



Review Article



Harnessing Natural Killer Cell-Mediated Innate Immune Responses for Cancer Treatment: Advances and Challenges

Anil Kumar¹, Adeleh Taghi Khani¹ and Srividya Swaminathan^{1,2*}

¹Department of Systems Biology, Beckman Research Institute of City of Hope, Monrovia CA, USA; ²Department of Hematological Malignancies, Beckman Research Institute of City of Hope, Monrovia CA, USA

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Abstract

Natural killer (NK) cells are a gatekeeper of the body's innate defense system against cancers and infections. A growing body of literature from us and others finds that NK cells promote anti-cancer immune surveillance, and that defects in NK cell development are associated with poor clinical prognosis of cancers. In preclinical studies, NK cells were found to drive tumor regression and delay tumor relapse. Because NK cells are potentially less damaging to the body and are easier to develop than T cell-based therapies, efforts are being made to improve NK cell cytotoxicity and *in vivo* persistence for use as an adoptive, off-the-shelf immunotherapy. In this review, we discuss how tumor-intrinsic and -extrinsic factors suppress NK cells in the cancer microenvironment. We also outline current strategies that restore NK surveillance in cancer and challenges facing the clinical use of NK cell-based therapies.

Natural killer cells form the first line of defense against cancer

Natural killer (NK) cells are innate lymphoid cells which account for 5–15% of cells in peripheral blood. NK cells develop in the

bone marrow and then undergo maturation to acquire different effector functions. During maturation, NK cells express chemokines and adhesion receptors which facilitate their migration from the bone marrow via the blood to different lymphoid and non-lymphoid tissues where they ultimately localize. NK cells are detected in lymphoid tissues including spleen, bone marrow, lymph nodes, and thymus and non-lymphoid tissues, including blood, lungs, liver, colon and uterus.^{1,2} The distribution of NK cells is not static and changes in response to myriad chemokines whose secretion is triggered by infection and malignancy.³

NK cells defend against pathogens and tumorigenesis by killing the infected or malignant cells in a sequence of steps. NK cells recognize target cells with absent or low major histocompatibility complex-I (MHC-I) expression.^{4–6} Activation of circulating or localized resting NK cells in tissues is triggered by an imbalance in the activation signals (natural killer cell p46 related protein (NKp46), natural killer group 2D (NKG2D), cluster of differentiation (CD) 16, and lymphocyte-function associated antigen-1, *etc.*) and inhibitory signals (NKG2A, killer cell lectin-like receptor G1, and T-cell immunoreceptor with immunoglobulin and ITIM domain (TIGIT), *etc.*) that originate from abnormal cells and the microenvironment.⁷ In addition to activating and inhibitory receptors, co-stimulatory receptors or adhesion molecules (2B4, CD27, CD28, CD226 *etc.*)^{8–11} can induce NK cell function. NK cells induce apoptosis of the target cell via tumor necrosis factor (TNF)-related apoptosis-inducing ligand and Fas ligand, and by directly releasing lytic granules such as perforin and granzyme-B.^{12,13} Another mechanism of NK-mediated killing is antibody-dependent

Keywords: Natural killer cells; Cancer; Tumor microenvironment; NK cell-based therapies.

Abbreviations: ADAM, a disintegrin and metalloproteinase; ADCC, antibody dependent cell-mediated cytotoxicity; ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia; BiKE, bi-specific natural killer cell engager; CAR, chimeric antigen receptor; CB, cord blood; CD, cluster of differentiation; CRISPR/cas9, clustered regularly interspaced short palindromic repeats/crispr associated protein 9; DC, dendritic cell; DNAM-1, DNAX accessory molecule-1; EBV, Epstein-Barr virus; GVHD, graft versus host disease; HLA-E, human leukocyte antigen class I alpha chain E; HPV, human papilloma virus; IFN, interferon; IFN- γ , type-I interferon; IL, interleukin; iPSC, induced pluripotent stem cell; KIR, killer immunoglobulin-like receptor; mAb, monoclonal antibody; MDSC, myeloid-derived suppressor cells; MHC-I, major histocompatibility class-I; MICA/B, MHC class-I chain related protein A/B; MM, multiple myeloma; NK, natural killer; NKG2D, natural killer group 2D; NKp46, natural killer cell related protein 46; PD-1, programmed cell death protein 1; PDC, plasmacytoid dendritic cell; PGE-2, prostaglandin E2; ScFv, single chain variable fragment; TGF- β , transforming growth factor-beta; Th1, T helper 1; TIGIT, T-cell immunoglobulin and ITIM domain; TME, tumor microenvironment; TriKE, tri-specific natural killer cell engager.

*Correspondence to: Srividya Swaminathan, Department of Systems Biology, Beckman Research Institute of City of Hope, 1218 S. Fifth Ave, Monrovia CA 91016, USA. ORCID: <https://orcid.org/0000-0002-3459-7488>. Tel: +1 626-218-5627, Fax: +1 626-218-7207, E-mail: sswaminathan@coh.org

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cell-mediated cytotoxicity (ADCC) where the Fc receptor CD16 (FcγRIIIa) on NK cells binds to antibody coated target cells and lyses them.¹⁴

Additionally, NK cells release cytokines and chemokines that activate surrounding immune cells.¹⁵ Dendritic cells (DC), plasmacytoid dendritic cells (PDC), mast cells, basophils, eosinophils, and neutrophils are shown to network with NK cells at the site of the inflammation. Crosstalk between NK cells and DCs generates interleukin (IL)-15, IL-12, IL-23, IL-27, and IL-18, which in turn further activates NK cells and interferon gamma (IFN-γ) production. IFN-γ produced by NK cells promotes DC activation and induces T helper 1 (Th1) polarization and CD8⁺ T cell-mediated memory responses.^{16–19}

Studies in humans and in experimental mouse models demonstrated the importance of NK cells in protecting against virus-induced tumorigenesis.²⁰ In virus-induced mouse models of prostate adenocarcinoma and lymphomas, cancer aggressiveness was shown to be positively associated with NKG2D deficiency.²¹ Tumors are caused by the ubiquitous murine polyomavirus in immunocompromised hosts.²² In immunocompetent hosts, NK cells and γδ T cells have been shown to delay murine polyomavirus-induced tumor formation.²³ Even in humans, NK cells resist the development of virus-induced tumors such as those driven by human papilloma virus, Epstein-Barr virus (EBV), Human T-cell leukemia virus, Kaposi sarcoma virus, Hepatitis B virus, Hepatitis C virus and Merkel cell polyomavirus.²²

Despite being innate immune cells, more recently, NK cells were shown to have immunological memory, a feature of adaptive immune cells. NK memory responses were observed in mice after infection, allergic reactions, and vaccination.^{24–26} Because NK cells are cytotoxic lymphocytes with immunological memory, they are attractive candidates for cell-based immunotherapy. Moreover, unlike B and T cells, NK cells do not cause graft-versus-host disease (GvHD) making them a potentially safe, off-the-shelf, living anticancer drug.

Mechanisms of suppression of NK surveillance in cancer

In murine tumor models, NK cells resist tumorigenesis and clear primary as well as metastatic tumor cells.^{27–29} The first evidence that established the importance of NK cells in anticancer immune surveillance came from a methylcholanthrene-induced fibrosarcoma mouse model in which depletion of NK cells resulted in faster tumor growth.³⁰ Due to their antitumorigenic effect, NK cells are actively suppressed in cancers. Therefore, to harness the full potential of NK cells, it is crucial to delineate the mechanisms of suppression of NK cell-mediated immune surveillance in cancers and develop therapeutic strategies to reverse this suppression.

In cancer patients, we and others have found NK surveillance to be suppressed by numerous mechanisms: NK cell numbers are reduced in the tumor microenvironment,³¹ NK cytotoxicity is reduced,^{32–35} tumor cells are rendered resistant to NK cell-mediated lysis,^{36,37} NK cells from other sites fail to infiltrate tumor tissue,³⁸ NK cell-mediated ADCC is defective,³⁹ other NK-interacting immune subsets such as DCs and T cells are suppressed in function,⁴⁰ and NK cells get exhausted due to hyperactivation.³²

In mouse models and patients with cancer, we and others have observed that reduction in NK cell numbers is caused by defective NK cell homeostasis, including perturbed cycling and maturation.^{32,33,41–43} Specifically, in patients with B- and T-cell acute lymphoblastic leukemia (ALL), we observed significantly increased frequencies of immature and poorly cytotoxic NK cells and a

concomitant reduction in the frequencies of functional cytotoxic NK cells.³² Similarly, in mouse models of solid tumors, including hepatocellular and breast cancer, others have observed a reduction in peripheral and tumor-infiltrating mature NK cells required for ADCC,^{33,44} and an increase in the expression of inhibitory receptors including CD85j on tumor cells that block ADCC.³⁹ Defective NK cell maturation impairs NK cell-mediated lysis of tumor cells because immature NK cells exhibit reduced expression of activation receptors such as DNAX accessory molecule-1 (DNAM-1), NKG2D, CD16, and NKp30, and increased expression of inhibitory receptors such as NKG2A.^{41,44–47}

In addition to defects in NK cells themselves, the resistance of tumor cells to NK cell-mediated cytotoxicity also abrogates anti-cancer NK surveillance. For example, cancer cells shed the NKG2D ligands and major histocompatibility complex class I chain-related proteins A and B (MICA/B), thus impairing NK cell activation.^{48,49} Shedding of these ligands is induced by endoplasmic reticulum protein 5-mediated cleavage by a disintegrin associated metalloproteinase (ADAM)10 or ADAM17 proteases, which are upregulated in cancers.⁵⁰ The restoration of NK surveillance at least partly explains why inhibitors targeting these proteases have shown anti-cancer efficacy in preclinical studies.⁵¹ Impaired binding of perforin to the tumor cell surface can also prevent NK cell-mediated killing of cancer cells.³⁶ Additionally, shedding of the target antigen can induce resistance to NK cell-based immunotherapies as is observed in the case of chimeric antigen receptor (CAR)-T cells.⁵²

Other mechanisms of NK suppression include exhaustion due to prolonged NK cell hyperactivation.^{32,53} Such hyperactivation-induced NK cell exhaustion can be countered using checkpoint blockade.^{54,55}

Finally, the above-described defective NK cell homeostasis can impact the anti-tumor immune surveillance by other immune cells. For example, migration of dendritic cells (DCs) to the tumor microenvironment (TME) is impacted when NK cells fail to produce chemokines, including C-C motif chemokine ligand 5 and X-C motif chemokine ligands 1 and 2 due to an increase in tumor-derived prostaglandin E2 (PGE2).⁵⁶ Other studies have shown that the production of the Fms-like tyrosine kinase 3 ligand cytokine by NK cells positively regulates DC abundance in the TME, improves response to checkpoint blockade, and leads to improved overall survival.⁵⁷ In a pancreatic ductal adenocarcinoma murine model, the Fms-like tyrosine kinase 3 ligand reduced frequencies of pathogenic Th17 cells and increased the numbers and effector functions of cytotoxic CD8⁺ T cells.⁵⁸ Therefore, defective NK surveillance in cancers can have far-reaching impacts on global anti-cancer host immunity.

