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Review

Review

Harnessing the power of memory-like NK cells to fight cancer

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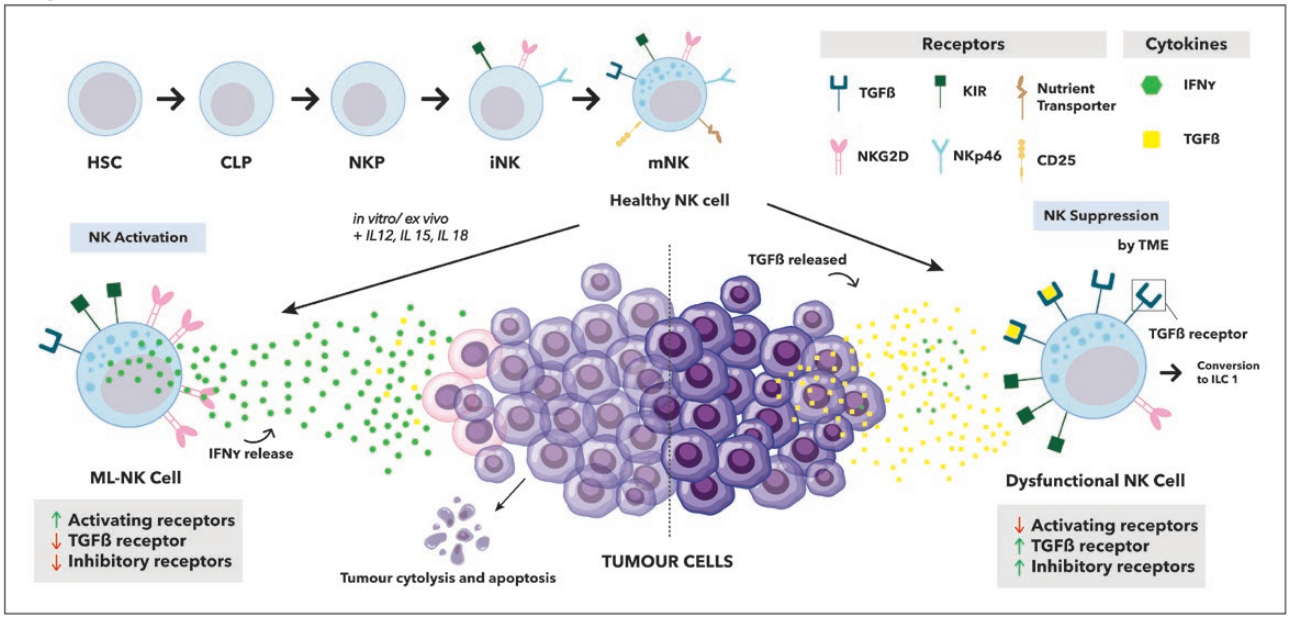
Summary

Natural killer (NK) cells possess the innate ability to eliminate cancerous cells effectively. Their crucial role in immunosurveillance has been widely recognized and exploited for therapeutic intervention. Despite the fast-acting nature of NK cells, NK adoptive cell transfer lacks favorable response in some patients. Patient NK cells often display diminished phenotype in preventing cancer progression resulting in poor prognosis. Tumor microenvironment plays a significant role in causing the downfall of NK cells in patients. The release of inhibitory factors by tumor microenvironment hinders normal function of NK cells against tumor. To overcome this challenge, therapeutic strategies such as cytokine stimulation and genetic manipulation are being investigated to improve NK tumor-killing capacity. One of the promising approaches includes generation of more competent NK cells via *ex vivo* cytokines activation and proliferation. Cytokine-induced ML-NK demonstrated phenotypic alterations such as enhanced expression of activating receptors which help elevate their antitumor response. Previous preclinical studies showed enhanced cytotoxicity and IFNγ production in ML-NK cells compared to normal NK cells against malignant cells. Similar effects are shown in clinical studies in which MK–NK demonstrated encouraging results in treating hematological cancer. However, there is still a lack of in-depth studies using ML-NK in treating different types of tumors and cancers. With convincing preliminary response, this cell-based approach could be used to complement other therapeutic modalities to achieve better clinical outcomes.

