

## REVIEW ARTICLE

**Prospect of Natural Killer Cells in Cancer Immunotherapy**Anna Meiliana<sup>1,2,\*</sup>, Nurrani Mustika Dewi<sup>2</sup>, Andi Wijaya<sup>1,2</sup><sup>1</sup>Postgraduate Program in Clinical Pharmacy, Padjadjaran University, Jl. Eijkman No.38, Bandung, Indonesia<sup>2</sup>Prodia Clinical Laboratory, Jl. Cisangkuy No.2, Bandung, Indonesia

\*Corresponding author. E-mail: anna.meiliana@prodia.co.id

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**Abstract**

**BACKGROUND:** Current understanding in molecular character of natural killer (NK) cell, its function and mechanisms, send people the ideas to develop a NK-cell-based immunotherapeutic strategies against human cancer.

**CONTENT:** Before being regards as a cell-based cellular therapy, NK cell have to be clinical proven. Early studies with NK cells infusions for acute myeloid leukemia and lung cancer showed a promising result. NK cells need simplified methods for enriching and expanding, in addition

to its transfection with chimeric antigen receptors (CARs). NK-92 arise as an assuring effector cells to augment monoclonal and bispecific antibody therapy. Thus, NK cells show a potential opportunity for cell engineering, outstep the era of T cells.

**SUMMARY:** It is believed that NK cells bring a bright hope for future cancer immunotherapies, either alone or in combination as a harmonious therapy.

**KEYWORDS:** NK-cells, NK-92 cells, immunotherapy, CAR

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**Introduction**

Current achievement in immunology promoted the immune cells as powerful therapeutic antigens, targeting tumor antigens with T cells expressing chimeric antigen receptors (CARs), especially in lymphoid malignancies patients.(1-3) However, a potentially fatal cytokine release syndrome was reported in a significant number of subjects and requiring intensive care unit (ICU) admission. Then, despite its promising results in treating cancer, this give a big challenge to enact it available for a larger number of patients.(4)

Increasingly considered as promising immunotherapeutic agents for cancer, Natural Killer (NK) cells have advantages because they are potentially broadly applicable and do not cause graft-versus-host disease such as other T cells counterparts.(5) NK cells have an important role in cancer immunosurveillance as a part of innate immune system.(6,7) They don't need peptide antigen to

be presented in major histocompatibility complex (MHC) molecules foregoing, because their activity were controlled by distinct germline-encoded cell surface receptors so the healthy and diseased tissue can be distinguished.(8,9) The problem is, in cancer patients, the immunosuppressive tumor microenvironment was generally impaired, so the NK cell responses were also impaired. That's why current NK cell therapies were commonly performed with allogeneic adoptive transfer from a suitable donor's NK cell which had expanded *ex vivo*.(10-12)

Primary NK cells usually have a limited of life span and expansion potential, but the NK cell line NK-92, with help of interleukin (IL)-2 can be continuously expanded in a good manufacturing practices (GMP)-compliant process. (13) Early phase clinical trials for safety and clinical activity of NK-92 cells for allogeneic therapy in patients with advanced malignancies showed a good result.(13-15) Preclinical studies using both NK-92 primary human NK cells and investigating the generating of cluster of

differentiation (CD)19-specific chimeric antigen receptors (CAR) NK-cells.(16-18) By that, compared than limited original T cells (17-21), CAR-engineered cells can be isolated and expanded from bulk of non-transduced cells (19-22). Thus, donor-derived allogeneic cells immunotherapy were being preferred for adoptive cancer because the cells do not recognize tumor cells as 'self', and can bypassing inhibitory signals.(10,11) This advantage may be extended to NK-92 cells that do not express most of the inhibitory killer cell immunoglobulin-like receptors (KIRs) and phenotypically resemble activated NK cells.(5,23) This approach could be potentially utilized for solid tumor or hemotological malignancies cases, where CAR-engineered NK-92 cells will target antigens expressed by cancer cells. (17,19,20,24-28)