Tumor-induced suppression of anti-cancer NK surveillance

The increased suppression of NK surveillance at a higher stage of the cancer and size of the tumor^{59,60} strongly suggests that defects in NK cell-mediated immune surveillance of cancers are triggered by signaling mechanisms from within the tumor. For example, tumor-derived soluble factors such as PGE2, indoleamine 2,3-dioxygenase, transforming growth factor-beta (TGF-β), BCL-2 associated athanogene cochaperone 6, and programmed cell death protein ligand 1 bind to NK activating receptors, thereby blocking their target cell-binding site and tilting the balance towards NK cell inhibitory response.^{44,61–64} Tumor-derived TGFβ1 downregulates NKp30 and NKG2D surface expression in human NK cells.⁶⁴ Tryptophan catabolism mediated by indoleamine 2,3-dioxygenase inhibits the surface expression of NKp46 and NKG2D activating

receptors.⁶⁵ In virus-induced cancers, viral factors such as EBV encoded microRNA-BART 2-5p, downregulate the NKG2D ligand MICB expression by binding to the 3' untranslated region of MICB, thereby impairing NK cell-mediated clearance of EBV infected cells.⁶⁶ The human papilloma virus encoded oncoproteins E6 and E7 inhibit IL-18 induced IFN- γ production in NK cells and contribute to immune escape of HPV or HPV-induced cervical cancer.⁶⁷ Similarly, human T-cell leukemia virus,⁶⁸ PGE2⁶⁹ and hepatitis A virus⁷⁰ interfere with type-I interferon (IFN-I), which is an essential factor for NK cell homeostasis, activation, and anti-cancer functions.⁷¹

Apart from the tumor secretome, expression of ligands to NK checkpoint molecules including programmed cell death protein ligand 1 on cancer cells has been shown to promote NK cell exhaustion by binding to programmed cell death protein 1 (PD-1) on CD56^{bright}CD16⁻ NK cells.⁷² Other mechanisms of suppression include expression of CD39 on patient breast cancer cells that bind to tumor-infiltrating regulatory NK cells expressing CD73. CD73⁺ NK cells upregulate IL-10 production via signal transducer and activator of transcription-3, and thereby, suppress CD4⁺ T cell proliferation and IFN- γ production resulting in immune tolerance.⁷³ Poliovirus receptor (CD155) is a common ligand for NK activation receptor DNAM-1 and inhibitory receptors T-cell immunoglobulin and ITIM domain (TIGIT) and CD96. CD155 is known to be highly expressed in many human cancers while absent in most healthy tissues.⁷⁴ CD155 expression on tumor cells downregulates DNAM-1 expression on NK cells and interferes with NK cell activation.⁷⁵ Similarly, the human leukocyte antigen (HLA) class I histocompatibility antigen, alpha chain E (HLA-E), a ligand for CD94/NKG2A inhibitory receptor on NK cells, is highly expressed in tumors.⁷⁶ NK cell recognition of HLA-E through CD94/NKG2A protects cancer cells from NK cell-mediated lysis.⁷⁷ Studying the remodeling of the immune microenvironment during primary tumorigenesis in transgenic mouse models of cancer, we and others have shown that overexpression of universal driver oncoproteins including, MYC and RAS lead to a reduction in NK cell numbers within the tumor microenvironment.^{43,78} In a MYC-driven T-cell lymphoblastic leukemia/lymphoma mouse model, we showed that tumor-intrinsic MYC inhibits the early development of NK cells in the bone marrow as well as the maturation of NK cells into cytotoxic effectors in the periphery by suppressing the transcription of a class of NK cell activating cytokines termed IFN-Is. In lung adenocarcinoma, MYC and RAS oncoproteins cooperate to drive the CCL9 and IL-23 mediated expulsion of B-, T- and NK cells. As in our T-ALL/lymphoma model after MYC in activation, deactivation of the driver oncogene MYC in lung adenocarcinoma induces NK cell-mediated tumor regression. The above studies confirm that tumor-driven signaling is the primary driver of NK cell suppression in cancers.

Strategies to restore NK surveillance in cancer

The above outlined severe suppression of NK cells in cancer highlights the importance of developing strategies to restore anti-tumor NK surveillance (Fig. 1). One such strategy is to enhance the functions of the suppressed NK cells in the tumor microenvironment. NK cell functions can be enhanced by modulating the activity of activating and inhibitory receptors, blockade of immune checkpoints and exhaustion markers on NK cells using therapeutic antibodies, administering single and bi-specific antibodies to enhance the ADCC, and engineering NK cells to secrete NK cell growth-promoting cytokines.

Suppression of signaling from NK cell inhibitory receptors,

which bind to tumor cells that have retained or upregulated their MHC-I, has been achieved by blocking the interaction between killer immunoglobulin like receptor and class-I MHC molecules. In preclinical studies, Irlumab or IPH2101 human mAb that binds to the inhibitory receptors killer immunoglobulin-like receptor 2 immunoglobulin domains long cytoplasmic tail-1, -2, and -3 amplified NK cell-mediated cytotoxicity of HLA-C expressing tumor cells, including acute myeloid leukemia (AML) and multiple myeloma (MM) cells while sparing the normal peripheral blood mononuclear cells.⁷⁹⁻⁸¹ Blocking of immune checkpoint molecules such as PD-1, TIGIT, and cytotoxic T lymphocyte associated protein-4 enhance NK cell-mediated tumor control by preventing NK cell exhaustion.⁸²⁻⁸⁴ Monoclonal antibodies such trastuzumab and rituximab, which target epidermal growth factor receptor and CD20, respectively, on tumor cells induce NK cell-mediated ADCC of tumor cells.⁸⁵ The therapeutic efficacy of NK cells can be enhanced by co-administering anti-cancer, but NK cell growth-promoting, cytokines such as IL-2, IL-12, IL-15, IL-21, and Type I IFNs.⁸⁶

Clinical relevance of developing NK cell-based therapies

Clinical development of NK cells was inspired by the ability of NK cells to exert the graft versus leukemia effect in HLA-mismatched hematopoietic stem cell transplants. The Ruggeri group demonstrated that alloreactive NK cells have the potential to eradicate leukemia and its relapse while at the same time protecting the patients from GVHD.^{87,88} Other groups showed that adoptive transfer of haploidentical NK cells combined with a low dose of IL-2 after effective lymphodepletion chemotherapy in patients with AML resulted in increased endogenous IL-15 production that promoted persistence and expansion of NK cells. The fact that haploidentical NK cells can persist and expand *in vivo* further strengthens the notion that they can be used alone or as hematopoietic stem cell transplants to treat blood cancers.⁸⁹

Subsequently, the importance of NK cell-based therapies has been further underscored by numerous findings that an increased degree of NK cell infiltration in the TME, as well as high peripheral blood NK cell counts, predict favorable clinical prognosis.^{43,90-94} Most recently, we found in patients with high-risk B- and T-ALL that, the activation and maturation status of the NK cells in the leukemia microenvironment, rather than the number of NK cells, independently predicts clinical prognosis.³² Specifically, we observed that an increased relative frequency of activated but immature and dysfunctional NK cells is associated with worse prognosis in patients with ALL.³² Our findings further strengthen the premise for developing allogeneic NK cell-based therapies for treating cancers instead of excessively engineering defective autologous NK cells.