Graphical Abstract



Keywords: memory-like, natural killer cells (NK), cancer, immunotherapy, cytokine induced

Abbreviations: ADAM: a disintegrin and metalloproteinase; ADCC: antibody-dependant cellular cytotoxicity; ALL: acute lymphoblastic leukaemia; AML: acute myeloid leukaemia; CAF: cancer-associated fibroblasts; CAR: chimeric antigen receptor; CD: cluster of differentiation; CRC: colorectal cancer; CRS: cytokine

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release syndrome; CTLA-4: cytotoxic T lymphocyte associated protein-4; DNAM: DNAX accessory molecule 1; ECM: extracellular matrix; GvDH: graft versus host disease; HCC: hepatocellular carcinoma; IFNγ: interferon gamma; IL: Interleukin; ILC: innate lymphoid cells; KIR: killer immunoglobulin-like receptor; MCMV: murine cytomegalovirus; MDS: myelodysplastic syndrome; MDSC: myeloid derived suppressor cells; MHC: major histocompatibility complex; miRNA: microRNA; ML-NK: memory-like natural killer cells; MMP: matrix metalloproteinase; NK: natural killer; NKG2A: natural killer group 2 member A; NSCLC: non-small cell lung cancer; PD-1: programmed cell death-1; PD-L1: programmed cell death ligand-1; RCC: renal cell carcinoma; SEMA7A: semaphorin 7A; TAM: tumor associated macrophages; TGFβ: transforming growth factor beta; TME: tumor microenvironment; TNFα: tumor necrosis factor alpha; TRAIL: tumor necrosis factor-related apoptosis inducing ligand; Treg: regulatory T cells.

Introduction

Cancer immunotherapy has been an emerging field over the last decade. The objective of immunotherapy is to enhance the ability of the immune system in recognizing and destroying malignant cells. Immunotherapy consists of several treatment modalities; including oncolytic therapy, cancer vaccines, cytokine therapy, adoptive cell therapy, and immune checkpoint inhibitors [1–5]. Each approach targets specific cancer cells by enhancing antitumor immunity in a unique way thus generating durable responses in some cancers. One example is the adoptive transfer of genetically altered T cells known as CAR-T which significantly improved clinical outcomes in patients with hematological malignancies by achieving remission rates of 81% and 71% in pediatric acute lymphoblastic leukemia (ALL) and adult B-cell acute lymphoblastic leukemia (B-ALL) patients, respectively [6–8]. Aside from that, immune checkpoint blockades comprising of monoclonal antibodies which target cytotoxic T lymphocyte associated protein-4 (CTLA-4), programmed cell death-1 (PD-1), and programmed cell death ligand-1 (PD-L1) also showed remarkable results in enhancing T-cell response against various tumor types; for instance, metastatic melanoma, non-small cell lung cancer (NSCLC), bladder cancer, renal cell carcinoma (RCC), and colorectal cancer (CRC) [5]. These approaches have benefited some patients in terms of achieving remission and preventing relapse. However, severe adverse effects such as cytokine release syndrome (CRS), neurotoxicity, and graft-versus-host disease (GvHD) are still major concerns despite promising outcomes [9].

Natural killer (NK) cells have been long known for their role in mediating immune response against tumor development and infections. Researchers believe that NK cells can be harnessed in developing alternative approaches to curtail cancer. Early clinical studies utilizing NK cells have shown safety and efficacy against hematological malignancies. Complete remission was reported in 5 out of 19 acute myeloid leukemia (AML) patients with poor prognosis after infusion of haploidentical allogeneic NK cells [10]. Another study on pediatric AML indicates all patients achieved complete remission and remained event-free for approximately 2 years [11]. No serious adverse effects (such as GvHD) with limited toxicities were reported in these studies suggesting that NK cell therapy is generally well-tolerated in patient [10, 12]. With this, NK-cell-based immunotherapy is gathering more attention revolving around its clinical potential and application. Currently, autologous or allogeneic NK cell transfers are being explored as a form of immunotherapy [13]. Clinical trials leveraged on NK cells show promising results which encourage further studies and continuous innovation [14]. Strategies for improving NK cell function such as cytokine induction and engineering exogenous receptors are currently ongoing [15, 16].

In this review, we briefly discuss the role of NK cells in eliminating cancer and how NK cell function becomes depleted in the tumor microenvironment (TME). We also provide an overview of cytokine-induced memory-like NK (ML-NK) cells and their key features in mediating antitumor response. We describe the changes in ML-NK cells, which attribute to their elevated responsiveness against tumor cells. Lastly, this review highlights the clinical significance and possible future applications of ML-NK cells in cancer immunotherapy.

NK cell biology and function

Defined as key mediators of the innate immunity, NK cells are the first responders against harmful infections and malignancies. NK cells arise from the bone marrow and are usually found in lymphoid tissue, spleen, and peripheral blood circulating at low frequency (5–15%). They are identified as large granular lymphocytes derived from a single lymphoid progenitor cell [14]. Known to exhibit powerful cytotoxic activity, NK cells have the capacity to recognize and eliminate a wide spectrum of cancer cells rapidly without prior sensitization. Unlike other immune cells, NK cells are able to mediate cytotoxicity without the need for antigen specificity. Human NK cells can be divided into two subpopulations based on the expression level of cluster of differentiation (CD) markers; namely $CD56^{bright}$ and $CD56^{dim}$ [17]. Each population has its distinctive properties and function. Based on previous research, $CD56^{bright}$ cells (roughly make-up 10% of the NK-cell population) are suggested to be more proliferative and have greater cytotoxicity when stimulated yet possess poor cytotoxicity during resting conditions [18]. Another research showed that $CD56^{dim}$ cells which make-up 90% of the NK-cell population are potently cytotoxic without prior stimulation and are able to produce cytokines after stimulation [19].