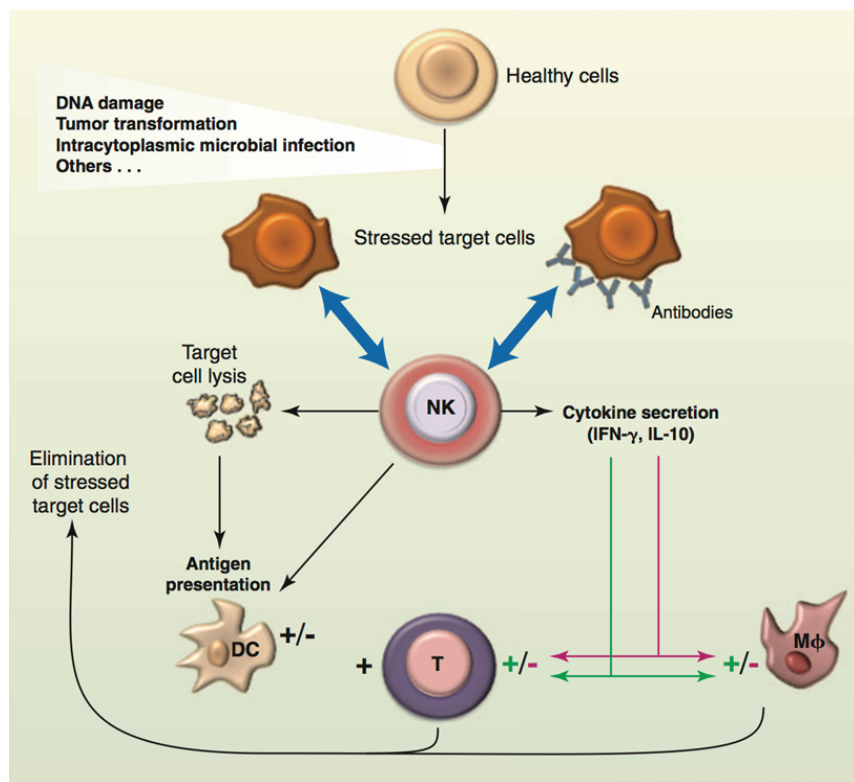
### Human Natural Killers Cells

The immune system is divided into two parts, called the acquired immune system and the innate immune system. Innate immunity was typically characterized with a broadly distributed variety of myeloid and lymphoid cells and the rapid responses through a defined repository of germline-encoded receptors. In the contrary, adaptive immunity in mammals was defined by two lymphocytes, T and B cells.

T cell receptor (TCR) and antibody/B cell receptor (BCR) produced by the site-specific somatic recombination of these cells.(29)

NK cells first illustrated as large granular lymphocytes, and has natural cytotoxicity to counter tumor cells. Later on people recognized NK cells as a separate lymphocyte lineage with cytokine-producing effector function besides its cytotoxicity (Figure 1).(29,30) NK cell activity regulation including the NK cell detection system involving the activation of a variety of cell surface and the inhibitory receptors. There will be an equilibrium between the integration of antagonistic pathways upon interaction with neighboring cells to decide the activation of NK cells to kill target cells.(31) When activated, NK cell receptors will detect a distress on cells ligands, for example the stress-induced self ligands which recognized by some slert molecules like the NK group 2D (NKG2D).(32) There are also alert molecules such as nonself ligands (the cytomegalovirus-encoded m157 recognized by Ly49H in the mouse) and Toll-like receptor (TLR) ligands. Naturally, NK-cells express several TLRs which can induces interferon (IFN)- $\gamma$  production and enhances cytotoxicity *in vitro*.(33)

Like any other lymphocytes, during their development NK cell acquire tolerance to self. This does not always occur but NK cell have the potential to attack normal self cells.(34) NK cells utilize the absence mensuration of consecutively



**Figure 1. The biological functions of NK-cells.** NK-cells can recognize a variety of stressed cells in the absence or in the presence of antibodies (blue arrows). NK-cell activation triggered by this recognition can lead to the lysis of the target cell and to the production of various cytokines and chemokines depending on the nature of the stimulation.(29) (Adapted with permission from American Association for the Advancement of Science).