Advantages of NK cells over other forms of cell-based immunotherapies

Over the last decade, NK cell-based immunotherapies have gained attention as a safer and off-the-shelf alternative to autologous CAR-T cell-based therapies. This is because, unlike T cells, NK cell functions are not HLA-restricted, they do not exhibit cytokine release syndrome, and have less neurotoxicity than CAR-T cells. Most importantly, allogeneic NK cells do not cause GVHD and have, in fact, been shown to reduce GVHD while concurrently enhancing graft versus tumor effects.^{95,96} These advantages of NK cells have made them particularly attractive as alternatives for

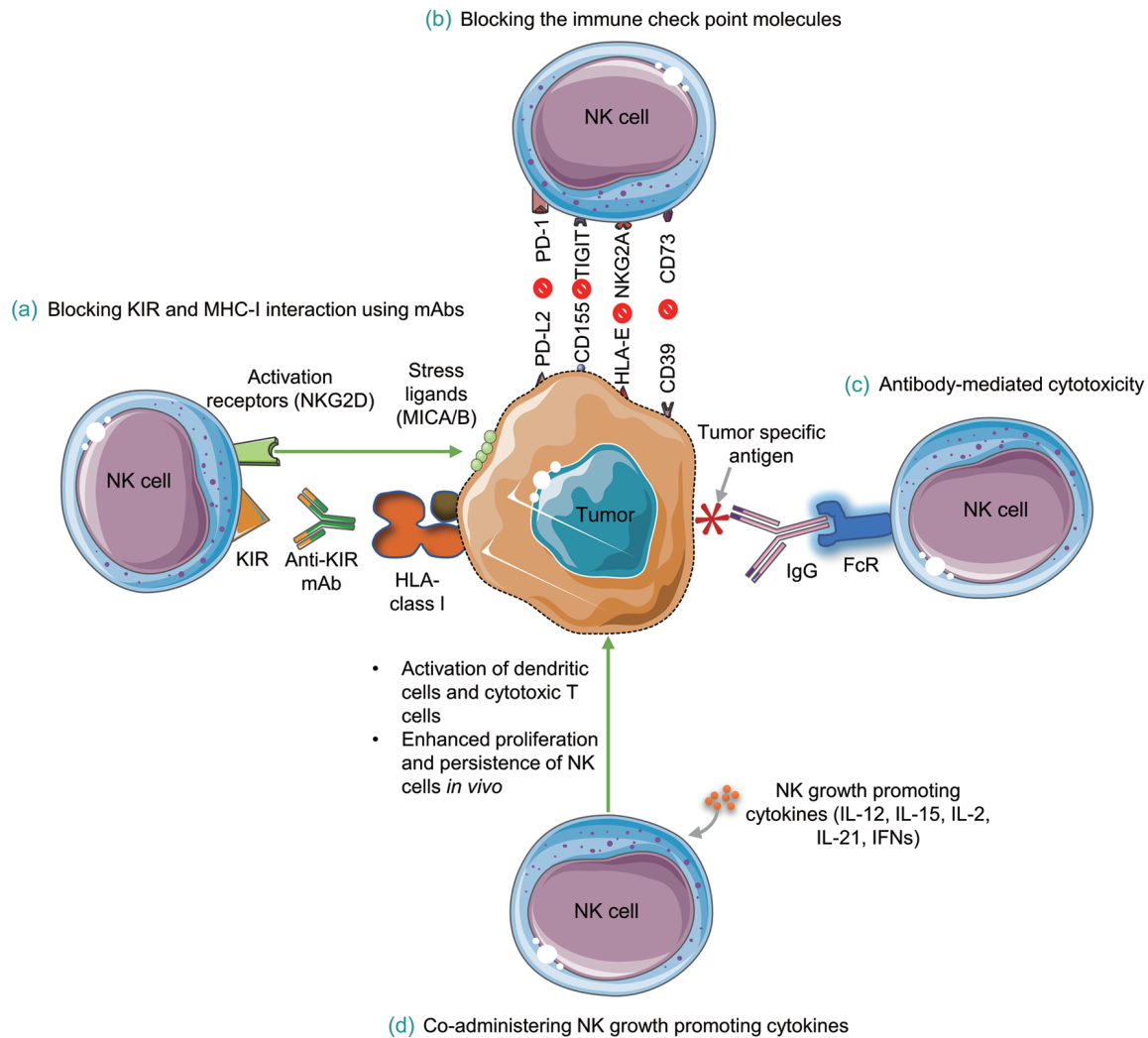


Fig. 1. Strategies employed to enhance anti-cancer Natural Killer surveillance in the clinic. (A) Therapeutic potential of natural killer (NK) cells can be enhanced by blocking the interaction between inhibitory killer immunoglobulin like receptors (KIR) and class-I major histocompatibility class (MHC) molecules using monoclonal antibodies targeting KIRs. (B) Blocking immune checkpoint molecules on NK cells such as, programmed cell death (PD-1), T-cell immunoreceptor with immunoglobulin and ITIM domain (TIGIT), cluster of differentiation (CD) 94/natural killer group NKG2A and CD73 to prevent NK cell exhaustion. (C) Using monoclonal antibodies targeting tumor-associated antigens to enhance antibody-dependent cell-mediated cytotoxicity (ADCC). (D) Co-administering NK cell growth-promoting cytokines such as interleukin (IL)-2, IL-12, IL-15, IL-21, and type I interferons (IFNs) to enhance NK cell proliferation and long-term persistence *in vivo*.

CAR-T cell-based therapies and accelerated the development of CAR-NK cells, described below.

NK cell-based therapies under clinical and pre-clinical development

Chimeric Antigen Receptor NK cells

The design of CAR NK cells is adapted from the design of CAR-T cells, currently used for the treatment of cancers. A CAR consists of three domains: an ectodomain, a transmembrane domain, and a cytoplasmic activation domain.⁹⁷ The ectodomain is a single-chain variable fragment (ScFv) that confers specificity. The ScFv is connected to a spacer (hinge) region to provide flexibility to

the ScFv fragment. CAR spacers are mostly derived from IgG, CD8 α , and CD28. The transmembrane domain links the ectodomain domain to the intracellular activation domain and anchors the receptor to cell membrane. The CD8 α and CD28 based transmembrane domain are used in primary CAR-NK cells whereas CD28 is frequently used for CAR-NK-92 cell lines.^{98,99} The cytoplasmic activation domain, which transduces the signal in response to target antigen binding in CAR-NK cells, is the CD3 receptor ζ -chain. To potentiate intracellular signaling and CAR-NK activation, the signaling domain is often coupled with a co-stimulatory domain such as CD28,¹⁰⁰ 2B4,¹⁰¹ 4-1BB,¹⁰² DAP10,¹⁰³ or DAP12.¹⁰⁴

CAR-NK cell-based therapies can be developed from NK cells derived from varied sources including peripheral blood (PB),^{105,106} cord blood (CB),¹⁰⁷ induced pluripotent stem cells (iPSCs),^{108,109} and NK cell lines.¹¹⁰ NK cells from each source have their pros and cons.¹¹¹ PB and CB NK cells are readily available but difficult

to engineer using conventional lentiviral vector-based transduction methods to introduce the CAR vector.¹¹² For this reason, a majority of the ongoing clinical trials have employed scalable NK cell lines such as the NK-92. However, cell lines such as NK-92 are malignant and have been derived from patients with NK cell neoplasms, thus requiring them to be irradiated before infusion. Such irradiation limits the *in vivo* persistence and effector functions of CAR-NK92 cells and calls for frequent infusions in a single patient.^{113,114} Contrary to PB and CB NK cells, iPSC-derived NK cells are homogenous and can be generated in larger quantities for off-the-shelf therapeutic applications.¹⁰⁹

IL-15 expressing NK cells

IL-15 is required for the development and function of NK cells. Mice deficient in the IL15 receptor alpha lack both functional T and NK cells in the spleen.^{115,116} Therefore, to support the long-term persistence and expansion of NK cells *in vivo*, NK cells have been engineered to express IL-15. CAR NK-92 cells engineered to express membrane-bound IL-15/IL-15 receptor alpha complex demonstrated superior proliferation under *in vitro* conditions.¹¹⁷ IL-15 CAR NK cells targeting epithelial cell adhesion molecule showed selective and increased cytolytic activity against NK-resistant epithelial cell adhesion molecule-expressing breast carcinoma cells.¹¹⁸ CB CD19-CAR-NK cells expressing IL-15 efficiently lysed primary B-ALL cells and cell lines *in vitro* and dramatically prolonged the survival of leukemia/lymphoma-bearing mice.¹⁰⁷ Ectopic expression of IL-15 in NKG2D CAR-NK cells restrained tumor growth and significantly enhanced survival of AML-bearing mice.¹¹⁹ However, a recent study showed that IL-15-secreting CAR-NK cells led to severe inflammation in AML-bearing mice due to the hyperproliferation of NK cells and high levels of proinflammatory cytokines.¹²⁰ Hence, fine-tuning of IL-15-expressing CAR-NK cells to reduce its systemic toxicity is required.

Gene-edited NK cell-based therapies

The clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated protein 9 (Cas9) gene-editing technology provides versatile and efficient gene manipulation capabilities to modulate various regulatory pathways in NK cells and fine-tune NK cell effector functions. Recently, Pomeroy *et al.*, successfully knocked out ADAM17 and programmed cell death protein D1 genes in primary human NK cells by CRISPR/Cas9 methods and demonstrated that these genetically edited NK cells induced significantly more cytotoxicity of cancer cells as compared to their unmodified counterparts.¹²¹ Cas9-mediated promoter insertion to reactivate the silenced genes CD16 and DNAM-1 (CD226) in NK-92 cells significantly improved their cytotoxicity against cancer cell lines *in vitro*.¹²² Our group recently pioneered the generation of stable CRISPR/Cas9, dCas9-KRAB and dCas9-VP64 NK-92 cell line platforms that allow knockout, transcriptional repression, and transcriptional activation of genes, respectively, in NK cells. Our platforms can be seamlessly modified for off-the-shelf therapeutic applications. We predict that our CRISPR-engineered NK cell lines will allow the fine tuning of effector functions of NK cells while developing them as therapies.¹²³

Combination of NK cells with other therapies, checkpoint inhibitors, and CAR-T cells

The therapeutic benefits of combining NK cell monotherapy with

other modalities of cancer treatment are currently being tested. For example, NK cells in conjunction with chemotherapy improved the clinical outcome in colon carcinoma patients.¹²⁴ In preclinical studies, CAR-NK cells combined with a low dose of CAR-T cells delayed tumor progression and enhanced the survival of tumor bearing animals.¹²⁵ In a gastric cancer mouse model, NK cells combined with anti-PD1 improved NK cell infiltration in the TME resulting in reduced tumor growth.¹²⁶