In general, NK cells can destroy numerous target cells in the absence of antibodies or via antibody-dependant cellular cytotoxicity (ADCC) [20]. Cytotoxic action occurs either through the direct release of perforin and granzymes which then bind to the target cell resulting in cell lysis or through the engagement of death ligands such as Fas ligand and TNF-related apoptosis inducing ligand (TRAIL) to their respective receptors to initiate apoptosis following caspase activation. Besides that, NK cells can indirectly mediate antitumor immunity through their role as immunoregulatory cells. The release of tumor necrosis factor alpha (TNFα) and interferon gamma (IFNγ) by NK cells promotes activation and recruitment of other immune cells, enabling the action of adaptive immunity against malignancy [21–23]. Additionally, NK cells can detect target cells in the presence of antibodies by forming ADCC complexes upon the engagement of CD16 receptors to those target cells, resulting in cell lysis and cytokine release [20, 24].

NK-cell activation is strictly regulated by the expression of inhibitory, activating, and cytokine receptors on NK cells [23]. Surface expression of human killer immunoglobulin-like receptors (KIR and CD94/NKG2A inhibitory receptors allows recognition of major histocompatibility complex

(MHC) class I commonly expressed by normal healthy cells, sparing these healthy cells from NK-cell-mediated killing. In the event of decrease or lack of MHC class I expression in tumor cells, NK cells will recognize tumor cells as "non self", distinguishing tumor cells from MHC class I expressing normal healthy cells. The lack of NK inhibitory signals unleashes the brakes for NK-cell activation and permits the release of inflammatory cytokines that selectively destroy tumor cells. At the same time, cancer or virus-induced stress stimulates NK activating receptors (namely NKp46, NKp44 and NKp30, CD16, NKG2D, and DNAM-1) facilitating the engagement of NK cells to the ligands of target cancer cells and exerting killing mission. Consequently, NK cells will produce inflammatory cytokines and perform cytotoxic killing of tumor cells. The equilibrium between activating and inhibitory receptors and the interaction with target cells are crucial in directing NK cells to proceed or abort their killing mission [21–23].

NK cells in cancer

NK cell deficiency

Previous case studies have shown that patients with poor prognoses in cancer are correlated to relatively low levels of NK cells in their body [25, 26], suggesting that NK cells are possibly vital in cancer prevention. This is in line with a preclinical study by Mamessier *et al*. where the NK cells isolated from different stages of breast cancer showed reduced functionality against leukemia cell line [27]. The expression of activating and inhibitory receptors was significantly altered in the collected samples, in addition to that, more severe impairment is observed in NK cells from the advanced stage cancer group. In another correlation study by Bachanova *et al*., NK cells from Non-Hodgkin's Lymphoma patients and healthy subjects were evaluated for their antitumor effect [28]. NK cells from cancer patients demonstrated poor effector function *in vitro* including poor degranulation, impaired cytotoxicity, and cytokine release compared to healthy controls. Similarly, Zhang *et al*.'s study have shown that patients with myelodysplastic syndrome (MDS) had a decreased in $CD16^+$ NK-cell population in comparison to healthy controls [29]. Despite the alteration in the expression of activating and inhibitory receptors, patient NK cells failed to produce sufficient perforin and granzyme. These findings imply that NK cells have impaired cytotoxic and antibody-mediated killing mechanisms which led to an ineffective antitumor response in cancer patients.

Additionally, functional and metabolic defects were evident in NK cells retrieved from metastatic breast cancer patients who undergo chemotherapy or treatment-free for a year [30]. Apart from impaired cytotoxicity and cytokine production, this study also reported significant mitochondrial alterations, reduced glycolysis, and oxidative phosphorylation in cancer patients. Such metabolic changes hinder normal functioning of NK cells, hence contributing to uncontrollable tumor growth and immune evasion. Strategies to augment and restore NK cells are needed to overcome challenges imposed by tumors. To achieve this, we must first address the root cause of the inhibition or dampening of NK-cell activity.