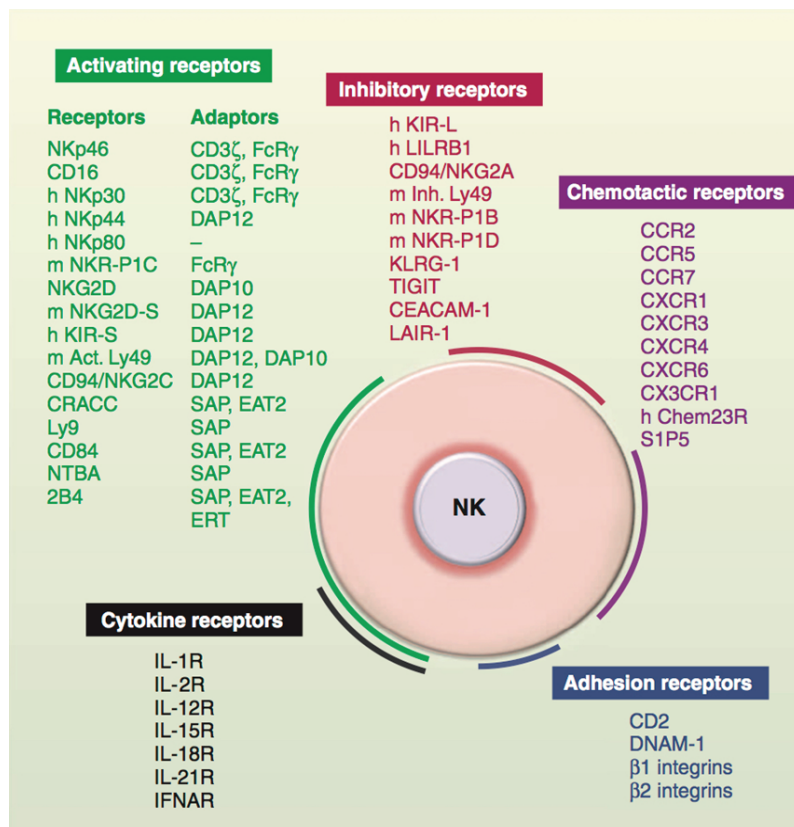
expression of self's molecule on susceptible target cells. In several *in vitro* and *in vivo* models, MHC class I-specific receptors expressed by NK cells lose its inhibitory signals when encountering hematopoietic cells with MHC class I-deficient. This usually happened when cells were flustered due to viral infection or cellular transformation.(35,36) The fallout effects, this missing self on hematopoietic cells will be recognized by NK cells.(37) This recognition happened due to the NK cells inhibitor receptors specific to MHC class I, including KIRs in humans, lectin-like Ly49 molecules in mice, and CD94/NKG2A heterodimers in both species. (38,39) The MHC class I-specific inhibitory receptors include the KIRs in humans, the lectin-like Ly49 dimers in the mouse and the lectin-like CD94-NKG2A heterodimers in both species (Figure 2).(35,36)

Intracytoplasmic Immunoreceptor tyrosine-based inhibitions motifs (ITIMs) mediates the signal to activate the large family of inhibitory receptors including MHC class I receptors.(31) Therefore NK cell can distinguish and spare healthy cells which express self MHC class I molecules accompanied by low amounts of stress-induced self molecules, while dejected cells with down-regulated MHC class I molecules and/or high level of stress-induced

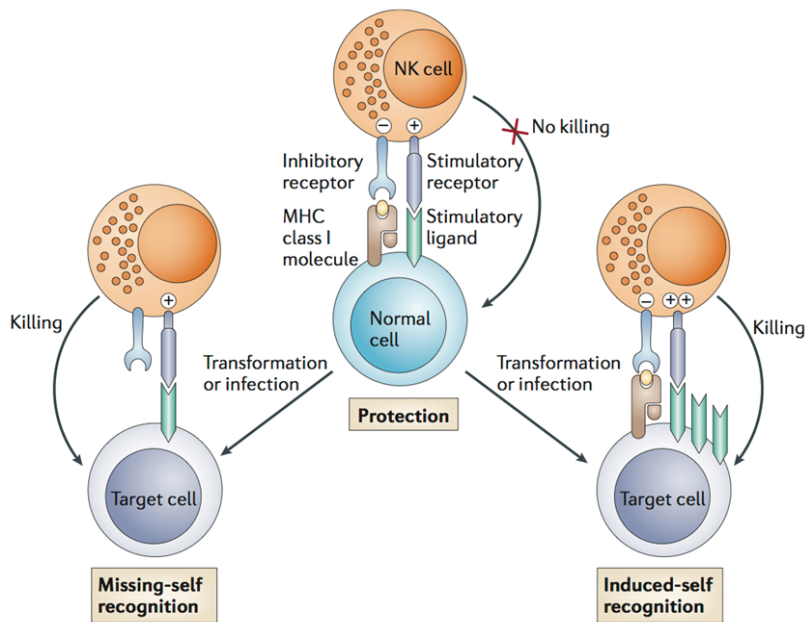
self molecules, such as NKG2D ligands.(40) Figure 3 shows a diagram describing balance of inhibitory and stimulatory signals received by a natural killer.