Natural killer cell engagers

As discussed previously, NK cells express a wide array of activation receptors, including CD16, signaling lymphocyte activation molecule family receptors, NKG2D, DNAM-1, and the natural cytotoxicity receptors NKp30, NKp44, and NKp46.¹²⁷ These receptors have been exploited in the clinic to induce ADCC using bi- and tri-specific Natural Killer cell engagers (BiKEs, TriKEs). BiKEs are constructed by joining ScFv targeting activation receptors on NK cells with an ScFv specific for a tumor antigen (BiKE) or two different tumor antigens (TriKE).¹²⁸

The development of BiKEs and TriKEs for cancer treatment gained momentum after multiple studies demonstrated the effectiveness of bi-specific antibodies in engaging immune effector cells with tumor cells.^{129–131} Soon after, bi- and tri-specific antibodies were generated and tested in a wide range of tumors including non-Hodgkin's lymphoma, mixed lineage leukemia, metastatic breast cancer, and ovarian cancers.¹³² The first clinical trial with NK cell activating bispecific monoclonal Ab (anti-CD16/CD30) was conducted in patients with refractory Hodgkin's disease.¹³³ A CD16xCD33 (1633) BiKE was tested *in vitro* using NK cells from patients with myelodysplastic syndromes to target CD33⁺ myeloid-derived suppressor cells (MDSCs). The CD16xCD33 BiKE not only reversed MDSC-driven immunosuppression of NK cells but also induced MDSC cell lysis.¹³⁴ Subsequently, Valleria and colleagues introduced the IL-15 gene into the 1633 BiKE to make 161533 TriKE, which showed improved cytotoxicity, degranulation, and cytokine production against CD33⁺ human acute promyelocytic leukemia cell line, HL-60.¹³⁵ More recently, the Vivier group developed a TriKE that targets two activation receptors, NKp46 and CD16 on NK cells and a tumor antigen which exhibited superior killing potency as compared to BiKEs *in vitro* and *in vivo*.¹³⁶

Challenges facing clinical use of NK cell-based therapies

Although NK cell-based therapies are highly attractive, multiple challenges must be overcome before NK therapies can be taken into the clinic. Some of the major challenges in developing NK cells as therapies include difficulty in expanding NK cells *ex vivo*, reduced *in vivo* persistence of NK cells after infusion into the patients, functional impairment of NK cells once within the TME, resistance of tumor cells to NK cell-mediated lysis, difficulty in engineering NK cells to modify their functional properties, and limited infiltration of NK cells into solid tumors. To overcome these challenges, development of convenient and cost-effective protocols and workflows for *ex vivo* expansion and genetic engineering of NK cells is imperative, as is improvement of NK cell persistence *in vivo*.

Future directions

To facilitate *ex vivo* NK cell expansion for therapeutic applica-

tions, iPSC-derived NK cells are being employed because they are easier to scale up, undergo genetic modification, and provide sufficient supply of NK cells for use as allogeneic off-the-shelf immunotherapies. To improve NK cell persistence *in vivo*, we and others are engineering NK cells to express key cytokines required for their survival and proliferation.^{119,123} Other strategies include combining NK cells with agents that neutralize NK suppressive soluble factors such as TGF- β ,¹³⁷ or combining NK cells with agents that prevent shedding of NKG2D ligand by tumor cells and promote cytotoxicity of NK cell-based therapies *in vivo*.¹³⁸ Strategies are being investigated to enhance NK cell infiltration into tumors by modifying chemokines and chemokine receptors on NK cells.¹³⁹

Conclusions

Despite the roadblocks detailed earlier, the recent advances in developing NK cells for therapeutic applications are anticipated to accelerate the use of these therapies in the clinic in the near future.

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Conflict of interest

Dr. Srividya Swaminathan has been an editorial board member of *Exploratory Research and Hypothesis* since November 2021. The authors have no other conflicts of interest to note.

Author contributions

Drafting of the manuscript (S.S.), critical revision of the manuscript for important intellectual content (A.K., A.T.K., S.S.), administrative, technical, or material support (S.S.), and study supervision (S.S.). All authors have made a significant contribution to this study and have approved the final manuscript.

References

- Campbell KS, Hasegawa J. Natural killer cell biology: an update and future directions. *J Allergy Clin Immunol* 2013;132(3):536–544. doi:10.1016/j.jaci.2013.07.006, PMID:23906377.
- Shi FD, Ljunggren HG, La Cava A, Van Kaer L. Organ-specific features of natural killer cells. *Nat Rev Immunol* 2011;11(10):658–671. doi:10.1038/nri3065, PMID:21941294.
- Bekiaris V, Lane PJL. The localization and migration of natural killer cells in health and disease. In: Lotze MT, Thomson AW (eds). *Natural Killer Cells*. Academic Press. 2010:137–153. doi:10.1016/B978-0-12-370454-2.00010-7.
- Ljunggren HG, Kärre K. Host resistance directed selectively against H-2-deficient lymphoma variants. Analysis of the mechanism. *J Exp Med* 1985;162(6):1745–1759. doi:10.1084/jem.162.6.1745, PMID:3877776.
- Kärre K, Ljunggren HG, Piontek G, Kiessling R. Selective rejection of H-2-deficient lymphoma variants suggests alternative immune defence strategy. *Nature* 1986;319(6055):675–678. doi:10.1038/319675a0, PMID:3951539.
- van den Broek MF, Kägi D, Zinkernagel RM, Hengartner H. Perforin dependence of natural killer cell-mediated tumor control *in vivo*. *Eur J Immunol* 1995;25(12):3514–3516. doi:10.1002/eji.1830251246, PMID:8566046.
- Abel AM, Yang C, Thakar MS, Malarkannan S. Natural Killer Cells: Development, Maturation, and Clinical Utilization. *Front Immunol* 2018;9:1869. doi:10.3389/fimmu.2018.01869, PMID:30150991.
- Sivori S, Parolini S, Falco M, Marcenaro E, Biassoni R, Bottino C, *et al*. 2B4 functions as a co-receptor in human NK cell activation. *Eur J Immunol* 2000;30(3):787–793. doi:10.1002/1521-4141(200003)30:3<787::AID-IMMU787>3.0.CO;2-I, PMID:10741393.
- Kelly JM, Takeda K, Darcy PK, Yagita H, Smyth MJ. A role for IFN- γ in primary and secondary immunity generated by NK cell-sensitive tumor-expressing CD80 *in vivo*. *J Immunol* 2002;168(9):4472–4479. doi:10.4049/jimmunol.168.9.4472, PMID:11970991.
- Pende D, Spaggiari GM, Marcenaro S, Martini S, Rivera P, Capobianco A, *et al*. Analysis of the receptor-ligand interactions in the natural killer-mediated lysis of freshly isolated myeloid or lymphoblastic leukemias: evidence for the involvement of the Poliovirus receptor (CD155) and Nectin-2 (CD112). *Blood* 2005;105(5):2066–2073. doi:10.1182/blood-2004-09-3548, PMID:15536144.
- de Andrade LF, Smyth MJ, Martinet L. DNAM-1 control of natural killer cells functions through nectin and nectin-like proteins. *Immunol Cell Biol* 2014;92(3):237–244. doi:10.1038/icb.2013.95, PMID:24343663.
- Zamai L, Ahmad M, Bennett IM, Azzoni L, Alnemri ES, Perussia B. Natural killer (NK) cell-mediated cytotoxicity: differential use of TRAIL and Fas ligand by immature and mature primary human NK cells. *J Exp Med* 1998;188(12):2375–2380. doi:10.1084/jem.188.12.2375, PMID:9858524.
- Prager I, Watzl C. Mechanisms of natural killer cell-mediated cellular cytotoxicity. *J Leukoc Biol* 2019;105(6):1319–1329. doi:10.1002/JLB.MR0718-269R, PMID:31107565.
- Tremblay RE, Loeber R, Gagnon C, Charlebois P, Larivée S, LeBlanc M. Disruptive boys with stable and unstable high fighting behavior patterns during junior elementary school. *J Abnorm Child Psychol* 1991;19(3):285–300. doi:10.1007/BF00911232, PMID:1865046.
- Vivier E, Raulet DH, Moretta A, Caligiuri MA, Zitvogel L, Lanier LL, *et al*. Innate or adaptive immunity? The example of natural killer cells. *Science* 2011;331(6013):44–49. doi:10.1126/science.1198687, PMID:21212348.
- Zwirner NW, Ziblat A. Regulation of NK Cell Activation and Effector Functions by the IL-12 Family of Cytokines: The Case of IL-27. *Front Immunol* 2017;8:25. doi:10.3389/fimmu.2017.00025, PMID:28154569.
- Walzer T, Dalod M, Robbins SH, Zitvogel L, Vivier E. Natural-killer cells and dendritic cells: “l’union fait la force”. *Blood* 2005;106(7):2252–2258. doi:10.1182/blood-2005-03-1154, PMID:15933055.
- Martín-Fontecha A, Thomsen LL, Brett S, Gerard C, Lipp M, Lanzavecchia A, *et al*. Induced recruitment of NK cells to lymph nodes provides IFN- γ for T(H)1 priming. *Nat Immunol* 2004;5(12):1260–1265. doi:10.1038/ni1138, PMID:15531883.
- Uzhachenko RV, Shanker A. CD8+ T Lymphocyte and NK Cell Network: Circuitry in the Cytotoxic Domain of Immunity. *Front Immunol* 2019;10:1906. doi:10.3389/fimmu.2019.01906, PMID:31456803.
- Lee SH, Miyagi T, Biron CA. Keeping NK cells in highly regulated antiviral warfare. *Trends Immunol* 2007;28(6):252–259. doi:10.1016/j.it.2007.04.001, PMID:17466596.
- Guerra N, Tan YX, Joncker NT, Choy A, Gallardo F, Xiong N, *et al*. NKG2D-deficient mice are defective in tumor surveillance in models of spontaneous malignancy. *Immunity* 2008;28(4):571–580. doi:10.1016/j.

