. Klingemann, Comparison of mRNA and lentiviral based transfection of natural killer cells with chimeric antigen receptors recognizing lymphoid antigens, Leuk. Lymphoma 53 (5) (2012) 958–965.
[202] L. Boissel, M. Betancura, W.S. Wels, H. Tuncer, H. Klingemann, Transfection with mRNA for CD19 specific chimeric antigen receptor restores NK cell mediated killing of CLL cells, Leuk. Res. 33 (9) (2009) 1255–1259.
[203] A. Romanski, C. Uherek, G. Bug, E. Seifried, H. Klingemann, W.S. Wels, O.G. Ottmann, T. Tonn, CD19-CAR engineered NK-92 cells are sufficient to overcome NK cell resistance in B-cell malignancies, J. Cell. Mol. Med. 20 (7) (2016) 1287–1294.
[204] N. Shimasaki, H. Fujisaki, D. Cho, M. Masselli, T. Lockey, P. Eldridge, W. Leung, D. Campana, A clinically adaptable method to enhance the cytotoxicity of natural killer cells against B-cell malignancies, Cytotherapy 14 (7) (2012) 830–840.
[205] T. Muller, C. Uherek, G. Maki, K.U. Chow, A. Schimpf, H.G. Klingemann, T. Tonn, W.S. Wels, Expression of a CD20-specific chimeric antigen receptor enhances cytotoxic activity of NK cells and overcomes NK-resistance of lymphoma and leukemia cells, Cancer Immunol. Immunother. 57 (3) (2008) 411–423.
[206] Y. Chu, J. Hochberg, A. Yahr, J. Ayello, C. van de Ven, M. Barth, M. Czuczman, M.S. Cairo, Targeting CD20(+) aggressive B-cell non-Hodgkin lymphoma by anti-CD20 CAR mRNA-modified expanded natural killer cells in vitro and in NSG mice, Cancer Immunol. Res. 3 (4) (2015) 333–344.
[207] J. Chu, Y. Deng, D.M. Benson, S. He, T. Hughes, J. Zhang, Y. Peng, H. Mao, L. Yi, K. Ghoshal, X. He, S.M. Devine, X. Zhang, M.A. Caligiuri, C.C. Hofmeister, J. Yu, CS1-specific chimeric antigen receptor (CAR)-engineered natural killer cells enhance in vitro and in vivo antitumor activity against human multiple myeloma, Leukemia 28 (4) (2014) 917–927.
[208] H. Jiang, W. Zhang, P. Shang, H. Zhang, W. Fu, F. Ye, T. Zeng, H. Huang, X. Zhang, W. Sun, D.M.-Y. Sze, Q. Yi, J. Hou, Transfection of chimeric anti-CD 138 gene enhances natural killer cell activation and killing of multiple myeloma cells, Mol. Oncol. 8 (2) (2014) 297–310.
[209] D.V. Tassev, M. Cheng, N.K. Cheung, Retargeting NK92 cells using an HLA-A2-restricted, EBNA3C-specific chimeric antigen receptor, Cancer Gene Ther. 19 (2) (2012) 84–100.
[210] G. Zhang, R. Liu, X. Zhu, L. Wang, J. Ma, H. Han, X. Wang, G. Zhang, W. He, W. Wang, C. Liu, S. Li, M. Sun, B. Gao, Retargeting NK-92 for anti-melanoma activity by a TCR-like single-domain antibody, Immunol. Cell Biol. 91 (10) (2013) 615–624.
[211] C. Sahm, K. Schonfeld, W.S. Wels, Expression of IL-15 in NK cells results in rapid enrichment and selective cytotoxicity of gene-modified effectors that carry a tumor-specific antigen receptor, Cancer Immunol. Immunother. 61 (9) (2012) 1451–1461.
[212] A. Kruschinski, A. Moosmann, I. Poschke, H. Norell, M. Chmielewski, B. Seliger, R. Kiessling, T. Blankenstein, H. Abken, J. Charo, Engineering antigen-specific primary human NK cells against HER-2 positive carcinomas, Proc. Natl. Acad. Sci. U. S. A. 105 (45) (2008) 17481–17486.
[213] K. Schonfeld, C. Sahm, C. Zhang, S. Naundorf, C. Brendel, M. Odendahl, P. Nowakowska, H. Bonig, U. Kohl, S. Kloess, S. Kohler, H. Holtgreve-Grez, A. Jauch, M. Schmidt, R. Schubert, K. Kuhlcke, E. Seifried, H.G. Klingemann, M.A. Rieger, T. Tonn, M. Grez, W.S. Wels, Selective inhibition of tumor growth by clonal NK cells expressing an ErbB2/HER2-specific chimeric antigen receptor, Mol. Ther. 23 (2) (2015) 330–338.
[214] X. Chen, J. Han, J. Chu, L. Zhang, J. Zhang, C. Chen, L. Chen, Y. Wang, H. Wang, L. Yi, J.B. Elder, Q.-E. Wang, X. He, B. Kaur, E.A. Chiocca, J. Yu, A combinational therapy of EGFR-CAR NK cells and oncolytic herpes simplex virus 1 for breast cancer brain metastases, Oncotarget 7 (19) (2016) 27764–27777.
[215] J. Han, J. Chu, W.K. Chan, J. Zhang, Y. Wang, J.B. Cohen, A. Victor, W.H. Meisen, S.-h. Kim, P. Grandi, Q.-E. Wang, X. He, I. Nakano, E.A. Chiocca, J.C. Glorioso III, B. Kaur, M.A. Caligiuri, J. Yu, CAR-engineered NK cells targeting wild-type EGFR and EGFRvIII enhance killing of glioblastoma and patient-derived glioblastoma stem cells, Sci. Rep. 5 (2015).
[216] P.D. Kottaridis, J. North, M. Tsirogianni, C. Marden, E.R. Samuel, S. Jide-Banwo, S. Grace, M.W. Lowdell, Two-stage priming of allogeneic natural killer cells for the treatment of patients with acute myeloid leukemia: a phase I trial, PLoS One 10 (6) (2015) e0123416.
[217] H. Klingemann, C. Grodman, E. Cutler, M. Duque, D. Kadidlo, A.K. Klein, K.A. Sprague, K.B. Miller, R.L. Comenzo, T. Kewalramani, N. Yu, R.A. Van Etten, D.H. McKenna, Autologous stem cell transplant recipients tolerate haploidentical related-donor natural killer cell-enriched infusions, Transfusion (Paris) 53 (2) (2013) 412–418 (quiz 411).
[218] A. Perez-Martinez, L. Fernandez, J. Valentin, I. Martinez-Romera, M.D. Corral, M. Ramirez, L. Abad, S. Santamaria, M. Gonzalez-Vicent, S. Sirvent, J. Sevilla, J.L. Vicario, I. de Prada, M.A. Diaz, 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 17 (11) (2015) 1594–1603.