So we've figure out that NK cells can influence the host's immune response via cytokine and chemokine and kill certain infected or transformed cells through perforin/granzyme or death receptors-related pathways such as Fas, and tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL).(40-46) The prototypic cytokine secreted by NK cells is IFN- $\gamma$ . It has the ability to shape the Th1 immune response (44), activate antigen-presenting cell (APC) for MHC class I further upregulate expression (47), activate macrophages to kill the restricted intracellular pathogens (48), and anti-proliferating effect on viral and malignant-transformed cells (49).

NK cells in human is phenotypically and functionally heterogeneous. NK cells which were rich with CD56<sup>+</sup> surface molecules were primarily cytokine-secreting cells, found about 10% NK cells found in blood, and almost 100% in secondary lymphoid tissues. They can be activated within minutes to secrete cytokines and chemokines, but these CD3<sup>-</sup>CD56<sup>+</sup>NKp46<sup>+</sup> cells barely can spontaneously kill tumor cell targets.(50) Otherwise, Circulating NK



**Figure 2. NK-cell receptors.** NK-cells express many cell surface receptors that can be grouped into activating (green), inhibitory (red), adhesion (blue), cytokine (black) and chemotactic receptors (purple). In addition to MHC class I-specific receptors, other NK-cell inhibitory receptors specific for non-MHC ligands also regulate NK-cell reactivity.(29) (Adapted with permission from American Association for the Advancement of Science).



**Figure 3. The balance of inhibitory and stimulatory signals received by a natural killer cell determines the outcome of interactions with target cells.(37)** (Adapted with permission from Springer International Publishing AG).

cells with low surface density (called dim expression of CD56 and NKp46) does not express cytokines as agile as the other type but some, but not all, have the ability to lyse susceptible tumor cell targets sprightly.(51) however both types have relatively distinct and balanced important roles during human immune response.(52-55) despite its cytokine secretion or cytotoxicity, NK-cell was tightly regulated by the balancing of its activation and inhibition signals which are integrated in a complex network expressed from its cell surface receptors, and we have the ideas that the immune system can be engineered to cure some forms of cancer.(56)

### Targeting NK-Cells in Cancer Immunotherapy

Formerly, people pit most attention at T-lymphocytes for immunotherapy strategies, but new discoveries showed the potential of NK cells as better effector cells for treating cancer. The cytotoxicity effects, together with cytokine secretion, especially IFN and granulocyte-macrophage colony-stimulating factor, assign them at the crossroads of innate and adaptive immunity. Through antibody-mediated cellular cytotoxicity, the activity of monoclonal antibody can be enhancing and can be transfected with chimeric antigen receptors.(57) As their name suggests, NK-cells spontaneously kill cells deemed to be dangerous to the host (cancer, foreign or virus-infected cells) and thus are presumed to be key effectors in cancer immunosurveillance, transplantation rejection and early viral immunity.(33)

NK-cells are usually defined as CD3<sup>-</sup>CD56<sup>+</sup> cells in humans and CD3<sup>-</sup>NK1.1<sup>+</sup> or CD3<sup>-</sup>NKp46<sup>+</sup> cells in mice. CD56<sup>lo</sup>CD16<sup>+</sup> NK-cells with high cytotoxic potential mostly found in human blood, whereas the immunomodulatory CD56<sup>hi</sup>CD16<sup>-</sup> subset found more in lymph nodes. NK cells serve as 5-15% of human circulating lymphocytes, classified as different population based in their maturation and specific function.(58,59)