Tumour microenvironment (TME) suppression

Generally, tumor microenvironment (TME) is the main contributing factor in impeding NK-cell activity. TME disrupts antitumor immunity through the formation of an extracellular matrix (ECM), creating oxygen and nutrient-depleted environments, the release of soluble factors, and producing an immunosuppressive cell population [31–34]. Disruption in cellular immunity not only perturbs NK cells but also limits the efficacy of other therapeutic strategies [33]. To facilitate the disruption, TME comprises immunosuppressive cells such as regulatory T cells (Treg), stromal cells, tumor associated macrophages (TAMs), cancer-associated fibroblasts (CAFs), and myeloid-derived suppressor cells (MDSC) which interfere NK-cell interaction with cancer cells that promote tumor progression and metastasis [33, 35, 36]. The ECM components such as matrix metalloproteinase (MMP) as well as a disintegrin and metalloproteinase (ADAM) form a tissue barrier surrounding the tumor, preventing NK cells from penetrating its core and restricting tumor infiltration [32]. TME creates an inconducive environment consisting of hypoxia, an acidic condition with low nutrient levels that reduces NK-cell survival and expansion [34]. Besides that, these components release soluble factors and immunosuppressive cytokines such as transforming growth factor beta (TGFβ) and Interleukin 10 (IL-10) which restricts NK effector and cytotoxic function [35, 36].

TGFβ is a pleiotropic factor involved in the development and progression of tumors. Expressed in either soluble or membrane-bound form, TGFβ is an essential cytokine in modulating inflammation and preventing tumor formation in healthy tissues. However, tumor suppressive effects of TGFβ are being compromised in cancer after acquiring mutation leading to dysfunctionality. Instead of suppressing tumor growth, TGFβ may interfere NK-cell activity by promoting or repressing specific mechanisms, which disrupt activating receptor expression, manipulate cell metabolism, and convert NK cells to less cytolytic ILC1 as described in Fig. 1 [37–39]. At the transcriptional level, TGFβ reduces the T-box transcription factors (T-bet and Eomes) responsible for NK-cell development and recruitment [37, 38]. This occurrence is demonstrated in Kiekens et al.'s experiment, whereby a reduction of NK cells and immature NK phenotypes were observed in T-bet and Eomes deficient mice [40]. Besides that, TGFβ also plays a role in reducing cytokine production in NK cells by acting as a repressor of IFNγ [38]. In terms of metabolic alteration, TGFβ inhibits mTOR pathway essential in NK-cell proliferation [41]. This leads to a decrease in oxidative phosphorylation and glycolysis as well as developing mitochondrial defects evidently shown in NK cells from breast cancer patients where fragmented and elongated morphology was observed [30]. Without these essential metabolic processes, NK cells are incompetent to expand, proliferate, and execute their killing mechanism.

In addition to that, TGFβ could mediate miRNAs to perturb the expression of activating receptors at post-transcriptional level [42]. Overexpression of different miRNAs (such as miR-1245, 146a, and 183) causes downregulation of NKG2D, NKG2C, KIR, and NKp44 surface expression affecting cytokine production [43–45]. Decline in activating signals contributes to the impairment of NK-cell recognition [37, 46]. Therefore, TGFβ exploits miRNA machinery to repress NK-cell activity. Furthermore, TGFβ also promotes the transition of NK cells to Group 1 innate lymphoid cells (ILC1s) which are less effective against tumors. Despite having similar functions in IFNγ

production, ILC1s lack perforin-dependant cytotoxicity and overall capacity to reduce tumor burden [39]. Consequently, the release of TGFβ by tumor and their surrounding cells have the ability to counteract immune cells which promotes tumor immune escape as shown in Fig. 2 [37, 38]. On the whole, TME is not the sole factor in driving NK-cell suppression. Multiple factors such as the immunosuppressive effects of chemotherapy, radiation, surgery, and cancer itself may also be responsible for the deterioration of NK cells affecting the overall antitumor immunity [47].

NK-cell therapy

Since NK cells in cancer patients are mostly compromised by the effects of TME, researchers have been looking for ways to restore NK-cell function. According to Miller *et al.*'s study, infused donor NK cells persisted *in vivo* and induced an antitumor effect [10]. Five out of 19 AML patients from this study have achieved complete remission while previous studies using autologous NK cells reported having no significant antitumor effects [48, 49]. Another study conducted on patients with refractory Non-Hodgkin's lymphoma