- immuni.2008.02.016, PMID:18394936.
- [22] Delbue S, Comar M, Ferrante P. Review on the relationship between human polyomaviruses-associated tumors and host immune system. *Clin Dev Immunol* 2012;2012:542092. doi:10.1155/2012/542092, PMID:22489251.
 - [23] Mishra R, Chen AT, Welsh RM, Szomolanyi-Tsuda E. NK cells and gamma-delta T cells mediate resistance to polyomavirus-induced tumors. *PLoS Pathog* 2010;6(5):e1000924. doi:10.1371/journal.ppat.1000924, PMID:20523894.
 - [24] Cooper MA, Colonna M, Yokoyama WM. Hidden talents of natural killers: NK cells in innate and adaptive immunity. *EMBO Rep* 2009;10(10):1103–1110. doi:10.1038/embor.2009.203, PMID:19730434.
 - [25] Pahl JHW, Cerwenka A, Ni J. Memory-Like NK Cells: Remembering a Previous Activation by Cytokines and NK Cell Receptors. *Front Immunol* 2018;9:2796. doi:10.3389/fimmu.2018.02796, PMID:30546366.
 - [26] Nikzad R, Angelo LS, Aviles-Padilla K, Le DT, Singh VK, Bimler L, *et al*. Human natural killer cells mediate adaptive immunity to viral antigens. *Sci Immunol* 2019;4(35):eaat8116. doi:10.1126/sciimmunol.aat8116, PMID:31076527.
 - [27] Riccardi C, Santoni A, Barlozzari T, Puccetti P, Herberman RB. In vivo natural reactivity of mice against tumor cells. *Int J Cancer* 1980;25(4):475–486. doi:10.1002/ijc.2910250409, PMID:6154658.
 - [28] Gorelik E, Rosen B, Copeland D, Weatherly B, Herberman RB. Evaluation of role of natural killer cells in radiation-induced leukemogenesis in mice. *J Natl Cancer Inst* 1984;72(6):1397–1403. PMID:6374239.
 - [29] Barlozzari T, Reynolds CW, Herberman RB. In vivo role of natural killer cells: involvement of large granular lymphocytes in the clearance of tumor cells in anti-asialo GM1-treated rats. *J Immunol* 1983;131(2):1024–1027. PMID:6863925.
 - [30] Smyth MJ, Thia KY, Street SE, Cretney E, Trapani JA, Taniguchi M, *et al*. Differentiated tumor surveillance by natural killer (NK) and NKT cells. *J Exp Med* 2000;191(4):661–668. doi:10.1084/jem.191.4.661, PMID:10684858.
 - [31] Halama N, Braun M, Kahlert C, Spille A, Quack C, Rahbari N, *et al*. Natural killer cells are scarce in colorectal carcinoma tissue despite high levels of chemokines and cytokines. *Clin Cancer Res* 2011;17(4):678–689. doi:10.1158/1078-0432.CCR-10-2173, PMID:21325295.
 - [32] Duault C, Kumar A, Taghi Khani A, Lee SJ, Yang L, Huang M, *et al*. Activated natural killer cells predict poor clinical prognosis in high-risk B- and T-cell acute lymphoblastic leukemia. *Blood* 2021;138(16):1465–1480. doi:10.1182/blood.2020009871, PMID:34077953.
 - [33] Cai L, Zhang Z, Zhou L, Wang H, Fu J, Zhang S, *et al*. Functional impairment in circulating and intrahepatic NK cells and relative mechanism in hepatocellular carcinoma patients. *Clin Immunol* 2008;129(3):428–437. doi:10.1016/j.clim.2008.08.012, PMID:18824414.
 - [34] Whiteside TL. The tumor microenvironment and its role in promoting tumor growth. *Oncogene* 2008;27(45):5904–5912. doi:10.1038/onc.2008.271, PMID:18836471.
 - [35] Weber R, Fleming V, Hu X, Nagibin V, Groth C, Altevogt P, *et al*. Myeloid-Derived Suppressor Cells Hinder the Anti-Cancer Activity of Immune Checkpoint Inhibitors. *Front Immunol* 2018;9:1310. doi:10.3389/fimmu.2018.01310, PMID:29942309.
 - [36] Lehmann C, Zeis M, Schmitz N, Uharek L. Impaired binding of perforin on the surface of tumor cells is a cause of target cell resistance against cytotoxic effector cells. *Blood* 2000;96(2):594–600. PMID:10887123.
 - [37] Costello RT, Sivori S, Marcenaro E, Lafage-Pochitaloff M, Mozziconacci MJ, Reviron D, *et al*. Defective expression and function of natural killer cell-triggering receptors in patients with acute myeloid leukemia. *Blood* 2002;99(10):3661–3667. doi:10.1182/blood.v99.10.3661, PMID:11986221.
 - [38] C  zar B, Greppi M, Carpentier S, Narni-Mancinelli E, Chiossone L, Vivier E. Tumor-Infiltrating Natural Killer Cells. *Cancer Discov* 2021;11(1):34–44. doi:10.1158/2159-8290.CD-20-0655, PMID:33277307.
 - [39] Roberti MP, Juli   EP, Rocca YS, Amat M, Bravo AI, Loza J, *et al*. Overexpression of CD85j in TNBC patients inhibits Cetuximab-mediated NK-cell ADCC but can be restored with CD85j functional blockade. *Eur J Immunol* 2015;45(5):1560–1569. doi:10.1002/eji.201445353, PMID:25726929.
 - [40] Fu C, Jiang A. Dendritic Cells and CD8 T Cell Immunity in Tumor Microenvironment. *Front Immunol* 2018;9:3059. doi:10.3389/fimmu.2018.03059, PMID:30619378.
 - [41] Pazina T, MacFarlane AW, Bernabei L, Dulaimi E, Kotcher R, Yam C, *et al*. Alterations of NK Cell Phenotype in the Disease Course of Multiple Myeloma. *Cancers (Basel)* 2021;13(2):226. doi:10.3390/cancers13020226, PMID:33435153.
 - [42] Mundy-Bosse BL, Scoville SD, Chen L, McConnell K, Mao HC, Ahmed EH, *et al*. MicroRNA-29b mediates altered innate immune development in acute leukemia. *J Clin Invest* 2016;126(12):4404–4416. doi:10.1172/JCI85413, PMID:27755550.
 - [43] Swaminathan S, Hansen AS, Heftdal LD, Dhanasekaran R, Deutzmann A, Fernandez WDM, *et al*. MYC functions as a switch for natural killer cell-mediated immune surveillance of lymphoid malignancies. *Nat Commun* 2020;11(1):2860. doi:10.1038/s41467-020-16447-7, PMID:32503978.
 - [44] Mameessier E, Sylvain A, Thibault ML, Houvenaeghel G, Jacquemier J, Castellano R, *et al*. Human breast cancer cells enhance self tolerance by promoting evasion from NK cell antitumor immunity. *J Clin Invest* 2011;121(9):3609–3622. doi:10.1172/JCI45816, PMID:21841316.
 - [45] Sabry M, Zubiak A, Hood SP, Simmonds P, Arellano-Ballesterio H, Cournoyer E, *et al*. Tumor- and cytokine-primed human natural killer cells exhibit distinct phenotypic and transcriptional signatures. *PLoS One* 2019;14(6):e0218674. doi:10.1371/journal.pone.0218674, PMID:31242243.
 - [46] Wang F, Lau JKC, Yu J. The role of natural killer cell in gastrointestinal cancer: killer or helper. *Oncogene* 2021;40(4):717–730. doi:10.1038/s41388-020-01561-z, PMID:33262461.
 - [47] B  hr I, Goritz V, Doberstein H, Hiller GG, Rosenstock P, Jahn J, *et al*. Diet-Induced Obesity Is Associated with an Impaired NK Cell Function and an Increased Colon Cancer Incidence. *J Nutr Metab* 2017;2017:4297025. doi:10.1155/2017/4297025, PMID:28357137.
 - [48] Jinushi M, Takehara T, Tatsumi T, Hiramatsu N, Sakamori R, Yamaguchi S, *et al*. Impairment of natural killer cell and dendritic cell functions by the soluble form of MHC class I-related chain A in advanced human hepatocellular carcinomas. *J Hepatol* 2005;43(6):1013–1020. doi:10.1016/j.jhep.2005.05.026, PMID:16168521.
 - [49] Ferrari de Andrade L, Tay RE, Pan D, Luoma AM, Ito Y, Badrinath S, Tsoucas D, *et al*. Antibody-mediated inhibition of MICA and MICB shedding promotes NK cell-driven tumor immunity. *Science* 2018;359(6383):1537–1542. doi:10.1126/science.aao0505, PMID:29599246.
 - [50] Waldhauer I, Goehlsdorf D, Gieseke F, Weinschenk T, Wittenbrink M, Ludwig A, *et al*. Tumor-associated MICA is shed by ADAM proteases. *Cancer Res* 2008;68(15):6368–6369. doi:10.1158/0008-5472.CAN-07-6768, PMID:18676862.
 - [51] Duffy MJ, Mullooly M, O'Donovan N, Sukor S, Crown J, Pierce A, *et al*. The ADAMs family of proteases: new biomarkers and therapeutic targets for cancer? *Clin Proteomics* 2011;8(1):9. doi:10.1186/1559-0275-8-9, PMID:21906355.
 - [52] Xu X, Sun Q, Liang X, Chen Z, Zhang X, Zhou X, *et al*. Mechanisms of Relapse After CD19 CAR T-Cell Therapy for Acute Lymphoblastic Leukemia and Its Prevention and Treatment Strategies. *Front Immunol* 2019;10:2664. doi:10.3389/fimmu.2019.02664, PMID:31798590.
 - [53] Carrega P, Morandi B, Costa R, Frumento G, Forte G, Altavilla G, *et al*. Natural killer cells infiltrating human non-small-cell lung cancer are enriched in CD56 bright CD16(-) cells and display an impaired capability to kill tumor cells. *Cancer* 2008;112(4):863–875. doi:10.1002/cncr.23239, PMID:18203207.
 - [54] Kim N, Lee DH, Choi WS, Yi E, Kim H, Kim JM, *et al*. Harnessing NK cells for cancer immunotherapy: immune checkpoint receptors and chimeric antigen receptors. *BMB Rep* 2021;54(1):44–58. doi:10.5483/BMBRep.2021.54.1.214, PMID:33298244.
 - [55] Zhang Q, Bi J, Zheng X, Chen Y, Wang H, Wu W, *et al*. Blockade of the checkpoint receptor TIGIT prevents NK cell exhaustion and elicits potent anti-tumor immunity. *Nat Immunol* 2018;19(7):723–732. doi:10.1038/s41590-018-0132-0, PMID:29915296.
 - [56] B  ttcher JP, Bonavita E, Chakravarty P, Blees H, Cabeza-Cabreri   M, Sammiceli S, *et al*. NK Cells Stimulate Recruitment of cDC1 into the Tumor Microenvironment Promoting Cancer Immune Control. *Cell* 2018;172(5):1022–1037.e14. doi:10.1016/j.cell.2018.01.004, PMID:29429633.
 - [57] Barry KC, Hsu J, Broz ML, Cueto FJ, Binnewies M, Combes AJ, *et al*. A natural killer-dendritic cell axis defines checkpoint therapy-respon-