NK-cells have developed several mechanisms for distinguishing healthy cells from target cells. These mechanisms form the basis of NK-cell activation and cannot be considered in isolation but instead must be considered as complex integration of signals from an array of receptors. By binding with self MHC class I, NK cells tolerate to self-tissue and allows the licensing of NK cells by the engagement with MHC class I. Cells with downregulated expression of MHC class I such as in malignant cells, will put the NK cells inhibitory signals off and permit their function.(37,38)

Granzyme B and perforin are the core molecules required for NK-cell-mediated tumor killing (60), although death-receptor pathways (involving FasL and TRAIL) are sometimes used. Furthermore, NK-cells secrete pro-inflammatory cytokines and chemokines (such as IFN- $\gamma$ , TNF, IL-6, granulocyte-macrophage colony-stimulating factor (GM-CSF) and chemokine C-C motif ligand 5 (CCL5)) that might exert direct anti-tumor activity in addition to promoting innate and adaptive responses. Thus, NK-cells are not only killers but also immunoregulatory cells that can positively or negatively influence anti-cancer responses by modulating the responses of dendritic cells (DCs) and T cells.



(61) Another mechanism linked to the immunosurveillance of cancer by NK-cells involves the elimination of senescent cells. Indeed, the cytokines and chemokines associated with the senescence-associated secretory phenotype mobilize effective NK-cell responses (62).

NK-cells can be derived from various sources and can be obtained from the patient (autologous setting) or from a healthy donor (allogeneic setting). Few NK-cells circulate in human blood, and naive NK-cells exhibit limited cytotoxic activity. For these reasons, several protocols have been designed to expand large numbers of NK-cells with full anti-tumor functions. These methods have been discussed before.(63,64) Previous trials showed that NK cells autologous infusion are clinically ineffective, even with concomitant administration of cytokines such as IL-2. (65-67) Thus the focus shifted to allogeneic administration from donor, usually obtained by apheresis to obtain mononuclear cells, majority peripheral blood mononuclear cells (PBMC). There are still challenges including to consistently obtain sufficient numbers of cells for patient treatment, as we know NK cells only present approximately 10% of all lymphocytes in the blood, and the other issue is to remove T cells which can induce Graft versus host disease (GvHD) in the recipient, commonly using negative CD3 depletion using immunomagnetic antibody tagging with subsequent absorption of the cells over a magnetic column (*i.e.*, the Miltenyi CliniMACS system, Bergisch-Gladbach, Germany).(68,69)

Recently the interest shifted to NK-cells from umbilical cord blood which is readily available with no risk or aggravation for donor. Frozen cord blood cells either cryopreserved or not, can serve as starting material. NK cells in cord blood are immature, so they must be cultured for several weeks with a cocktail of cytokines which contains a combination of IL-2, IL-15, FMS-like tyrosine kinase 3 ligand (Flt-3L) and IL-3 on positively selected cord blood NK-cells were placed on a feeder layer of mesenchymal stromal cells, until reach an acceptable NK-cell numbers, average at a median of 60-fold.(57,70)

However, this expansion process is costly and burdensome, restricted process in a GMP-standard laboratory, performed in gas-permeable bags, in flasks or in bioreactors that expand larger cell numbers on a smaller footprint. To reduce the cost, any manipulation must be done before culture expansion, so the material and time cost will be less. The bag system is a good choice for it does not need wide spaces for sterile and closed system. Some company offers automated technology for cell expansion including Miltenyi's Prodigy (Miltenyi Biotec GmbH, Bergisch-

Gladbach, Germany) with their closed system in constant medium and cytokine feed as per program, and the G-Rex bioreactor (Wilson Wolf Manufacturing, New Brighton, MN, USA) expands NK-cells well and simplified medium change as the cells collect at the bottom of the flask.(71)

Furthermore, with the help of cytokines and certain growth factors, adoptively transferred NK-cells can be expanded and activated *in vivo* in conditioned patients, or may also combined with other treatment modalities to reach a synergistic antitumor activities.(72)