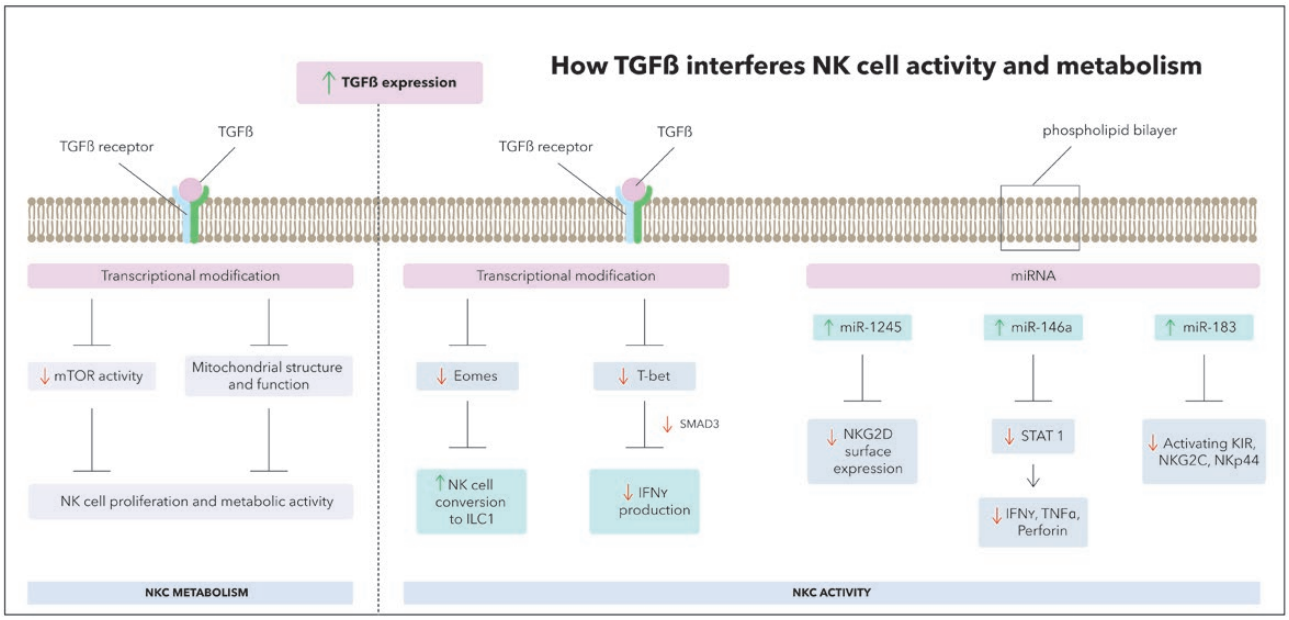

Figure 1. TGFβ-mediated inhibition of various pathways controlling NKC metabolism and activity. TGFβ induce changes at transcriptional level and thru manipulation of miRNA system. Changes in NKC activity include alteration in expression of surface receptors, reduced IFNγ production and NK cell conversion to ILC1. Reduction of mTOR activity and mitochondrial defects impair NKC metabolism.