- sive tumor microenvironments. *Nat Med* 2018;24(8):1178–1191. doi:10.1038/s41591-018-0085-8, PMID:29942093.
- [58] Hegde S, Krisnawan VE, Herzog BH, Zuo C, Breden MA, Knolhoff BL, *et al*. Dendritic Cell Paucity Leads to Dysfunctional Immune Surveillance in Pancreatic Cancer. *Cancer Cell* 2020;37(3):289–307.e9. doi:10.1016/j.ccell.2020.02.008, PMID:32183949.
- [59] Hersey P, Edwards A, McCarthy WH. Tumour-related changes in natural killer cell activity in melanoma patients. Influence of stage of disease, tumour thickness and age of patients. *Int J Cancer* 1980;25(2):187–194. doi:10.1002/ijc.2910250204, PMID:6993374.
- [60] Konjević G, Jurisic V, SpuziĆ I. Association of NK cell dysfunction with changes in LDH characteristics of peripheral blood lymphocytes (PBL) in breast cancer patients. *Breast Cancer Res Treat* 2001;66(3):255–263. doi:10.1023/a:1010602822483, PMID:11510697.
- [61] Vitale M, Antoni C, Pietra G, Mingari MC, Moretta L. Effect of tumor cells and tumor microenvironment on NK-cell function. *Eur J Immunol* 2014;44(6):1582–1592. doi:10.1002/eji.201344272, PMID:24777896.
- [62] Reiners KS, Topolar D, Henke A, Simhadri VR, Kessler J, Sauer M, *et al*. Soluble ligands for NK cell receptors promote evasion of chronic lymphocytic leukemia cells from NK cell anti-tumor activity. *Blood* 2013; 121(18):3658–3665. doi:10.1182/blood-2013-01-476606, PMID:23509156.
- [63] Li T, Yang Y, Hua X, Wang G, Liu W, Jia C, *et al*. Hepatocellular carcinoma-associated fibroblasts trigger NK cell dysfunction via PGE2 and IDO. *Cancer Lett* 2012;318(2):154–161. doi:10.1016/j.canlet.2011.12.020, PMID:22182446.
- [64] Castriconi R, Antoni C, Della Chiesa M, Vitale M, Marcenaro E, Conte R, *et al*. Transforming growth factor beta 1 inhibits expression of Nkp30 and NKG2D receptors: consequences for the NK-mediated killing of dendritic cells. *Proc Natl Acad Sci U S A* 2003;100(7):4120–4125. doi:10.1073/pnas.0730640100, PMID:12646700.
- [65] Della Chiesa M, Carlomagno S, Frumento G, Balsamo M, Antoni C, Conte R, *et al*. The tryptophan catabolite L-kynurenine inhibits the surface expression of Nkp46- and NKG2D-activating receptors and regulates NK-cell function. *Blood* 2006;108(13):4118–4125. doi:10.1182/blood-2006-03-006700, PMID:16902152.
- [66] Nachmani D, Stern-Ginossar N, Sarid R, Mandelboim O. Diverse herpesvirus microRNAs target the stress-induced immune ligand MICB to escape recognition by natural killer cells. *Cell Host Microbe* 2009;5(4):376–385. doi:10.1016/j.chom.2009.03.003, PMID:19380116.
- [67] Lee SJ, Cho YS, Cho MC, Shim JH, Lee KA, Ko KK, *et al*. Both E6 and E7 oncoproteins of human papillomavirus 16 inhibit IL-18-induced IFN-gamma production in human peripheral blood mononuclear and NK cells. *J Immunol* 2001;167(1):497–504. doi:10.4049/jimmunol.167.1.497, PMID:11418688.
- [68] Ollière S, Hernandez E, Lézin A, Arguello M, Douville R, Nguyen TL, *et al*. HTLV-1 evades type I interferon antiviral signaling by inducing the suppressor of cytokine signaling 1 (SOCS1). *PLoS Pathog* 2010;6(11):e1001177. doi:10.1371/journal.ppat.1001177, PMID:21079688.
- [69] Xu Y, Hu Y, Shi B, Zhang X, Wang J, Zhang Z, *et al*. HBsAg inhibits TLR9-mediated activation and IFN-alpha production in plasmacytoid dendritic cells. *Mol Immunol* 2009;46(13):2640–2646. doi:10.1016/j.molimm.2009.04.031, PMID:19501403.
- [70] Fensterl V, Grotheer D, Berk I, Schlemminger S, Vallbracht A, Dotzauer A. Hepatitis A virus suppresses RIG-I-mediated IRF-3 activation to block induction of beta interferon. *J Virol* 2005;79(17):10968–11077. doi:10.1128/JVI.79.17.10968-10977.2005, PMID:16103148.
- [71] Swann JB, Hayakawa Y, Zerafa N, Sheehan KC, Scott B, Schreiber RD, *et al*. Type I IFN contributes to NK cell homeostasis, activation, and antitumor function. *J Immunol* 2007;178(12):7540–7549. doi:10.4049/jimmunol.178.12.7540, PMID:17548588.
- [72] Alinari L. Awakening exhausted NK cells in lymphomas. *Blood* 2018;131(16):1768–1769. doi:10.1182/blood-2018-03-835173, PMID:29674350.
- [73] Neo SY, Yang Y, Record J, Ma R, Chen X, Chen Z, *et al*. CD73 immune checkpoint defines regulatory NK cells within the tumor microenvironment. *J Clin Invest* 2020;130(3):1185–1198. doi:10.1172/JCI128895, PMID:31770109.
- [74] Kućan Brlić P, Lenac Roviš T, Cinamon G, Tsukerman P, Mandelboim O, Jonjić S. Targeting PVR (CD155) and its receptors in anti-tumor therapy. *Cell Mol Immunol* 2019;16(1):40–52. doi:10.1038/s41423-018-0168-y, PMID:30275538.
- [75] Carlsten M, Norell H, Bryceson YT, Poschke I, Schedvins K, Ljunggren HG, *et al*. Primary human tumor cells expressing CD155 impair tumor targeting by down-regulating DNAM-1 on NK cells. *J Immunol* 2009;183(8):4921–4930. doi:10.4049/jimmunol.0901226, PMID:19801517.
- [76] Marín R, Ruiz-Cabello F, Pedrinaci S, Méndez R, Jiménez P, Geraghty DE, *et al*. Analysis of HLA-E expression in human tumors. *Immunogenetics* 2003;54(11):767–775. doi:10.1007/s00251-002-0526-9, PMID:12618909.
- [77] Lo Monaco E, Tremante E, Cerboni C, Melucci E, Sibilio L, Zingoni A, *et al*. Human leukocyte antigen E contributes to protect tumor cells from lysis by natural killer cells. *Neoplasia* 2011;13(9):822–830. doi:10.1593/neo.101684, PMID:21969815.
- [78] Kortlever RM, Sodir NM, Wilson CH, Burkhardt DL, Pellegrinet L, Brown Swigart L, *et al*. Myc Cooperates with Ras by Programming Inflammation and Immune Suppression. *Cell* 2017;171(6):1301–1315.e14. doi:10.1016/j.cell.2017.11.013, PMID:29195074.
- [79] Romagné F, André P, Spee P, Zahn S, Anfossi N, Gauthier L, *et al*. Preclinical characterization of 1-7F9, a novel human anti-KIR receptor therapeutic antibody that augments natural killer-mediated killing of tumor cells. *Blood* 2009;114(13):2667–2677. doi:10.1182/blood-2009-02-206532, PMID:19553639.
- [80] Benson DM Jr, Hofmeister CC, Padmanabhan S, Suvannasankha A, Jagannath S, Abonour R, *et al*. A phase 1 trial of the anti-KIR antibody IPH2101 in patients with relapsed/refractory multiple myeloma. *Blood* 2012;120(22):4324–4333. doi:10.1182/blood-2012-06-438028, PMID:23033266.
- [81] Benson DM Jr, Cohen AD, Jagannath S, Munshi NC, Spitzer G, Hofmeister CC, *et al*. A Phase I Trial of the Anti-KIR Antibody IPH2101 and Lenalidomide in Patients with Relapsed/Refractory Multiple Myeloma. *Clin Cancer Res* 2015;21(18):4055–4061. doi:10.1158/1078-0432.CCR-15-0304, PMID:25999435.
- [82] Benson DM Jr, Bakan CE, Mishra A, Hofmeister CC, Efebera Y, Becknell B, *et al*. The PD-1/PD-L1 axis modulates the natural killer cell versus multiple myeloma effect: a therapeutic target for CT-011, a novel monoclonal anti-PD-1 antibody. *Blood* 2010;116(13):2286–2294. doi:10.1182/blood-2010-02-271874, PMID:20460501.
- [83] Sanseviero E, O'Brien EM, Karras JR, Shabaneh TB, Aksoy BA, Xu W, *et al*. Anti-CTLA-4 Activates Intratumoral NK Cells and Combined with IL15/IL15Ra Complexes Enhances Tumor Control. *Cancer Immunol Res* 2019;7(8):1371–1380. doi:10.1158/2326-6066.CIR-18-0386, PMID:31239316.
- [84] Stanitsky N, Simic H, Arapovic J, Toporik A, Levy O, Novik A, *et al*. The interaction of TIGIT with PVR and PVRL2 inhibits human NK cell cytotoxicity. *Proc Natl Acad Sci U S A* 2009;106(42):17858–17863. doi:10.1073/pnas.0903474106, PMID:19815499.
- [85] Bakema JE, van Egmond M. Fc receptor-dependent mechanisms of monoclonal antibody therapy of cancer. *Curr Top Microbiol Immunol* 2014;382:373–392. doi:10.1007/978-3-319-07911-0_17, PMID:25116109.
- [86] Freund-Brown J, Chirino L, Kambayashi T. Strategies to enhance NK cell function for the treatment of tumors and infections. *Crit Rev Immunol* 2018;38(2):105–130. doi:10.1615/CritRevImmunol.2018025248, PMID:29953390.
- [87] Ruggeri L, Capanni M, Casucci M, Volpi I, Tosti A, Perruccio K, *et al*. Role of natural killer cell alloreactivity in HLA-mismatched hematopoietic stem cell transplantation. *Blood* 1999;94(1):333–339. PMID:10381530.
- [88] Ruggeri L, Capanni M, Urbani E, Perruccio K, Shlomchik WD, Tosti A, *et al*. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. *Science* 2002;295(5562):2097–2100. doi:10.1126/science.1068440, PMID:11896281.
- [89] Miller JS, Soignier Y, Panoskaltsis-Mortari A, McNearney SA, Yun GH, Fautsch SK, *et al*. Successful adoptive transfer and in vivo expansion of human haploidentical NK cells in patients with cancer. *Blood* 2005;105(8):3051–3057. doi:10.1182/blood-2004-07-2974, PMID:15632206.
- [90] Amouei S, Attaranzadeh A, Montazer M. Intratumoral CD68-, CD117-, CD56-, and CD1a-positive immune cells and the survival of Iranian patients with non-metastatic intestinal-type gastric carcinoma. *Pathol Res Pract*