### NK-92 Cell Line, "Off-The-Shelf" for Adoptive NK-Cells-based Cancer Immunotherapy

Recalling the cytotoxic features of NK cells as part of innate immunity system, they don't need prior sensitization to kill any virally infected cells and tumor cells. Adequate number of functional NK cells that are able to proliferate *in vivo* were needed for an effective therapy but are not over-stimulated by cytokines.(73,74) Some group study attempt to optimizing methods for NK cells *ex vivo* isolation, activation and expansion from peripheral blood, mainly for prevention and/or treatment of relapsed disease.(75,76) Another study using IL-2 and an artificial antigen presenting cell to expand umbilical cord blood NK-cells *ex vivo*.(78) Particularly, there are two ongoing clinical trials testing the feasibility (NCT01619761 and NCT01729091). Apheresis become the most preferred method for collecting PBMCs due to its ability to collect cells aseptically into a closed system and minimizing the contamination.(79)

Previous experiences demonstrated that concomitant administration of cytokines such as IL-2, for NK cells autologous infusion are clinically ineffective (65), then the focus shifted to allogeneic therapy. In this case, graft-versus-host disease in the recipient have to be prevented, by adding some manipulation steps in expansion process.(68) Substantial donor-dependent variability also found in terms of NK-cell yield, so a continuously growing NK-cell line is needed to provide predictable numbers of highly cytotoxic NK-cells on expansion.(15,68,69,80-82).

Domogala, *et al.*, tried to modify the NK cells differentiation protocol, and compared the mobilized peripheral blood stem cells (mPBSC), fresh CB CD34<sup>+</sup> cells and frozen umbilical cord blood CD34<sup>+</sup> cells to generate a functional, readily available off-the-shelf product of NK cells, and found that the best source for NK cells is frozen umbilical cord blood CD34<sup>+</sup> cells, suggested that

the cryopreservation procedure selected the most potent UCB CD34<sup>+</sup> cells, leading to a higher fold expansion (600 versus 200) therefore generated a higher NK cell numbers, not regarding the phenotype, cytokine production or cytotoxicity of the cells.(83,84)

NK cell proliferation *ex vivo* can generally be stimulated using a feeder cell such as monocytes which stimulate NK cell expansion by both humoral signals and direct cell-to-cell contact. That’s why some protocols suggest the using of PBMNCs as a source of feeder cells in the NK-cell culture.(65,85-87) Moreover, two allogeneic feeder cell lines commonly used in NK cell expansion including an Epstein-Barr virus-transformed lymphoblastoid cell line and an engineered leukemic cell line that express a membrane-bound form of IL-15 fused to the T-cell receptor CD8a and the 41BB ligand.(88-90) Using allogeneic feeder cell lines requires irradiation, meanwhile autologous feeder cell lines do not need it.(69,88-91)

NK-92 is a highly potent permanent NK-cell line (NantKwest, Culver City, CA, USA), which is now readily available, processed from a cGMP-compliant master cell bank, predictable and reproducible amplification of an extensively characterized potent NK-cell agent is at hand for clinical usage.(5) It is a permanent, IL-2-dependent NK-cell line, first obtained from the peripheral blood of a 50-year-old male patient with non-Hodgkin’s lymphoma at diagnosis in 1992, and can be kept in vented tissue culture asks.(92,93) NK-92 is characterized by the expression of CD56<sup>+</sup> and CD2, CD3<sup>-</sup>, CD8<sup>-</sup>, and and the low-affinity immunoglobulin G (IgG) fragment crystallizable region (Fc) receptor (FcγRIII), CD16.(23) A CD56<sup>+</sup>/CD16<sup>-</sup> low phenotype is typical for a minor subset of NK-cells in peripheral blood, which have immunomodulatory functions as cytokine producers.

However, NK-92 is a highly cytotoxic cell line. It expressed high levels of cytolytic pathway molecules, including perforin and granzyme B. NK-92 clone transformation may have emerged from an early undifferentiated NK-cell progenitor.(13,23) CD16 receptor is an important feature of NK cells that mediates binding to antibodies through the Fc portion of IgG so it may have an additional killing mechanism of antibody-dependent cell-mediated cytotoxicity (ADCC) in conjunction with tumor-targeting antibodies. Different with primary cytotoxic NK-cells, ADCC originally is not a cytotoxic mechanism naturally exerted by currently established NK-cell lines (Figure 4).(5) Uniquely, other established cytotoxic NK-cell lines, such as KHYG-1, NKL and YT, have inadequate of CD16 expression.(94)