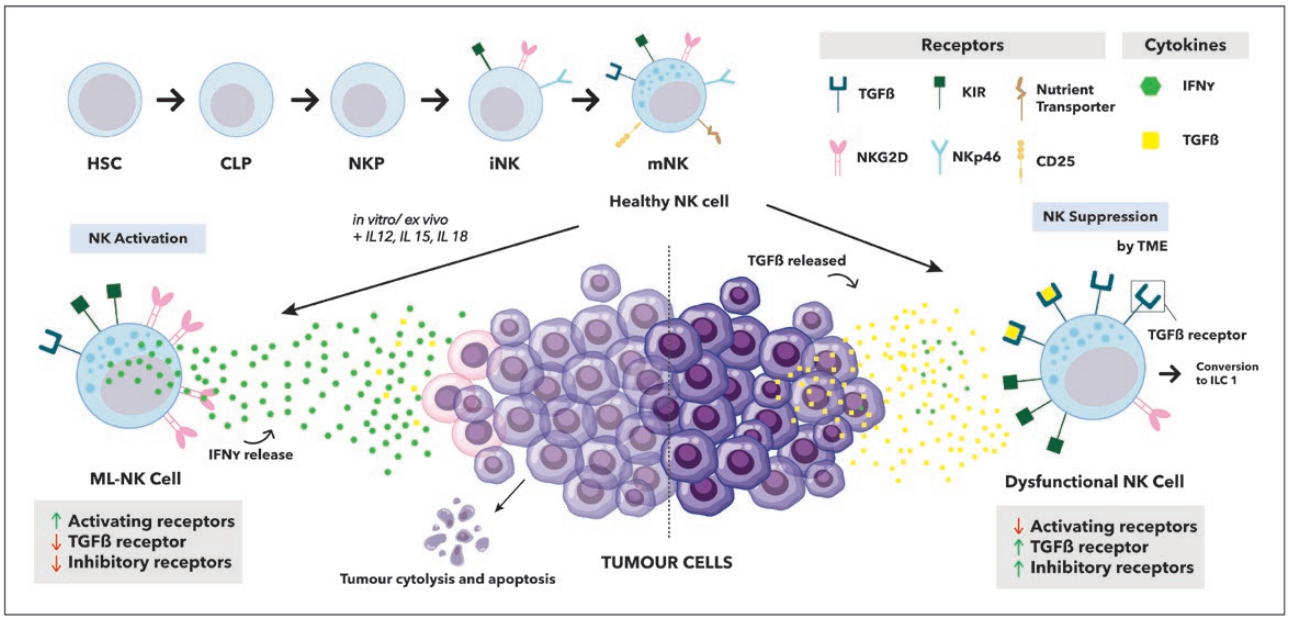

Figure 2. Schematic representation of exhausted NK cell (suppression) vs. ML-NK cell (activation) within the tumor microenvironment (TME). TME releasing TGFβ impairs normal NK cell activity by reducing activating signals and converting NK cells to ILC1 cells. Following cytokine induction, NK cells attain enhanced effector function as shown by elevated activating signals and IFNγ production, successfully transformed into memory-like NK cells.

reported notable improvement using allogeneic NK cells [28]. According to that study, transplantation of allogeneic NK cells was well tolerated without inducing severe adverse effects, even in an HLA-mismatch setting. The study reported that 4 out of 15 had objective responses at 2 months. Additionally, a clinical study using NK-cell therapy has successfully prolonged cancer remission and reduce the risk of relapse in children with AML [11]. On top of that, a phase 1 study performed on adult patients with ALL had also shown promising results in reducing relapse [50]. However, the study by Nguyen *et al*. on pediatric ALL did not produce similar results. The failure to respond to adoptive NK therapy may be due to the transient nature of pure NK cells and the low infusion dose given to this specific cohort [51]. From these findings, it is worth mentioning that the response rates toward NK-cell therapy vary among patients.

Based on previously mentioned studies, the preference for using donor cells compared to the patient's own NK cells for treating cancer is still debatable. Therefore, it is crucial for future studies to investigate the efficacy of allogeneic NK cells. Patient's own NK cells may be less sensitive to cytokine stimulation and are mostly functionally impaired. According to Terren *et al*.'s review, NK cells can be activated by many means including exposure to a tumor antigens [52]. Donor NK cells may have prior exposure to tumor antigen or carcinogens which could trigger the development of innate memory. When infused into cancer patients, these donor NK cells could recognize and attack cancer cells more effectively.

Memory-like NK cells

Recently, immense efforts have been made to improve NK-cell performance to become better fighters. Immunological memory in NK cells may confer the ability to recognize and react more rapidly against infection upon secondary encounter. It had been highlighted that the emergence of NK-cell memory can take place in two scenarios: antigen-dependent (hapten exposure and virus infection) or antigen-independent (cytokine induction) [53]. The characteristics, molecular mechanisms, and therapeutic opportunities of memory-like NK (ML-NK) cells are discussed in the second part of this review.

First discovered in mice deficient of T & B cells, results show that NK cells were able to mediate contact hypersensitivity response against specific haptens such as 2,4-dinitrofluorobenzene and oxazolone following secondary exposure [54]. Adoptive transfer of previously sensitized NK cells persisted about for 4 weeks. Besides their memory responses to haptens, NK cells also exhibit responses to viruses. In a murine cytomegalovirus (MCMV) model, NK cells demonstrated robust secondary expansion and protective immunity when re-encountered with viral antigen [55]. Prior exposure to viral antigen triggered a massive proliferation of preactivated NK cells, leading to the increased degranulation and cytokine release compared to naïve NK cells when rechallenged with virus. Theoretically, cytokine-mediated activation could generate NK cells that express similar memory traits. When subjected to cytokines, NK cells change the way they respond and transform into ML-NK cells [53, 56, 57]. This idea was shown in a previous experiment conducted by Cooper *et al*., pre-activated murine NK cells have shown enhanced IFNγ production against leukemia cells [58]. Consistent with Cooper *et al*.'s study, Jin *et al*. also suggested that cytokines are capable of inducing memory-like properties and these traits are passed down to the next generation [59]. Apart from that, increased expression of activating receptors was observed in cytokine-treated NK-cell population [60]. The transplantation of pre-activated NK cells successfully prolonged survival in leukemia mice. These studies support the notion that NK cells have the capabilities to develop memory-like features and recognize tumor or infected cells during reencounter [58–60].