- 2015;211(4):326–331. doi:10.1016/j.prp.2014.12.013, PMID:2559595.
- [91] Concha-Benavente F, Kansy B, Moskovitz J, Moy J, Chandran U, Ferris RL. PD-L1 Mediates Dysfunction in Activated PD-1+ NK Cells in Head and Neck Cancer Patients. *Cancer Immunol Res* 2018;6(12):1548–1560. doi:10.1158/2326-6066.CIR-18-0062, PMID:30282672.
- [92] Cursons J, Souza-Fonseca-Guimaraes F, Foroutan M, Anderson A, Holand F, Hediye-Zadeh S, *et al*. A Gene Signature Predicting Natural Killer Cell Infiltration and Improved Survival in Melanoma Patients. *Cancer Immunol Res* 2019;7(7):1162–1174. doi:10.1158/2326-6066.CIR-18-0500, PMID:31088844.
- [93] Muntasell A, Rojo F, Servitja S, Rubio-Perez C, Cabo M, Tamborero D, *et al*. NK Cell Infiltrates and HLA Class I Expression in Primary HER2+ Breast Cancer Predict and Uncouple Pathological Response and Disease-free Survival. *Clin Cancer Res* 2019;25(5):1535–1545. doi:10.1158/1078-0432.CCR-18-2365, PMID:30523021.
- [94] Klanova M, Oestergaard MZ, Trněný M, Hiddemann W, Marcus R, Sehn LH, *et al*. Prognostic Impact of Natural Killer Cell Count in Follicular Lymphoma and Diffuse Large B-cell Lymphoma Patients Treated with Immunotherapy. *Clin Cancer Res* 2019;25(15):4634–4643. doi:10.1158/1078-0432.CCR-18-3270, PMID:31053601.
- [95] Lu H, Zhao X, Li Z, Hu Y, Wang H. From CAR-T Cells to CAR-NK Cells: A Developing Immunotherapy Method for Hematological Malignancies. *Front Oncol* 2021;11:720501. doi:10.3389/fonc.2021.720501, PMID:34422667.
- [96] Olson JA, Leveson-Gower DB, Gill S, Baker J, Beilhack A, Negrin RS. NK cells mediate reduction of GVHD by inhibiting activated, alloreactive T cells while retaining GVT effects. *Blood* 2010;115(21):4293–4301. doi:10.1182/blood-2009-05-222190, PMID:20233969.
- [97] Zhang C, Liu J, Zhong JF, Zhang X. Engineering CAR-T cells. *Biomark Res* 2017;5:22. doi:10.1186/s40364-017-0102-y, PMID:28652918.
- [98] Stoiber S, Cadilha BL, Benmeharek MR, Lesch S, Endres S, Kobold S. Limitations in the Design of Chimeric Antigen Receptors for Cancer Therapy. *Cells* 2019;8(5):472. doi:10.3390/cells8050472, PMID:31108883.
- [99] Bashiri Dezfouli A, Yazdi M, Pockley AG, Khosravi M, Kobold S, Wagner E, *et al*. NK Cells Armed with Chimeric Antigen Receptors (CAR): Roadblocks to Successful Development. *Cells* 2021;10(12):3390. doi:10.3390/cells10123390, PMID:34943898.
- [100] Oelsner S, Friede ME, Zhang C, Wagner J, Badura S, Bader P, *et al*. Continuously expanding CAR NK-92 cells display selective cytotoxicity against B-cell leukemia and lymphoma. *Cytotherapy* 2017;19(2):235–249. doi:10.1016/j.jcyt.2016.10.009, PMID:27887866.
- [101] Xu Y, Liu Q, Zhong M, Wang Z, Chen Z, Zhang Y, *et al*. 2B4 costimulatory domain enhancing cytotoxic ability of anti-CD5 chimeric antigen receptor engineered natural killer cells against T cell malignancies. *J Hematol Oncol* 2019;12(1):49. doi:10.1186/s13045-019-0732-7, PMID:31097020.
- [102] Schönfeld K, Sahm C, Zhang C, Naundorf S, Brendel C, Odendahl M, *et al*. Selective inhibition of tumor growth by clonal NK cells expressing an ErbB2/HER2-specific chimeric antigen receptor. *Mol Ther* 2015;23(2):330–338. doi:10.1038/mt.2014.219, PMID:25373520.
- [103] Chang YH, Connolly J, Shimasaki N, Mimura K, Kono K, Campana D. A chimeric receptor with NKG2D specificity enhances natural killer cell activation and killing of tumor cells. *Cancer Res* 2013;73(6):1777–1786. doi:10.1158/0008-5472.CAN-12-3558, PMID:23302231.
- [104] Töpfer K, Cartellieri M, Michen S, Wiedemuth R, Müller N, Lindemann D, *et al*. DAP12-based activating chimeric antigen receptor for NK cell tumor immunotherapy. *J Immunol* 2015;194(7):3201–3212. doi:10.4049/jimmunol.1400330, PMID:25740942.
- [105] Herrera L, Santos S, Vesga MA, Anguita J, Martin-Ruiz I, Carrascosa T, *et al*. Adult peripheral blood and umbilical cord blood NK cells are good sources for effective CAR therapy against CD19 positive leukemic cells. *Sci Rep* 2019;9(1):18729. doi:10.1038/s41598-019-55239-y, PMID:31822751.
- [106] Liu E, Marin D, Banerjee P, Macapinlac HA, Thompson P, Basar R, *et al*. Use of CAR-Transduced Natural Killer Cells in CD19-Positive Lymphoid Tumors. *N Engl J Med* 2020;382(6):545–553. doi:10.1056/NEJMoa1910607, PMID:32023374.
- [107] Liu E, Tong Y, Dotti G, Shaim H, Savoldo B, Mukherjee M, *et al*. Cord blood NK cells engineered to express IL-15 and a CD19-targeted CAR show long-term persistence and potent antitumor activity. *Leukemia* 2018;32(2):520–531. doi:10.1038/leu.2017.226, PMID:28725044.
- [108] Ueda T, Kaneko S. Induced pluripotent stem cell-derived natural killer cells gene-modified to express chimeric antigen receptor-targeting solid tumors. *Int J Hematol* 2021;114(5):572–579. doi:10.1007/s12185-020-02951-5, PMID:32705572.
- [109] Knorr DA, Ni Z, Hermanson D, Hexum MK, Bendzick L, Cooper LJ, *et al*. Clinical-scale derivation of natural killer cells from human pluripotent stem cells for cancer therapy. *Stem Cells Transl Med* 2013;2(4):274–283. doi:10.5966/sctm.2012-0084, PMID:23515118.
- [110] Zhang C, Oberoi P, Oelsner S, Waldmann A, Lindner A, Tonn T, *et al*. Chimeric Antigen Receptor-Engineered NK-92 Cells: An Off-the-Shelf Cellular Therapeutic for Targeted Elimination of Cancer Cells and Induction of Protective Antitumor Immunity. *Front Immunol* 2017;8:533. doi:10.3389/fimmu.2017.00533, PMID:28572802.
- [111] Li Y, Yin J, Li T, Huang S, Yan H, Leavenworth J, *et al*. NK cell-based cancer immunotherapy: from basic biology to clinical application. *Sci China Life Sci* 2015;58(12):1233–1245. doi:10.1007/s11427-015-4970-9, PMID:26588912.
- [112] Mantesso S, Geerts D, Spanholtz J, Kučerová L. Genetic Engineering of Natural Killer Cells for Enhanced Antitumor Function. *Front Immunol* 2020;11:607131. doi:10.3389/fimmu.2020.607131, PMID:33391277.
- [113] Walcher L, Kistenmacher AK, Sommer C, Böhlen S, Ziemann C, Dehmel S, *et al*. Low Energy Electron Irradiation Is a Potent Alternative to Gamma Irradiation for the Inactivation of (CAR)-NK-92 Cells in ATMP Manufacturing. *Front Immunol* 2021;12:684052. doi:10.3389/fimmu.2021.684052, PMID:34149724.
- [114] Tang X, Yang L, Li Z, Nalin AP, Dai H, Xu T, *et al*. First-in-man clinical trial of CAR NK-92 cells: safety test of CD33-CAR NK-92 cells in patients with relapsed and refractory acute myeloid leukemia. *Am J Cancer Res* 2018;8(6):1083–1089. PMID:30034945.
- [115] Lodolce JP, Boone DL, Chai S, Swain RE, Dassopoulos T, Trettin S, *et al*. IL-15 receptor maintains lymphoid homeostasis by supporting lymphocyte homing and proliferation. *Immunity* 1998;9(5):669–676. doi:10.1016/S1074-7613(00)80664-0, PMID:9846488.
- [116] Kennedy MK, Glaccum M, Brown SN, Butz EA, Viney JL, Embers M, *et al*. Reversible defects in natural killer and memory CD8 T cell lineages in interleukin 15-deficient mice. *J Exp Med* 2000;191(5):771–780. doi:10.1084/jem.191.5.771, PMID:10704459.
- [117] Siegler EL, Wang P. Abstract A59: Expression of membrane-bound IL-15/IL-15Rα complex in chimeric antigen receptor-engineered natural killer cells for enhanced efficacy against solid tumors. *Cancer Immunol Res* 2018;6(Suppl 9):A59. doi:10.1158/2326-6074.TUMIMM17-A59.
- [118] Petsikas D, Mohamed F, Ricci M, Symes J, Guerraty A. Adenosine enhances left ventricular flow during 24-hour hypothermic perfusion of isolated cardiac allografts. *J Heart Transplant* 1990;9(5):543–547. PMID:2231093.
- [119] Du Z, Ng YY, Zha S, Wang S. piggyBac system to co-express NKG2D CAR and IL-15 to augment the in vivo persistence and anti-AML activity of human peripheral blood NK cells. *Mol Ther Methods Clin Dev* 2021;23:582–596. doi:10.1016/j.omtm.2021.10.014, PMID:34853803.
- [120] Christodoulou I, Ho WJ, Marple A, Ravich JW, Tam A, Rahnama R, *et al*. Engineering CAR-NK cells to secrete IL-15 sustains their anti-AML functionality but is associated with systemic toxicities. *J Immunother Cancer* 2021;9(12):e003894. doi:10.1136/jitc-2021-003894, PMID:34896980.
- [121] Pomeroy EJ, Hunziker JT, Kluesner MG, Lahr WS, Smeester BA, Crosby MR, *et al*. A Genetically Engineered Primary Human Natural Killer Cell Platform for Cancer Immunotherapy. *Mol Ther* 2020;28(1):52–63. doi:10.1016/j.ymthe.2019.10.009, PMID:31704085.
- [122] Huang RS, Shih HA, Lai MC, Chang YJ, Lin S. Enhanced NK-92 Cytotoxicity by CRISPR Genome Engineering Using Cas9 Ribonucleoproteins. *Front Immunol* 2020;11:1008. doi:10.3389/fimmu.2020.01008, PMID:32528479.
- [123] Kumar A, Lee SJ, Liu Q, Chan AKN, Pokharel SP, Yu J, *et al*. Generation and validation of CRISPR-engineered human natural killer cell lines for research and therapeutic applications. *STAR Protoc* 2021;2(4):100874. doi:10.1016/j.xpro.2021.100874, PMID:34746857.
- [124] Li L, Li W, Wang C, Yan X, Wang Y, Niu C, *et al*. Adoptive transfer of natural killer cells in combination with chemotherapy improves