NK-92 cells can be easily manipulated genetically for specific tumor antigens recognition, or to develop antibody-dependent cellular cytotoxicity. It has low risks and can be easily infused into patients, even in the advanced stage of cancer.(4) NK cell can be engineered to express many different CARs to prevent tumor resistance, by targeting for instance, CD19 or CD20 (anti-B cell malignancies), CD38 (anti-myeloma) or human epidermal growth factor receptor 2 (HER2; ErbB2; anti-epithelial cancers), with lower costs compared to autologous or allogeneic NK-cells and, particularly, CAR.T cells make it as a promising effective treatments in cancer.(5)

### CAR-expressing NK-Cells

On August 2017, the forst CAR-T therapy approved in United States was Kymriah (tisagenlecleucel) from Novartis. Without a phase 3 trial and within just six

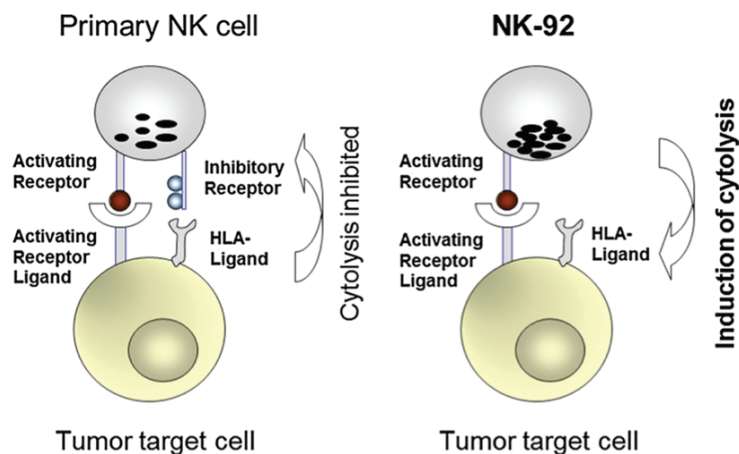


Figure 4. Simplified, schematic representation of NK-92 in a tumor target cell encounter compared to a primary NK-cell.(5) (Adapted with permission from Springer International Publishing AG).

month the approval was achieved, due to the astonishing patients' clinical responses. After three months' treatment with Kymriah, ~83% of pediatric and adult patients (up to 25 years old) 25 with relapsed or refractory B-cell acute lymphoblastic leukemia (ALL) showed sufficient remission and curative therapy are adequate. In that event, soon Kite Pharma was acquired by Gilead Sciences, as a sign the industry believe that Kymriah will lead to other CARs. For CAR-T itself, a successfully overcome immunosuppressive tumor microenvironments, address the heterogeneity of solid tumors and incorporate a wider range of antigens suitable for tumor targeting, to treat leukemia auspiciously.(95)

NK cells may serve as a better alternative than CAR-driven cytotoxicity. Allogeneic NK cells will induce the immune response and then be rejected after few days, while autologous NK cells will disappear after few days in line with its lifetime. Superior rather than T cells, NK cells' spontaneous cytotoxicity activity can trigger the demise of target cells in a tumor associated antigens (TAA)-unrestricted manner via specific natural cytotoxicity receptors (NCRs), including NCR3 (also known as NKp30), NCR2 (also known as NKp44), NCR1 (also known as NKp46), and killer cell lectin-like receptor subfamily K, member 1 (KLRK1, best known as NKG2D). NK cells express Fc fragment of IgG, low affinity III, receptor (FcγRIII), that binds the Fc fragment of antibodies to elicit ADCC. NK cells owned the CAR-expressing NK-cells and a TAA-specific monoclonal antibody, enable the combinations of two targeted therapies recognizing different (or the same) TAA(s).

The cytokines produced by NK cells are different than those from T cells, supporting the serial killing of NK cell, as the time-lapse videomicroscopy studies showed NK cells and NK-92 diligently move from one target to the next one, killing on as many as 7-10 cells.(96,97) CAR-modified NK-cells were in advance complementing the therapeutic option to CAR-expressing T cells, applied to fight against CD19, CD20, CD244, and HER2 as well as CAR-expressing NK-92 cells targeting a broader range of cancer antigens.