Epigenetic changes in NK cells

Epigenetic regulation in immune cells is not only crucial for cell growth, development, and function but it also plays a vital role in initiating innate and adaptive immune memory [61–63]. Few studies suggested that epigenetic remodeling contributes to the persistence and development of memory in NK cells [64–66]. Epigenetic changes can influence the states of NK cells varying "from 'at rest', 'active', 'memory', 'repressed' and 'exhausted'." [67]. Epigenetics depicts chromatin modifications that regulate gene expression without affecting the gene sequence. Epigenetic events include DNA methylation, histone modification, chromatin remodeling, and non-coding RNA activity [68]. Rapid and effective innate immune response is triggered upon repeated stimuli resulting from immune-related gene activation [65].

According to study by Lau *et al*., memory NK cells have distinct epigenetic profiles compared to naïve NK cells. The study pointed out that *Prf1* locus, involved in NK cell-mediated cytotoxicity has enhanced chromatin accessibility in memory NK cells. Apart from that, the study also revealed that NK cells have undergone chromatin remodeling of genes involved in innate and adaptive immune function when encountering with pathogen. The overlap of accessible chromatin regions between NK cells and CD8 + T cells is of interest, given that genes known to mediate memory formation in T cells such as *Bach2, Tcf7,* and *Zeb2* may be associated with memory formation in NK cells as well. Different experimental models of infection show distinct epigenetic rewiring in NK cells during infection (Table 1) [55, 64–66, 69–74]. Epigenetic changes appear to be stable in memory-like NK cells and memory phenotypes are maintained in daughter cells [64]. Despite the lack of understanding of the epigenetic molecular mechanism governing NK cells, cytokines may have an impact on the epigenetic remodeling of chromatin structure [75]. Cytokines are capable of improving the accessibility of transcription factors such as STAT proteins which are involved in numerous genes associated with NK-cell activation and immune function [68, 75]. Similarly, epigenetic mechanisms in exhausted CD8+ T cells may be repressed [76]. Treatment with IL-15 could induce epigenetic reconfiguration to revert its normal function. Hence, this concept may be applied to revert exhausted NK cells in cancer patients. Therefore, further studies to explore epigenetic signatures in regions associated with NK immune memory will be beneficial.

Cytokine-induced ML-NK cells

Early studies revealed that pre-activation with specific combination of cytokines is necessary to produce ML-NK cells and support expansion *in vitro* [16, 58, 77]. Cytokines such as IL-2, IL-12, IL-15, IL-18, and IL-21 are vital for the development, survival, activation, and promotion of effective antitumor response of NK cells [78–81]. Exposure to these cytokines has significant impact on NK cells as cytokines have

Table 1. Adoptive transfer of memory-like NK cells and how its epigenetic changes could modulate NK-cell antitumor immunity

Model	Epigenetic mechanism	Epigenetic changes	Results	References
Virus (CMV)	DNA methylation	Hypomethylation of IFNG locus at CNS1 region in murine and human modelsDemethylation of CpG on PRDMI/BLIMP-1, ZBTB32/TZFP genes in NKG2C$^+$ cells. Hypermethylation of *FCER1G* (EAT-2 promoter) detected in NKG2C$^+$ cells. Hypermethylation of SYK at CpG-rich promoter region.	Increase in transcription of IFNγ; when methylated, decrease in IFNγ transcripts Murine Ly49H$^+$ receptor recognizes MCMV m157 glycoproteinHuman NKG2C$^+$ cells respond to CMV peptidesMemory-like NKG2C$^+$ cells previously exposed to HCMV express higher levels of NKG2C than seronegative individualsEnhanced antibody-dependent response	[55, 66, 69, 72–74]
	Chromatin remodeling	Increase in chromatin accessibility at effecter genes in Ly49H$^+$ NK cells accessed by ATAC-seq	More accessible to IL10 and DMNT3A genes	[65]
Cytokines	Histone acetylation	p300, histone acetyltransferase found in enhancers and super-enhancers of highly upregulated genes		[70]
Endotoxin (LPS)	Histone methylation	H3K4me1 at enhancer region of IFNG locus	Increase in IFNγ production upon secondary infectionNK cells persisted 9 weeks and protected from *E. coli* infectionInhibition of methyltransferase activity by sinefungin (*Snfg*) causes NK-cell memory lost	[71]
Cytokine induced (tumor)	DNA methylation	Demethylation of CNS1 in IFNG locus of preactivated NK cells in mice	Increase in IFNγ transcripts and protein	[64]

the ability to stimulate immune effector function [78]. This indicates that direct exposure to cytokines can be used as a strategy to strengthen antitumor immunity. One example is applying Interleukin-2 (IL-2) in treatments for renal cancer and metastatic melanoma due to their capability of promoting proliferation, enhancing cytolytic action, and increasing cytokine release of NK cells [82]. Indeed IL-2 play a crucial function in activating NK cells through JAK1/3-STAT5 signaling pathway by binding to IL-2R comprising of subunits CD25, CD122, and CD132. Despite its vital role in promoting NK-cell growth, a major increase in IL-2 would most likely induce the expansion of regulatory T cells (Tregs) in turn may restrict NK-cell activity. Alternatively, IL-15 could mediate NK-cell development and activation in a similar manner as IL-2 [78]. It could activate NK cells through JAK-STAT5 and mTOR pathways without stimulating regulatory T-cells.