- outcomes of patients with locally advanced colon carcinoma. *Cytotherapy* 2018;20(1):134–148. doi:10.1016/j.jcyt.2017.09.009, PMID:29056549.
- [125] Li GN, Wu XM, Chan IH, Trager JB. A combination of CAR-NK and CAR-T cells results in rapid and persistent anti-tumor efficacy while reducing CAR-T cell mediated cytokine release and T-cell proliferation. *Cancer Res* 2020;80(Suppl 16):4235. doi:10.1158/1538-7445.AM2020-4235.
- [126] Abdolahi S, Ghazvinian Z, Muhammadnejad S, Ahmadvand M, Aghadaei HA, Ebrahimi-Barough S, *et al*. Adaptive NK Cell Therapy Modulated by Anti-PD-1 Antibody in Gastric Cancer Model. *Front Pharmacol* 2021;12:733075. doi:10.3389/fphar.2021.733075, PMID:34588986.
- [127] Kumar S. Natural killer cell cytotoxicity and its regulation by inhibitory receptors. *Immunology* 2018;154(3):383–393. doi:10.1111/imm.12921, PMID:29512837.
- [128] Rezvani K, Rouse RH. The Application of Natural Killer Cell Immunotherapy for the Treatment of Cancer. *Front Immunol* 2015;6:578. doi:10.3389/fimmu.2015.00578, PMID:26635792.
- [129] de Palazzo IG, Gercel-Taylor C, Kitson J, Weiner LM. Potentiation of tumor lysis by a bispecific antibody that binds to CA19-9 antigen and the Fc gamma receptor expressed by human large granular lymphocytes. *Cancer Res* 1990;50(22):7123–7128. PMID:2146012.
- [130] Garcia de Palazzo I, Holmes M, Gercel-Taylor C, Weiner LM. Antitumor effects of a bispecific antibody targeting CA19-9 antigen and CD16. *Cancer Res* 1992;52(20):5713–5719. PMID:1394194.
- [131] Silla LM, Chen J, Zhong RK, Whiteside TL, Ball ED. Potentiation of lysis of leukaemia cells by a bispecific antibody to CD33 and CD16 (Fc gamma RIII) expressed by human natural killer (NK) cells. *Br J Haematol* 1995;89(4):712–718. doi:10.1111/j.1365-2141.1995.tb08406.x, PMID:7772507.
- [132] Felices M, Lenvik TR, Davis ZB, Miller JS, Vallera DA. Generation of BiKEs and TriKEs to Improve NK Cell-Mediated Targeting of Tumor Cells. *Methods Mol Biol* 2016;1441:333–346. doi:10.1007/978-1-4939-3684-7_28, PMID:27177679.
- [133] Hartmann F, Renner C, Jung W, Deisting C, Juwana M, Eichentopf B, *et al*. Treatment of refractory Hodgkin's disease with an anti-CD16/CD30 bispecific antibody. *Blood* 1997;89(6):2042–2047. PMID:9058726.
- [134] Gleason MK, Ross JA, Warlick ED, Lund TC, Verneris MR, Wiernik A, *et al*. CD16xCD33 bispecific killer cell engager (BiKE) activates NK cells against primary MDS and MDSC CD33+ targets. *Blood* 2014;123(19):3016–3026. doi:10.1182/blood-2013-10-533398, PMID:24652987.
- [135] Vallera DA, Felices M, McElmurry R, McCullar V, Zhou X, Schmohl JU, *et al*. IL15 Trispecific Killer Engagers (TriKE) Make Natural Killer Cells Specific to CD33+ Targets While Also Inducing Persistence, In Vivo Expansion, and Enhanced Function. *Clin Cancer Res* 2016;22(14):3440–3450. doi:10.1158/1078-0432.CCR-15-2710, PMID:26847056.
- [136] Gauthier L, Morel A, Anceriz N, Rossi B, Blanchard-Alvarez A, Grondin G, *et al*. Multifunctional Natural Killer Cell Engagers Targeting NKp46 Trigger Protective Tumor Immunity. *Cell* 2019;177(7):1701–1713.e16. doi:10.1016/j.cell.2019.04.041, PMID:31155232.
- [137] Rouse RH, Shaim H, Sekine T, Weber G, Ballard B, Ku S, *et al*. The TGF- β /SMAD pathway is an important mechanism for NK cell immune evasion in childhood B-acute lymphoblastic leukemia. *Leukemia* 2016;30(4):800–811. doi:10.1038/leu.2015.327, PMID:26621337.
- [138] Hodgins JJ, Khan ST, Park MM, Auer RC, Ardolino M. Killers 2.0: NK cell therapies at the forefront of cancer control. *J Clin Invest* 2019;129(9):3499–3510. doi:10.1172/JCI129338, PMID:31478911.
- [139] Liu S, Galat V, Galat Y, Lee YKA, Wainwright D, Wu J. NK cell-based cancer immunotherapy: from basic biology to clinical development. *J Hematol Oncol* 2021;14(1):7. doi:10.1186/s13045-020-01014-w, PMID:33407739.