NK-92 cells offer an excellence chance to be an open platform CAR-based immunotherapy.(19) It has a higher efficiency in transfection and technically simple, giving better results while enriching, and also less risk of oncogene activation and insertional mutagenesis by avoiding viral vectors.(17,19,97) Continuously expanded CAR NK-92 can be clinical useful for treating cancer patients, especially when the suitable donors for CAR T cell was impossible. In phase I clinical trials, only a minority of cancer patients

treated by repeated infusion of parental NK-92 cells developed anti-HLA antibodies, and no adverse events related to immune responses to these cells were observed. (14,15) This advantage may also be seen with CAR NK-92 cells. NK-92 has a high and consistent cytotoxic activity expressing CD19-specific CARs that employ CD3 or CD28-CD3 signaling domains attacking established and primary B-cell lymphoma and leukemia cells more effectively compare to a similar CD137-CD3 CAR.(12)

CAR-modified NK-92 cells showed a potency of effectively treating any other types of cancer as CAR-engineered NK-cell lines (e.g., NK-92) glioblastoma for glioblastoma, GD2-CAR on NK-92 cells against neuroblastoma, CS1-CAR-expressing NK-92 cells to target multiple myeloma (MM) cells, and an adoptive transfer of CS1-CAR-modified NK-92 cells efficiently impeded the dissemination of human IM9 MM cells *in vivo* and prolonged the lifespan.(27,28) Retargeting of NK-92 cells to solid tumors-derived cells with a Her-2/neu-specific CAR resulted in efficient lysis of NK-resistant, ErbB2/HER2-expressing target cells *in vitro*, and improved tumor localization and anti-tumoral activity *in vivo*.(19,99) The ErbB2/HER2 specific CAR NK-92 cells has reached phase I clinical trials with cGMP requirements (20,100).

## Therapeutic Application of NK-Cells

Alloreactive NK-cells win the interest as suitable and powerful effector cells for cellular therapy of cancer after hematopoietic stem cells, dendritic cells, mesenchymal stromal cells, unselected T lymphocytes, and antigen-specific or regulatory T cells.(101) Have no risk of GvHD and having strong activity of cytotoxicity, NK cells efficiently and specifically redirected to recombine with CARs, which consist of a single-chain variable fragment (scFv; ectodomain) linked to intracellular signaling domains (endodomain).(101-104) The scFv binds to a defined target antigen on, *i.e.*, cancer cells and triggers effector cell activation upon target engagement.(98,105) Compared to cytokine-induced killer cells, that ovine more actively than NK cells, it killed both MHC-I-negative and -positive cancer cells, while NK cells destroyed MHC-I-negative cells only. Moreover, cytokine-induced killer (CIK) cells moved in all directions thus left longer tracks than did NK cells. CIK cells showed higher displacement and straightness scores than did NK cells, which indicates long-distance random migration of CIK cells.(106)

The potential NK cells currently has been translated into clinical-scale using cGMP using a variety of starting materials, technologies, and manipulations.(68) The cell line NK-92 as the most potent and promising therapeutic cell stand as master cell, thoroughly standardized and characterized.(82) After irradiation, it can be infused allogeneic to patients.(14) to ensure the product's safety, quality control become an important part include sterility testing, viability, gram stain, mycoplasma testing (for long-term cultures and products utilizing animal-derived reagents), endotoxin testing (LAL Kinetic-QCL, Lonza, Walkersville, MD) and sorting the cell phenotype as determined by flow cytometry.(68,107) Another challenge for successful therapy with NK cells in mainstream clinical applications include to find the most efficient and optimal method to yield enough cells that clinically relevant, *i.e.*, with highest tumor cytotoxicity level. The exciting future was saw as NK cells immunotherapy now marching from treating hematologic malignancy to solid tumor as well. The challenge ahead is how to optimally activate NK-cells endogenously without the use of a cell infusion, perhaps by utilizing IL-15 or any promising proteins.(108)

## Conclusion

NK cells as a part of immune system play a role in the first defense against diseases, including malignancies. NK cells provide an immediate natural response of cytolytic action. Recently, cell-based therapies increasingly favored and important as the promising treatment for cancer. An improved understanding of NK-cell character and mechanism will promote to a clinical applications of this approach. Eventually, we might even discover that both these cell types have their place in the multimodal approach that is required to eliminate cancer and control its recurrence.

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