An experiment conducted by Cooper *et al*. showed that brief exposure to human NK cells with IL12, 15, and 18 could trigger proliferation signals leading to NK-cell activation [58]. Upon receiving signals, these cells undergo expansion and "training" to become more efficient at targeting cancer cells. Profound effects such as enhanced proliferation and cytokine production were reported after restimulation *in vitro*. Similar results were observed in a study by Romee *et al*. where cytokine-induced ML-NK cells were co-cultured with leukemia cell lines [16]. Their subsequent study revealed that transplantation of cytokine-induced ML-NK cells displayed antitumor response in mouse models [77]. The synergistic effects of interleukins combination resulted in increased IFNγ production and cytotoxicity against leukemia targets in treated NK cells compared to untreated NK cells [16, 83]. Other studies have employed this method to characterize and potentially apply ML-NK cells for immunotherapy [59, 60, 83, 84].

Phenotypic modification of NK to ML-NK cells

Cytokine re-stimulation is able to induce a series of phenotypic alterations correlated with memory-like traits. This has been associated with observable changes including elevated expression of nutrient transporters (CD71, CD98, GLUT1, and GLUT3), activating receptors (NKG2D, NKp30, and NKp44), and downregulation of KIR and TGFβ receptors [16, 52, 57, 85, 86]. Elevated activating signals enable ML-NK cells to recognize tumor more rapidly compared to their naïve counterparts [57]. Further evidence suggested that these changes may contribute to the enhancement of cell differentiation, long-term persistence, effector function, and recall response observed in ML-NK cells [52, 57]. Up-regulation of potent stimulators such as Semaphorin 7A (SEMA7A) was also observed in ML-NK cells which promotes IFNγ release and differentiation of ML-NK cells [46]. Of note, increased IFNγ production in ML-NK cells mediates more cytotoxic killing and triggers a greater immune response against tumor cells [83]. The interaction between IFNγ and TME induces different pathways which halt the growth of cancer cells. For example, initiation of JAK/STAT1 signaling increases caspase activity resulting in apoptosis of tumor cells [87]. Apart from that, IFNγ triggers other pathways which inhibit tumor metastasis and angiogenesis, along with promoting tumor dormancy and senescence [88–91].

Preclinical and clinical studies

Following the success of generating ML-NK cells *ex vivo*, the antitumor effects of ML-NK cells are evaluated against a panel of cell lines and rodent models including HCC, melanoma, and ovarian cancer [83, 84, 92]. These preclinical studies have demonstrated robust responses against tumor targets as described in Table 2. *In vivo* study conducted by Ni *et al*. showed mice exposed to radiotherapy prior to the adoptive transfer of preactivated NK cells resulted in a significant reduction in tumor growth and prolonged lifespan in tumor-bearing mice [60]. Overall, these preclinical studies support the notion that cytokine-activated ML-NK cells inflict greater response against tumor cells compared to untreated counterparts. Enhanced IFNγ production and cytotoxic killing are evidence of enhanced antitumor response observed in ML-NK *in vitro* and *in vivo*.

In recent clinical studies, progress has been made in using ML-NK cells in providing therapeutic benefits to leukemia patients [86, 93]. Infusion of donor ML-NK cells is reported safe with limited toxicities, achieving robust expansion and proliferation post-infusion. Another pilot study that integrated chemotherapy and ML-NK cells for treating relapsed AML patients showed comparable outcomes [93]. Patients who received both treatment types showed a more favorable response than chemotherapy alone. ML-NK cells have safely induced remission with minimal toxicities in relapsed patients. Despite convincing preliminary results, there are still limited reported studies on treating hematological-related cancers and other cancer types with ML-NK cells. Having said that, ML-NK cell therapy could be further developed and potentially be applied to other cancers.

Future prospects

Current application of ML-NK cells is limited and mostly still undergoing clinical investigation. ML-NK cell therapy has great potential as an adjuvant or if used with other forms of treatment such as conventional therapy or monoclonal antibodies to boost antitumor effects with the goal of achieving significant clinical response [94–98]. Several clinical studies investigating the efficacy of NK cells in addition to their usual treatment regime to potentiate the therapeutic effects. For example, Ishikawa *et al*. used both adoptive NK-cell therapy and monoclonal antibodies for treating patients with advanced colorectal and gastric cancer [94]. Interestingly, encouraging results were obtained in four out of eight patients treated with combination therapy (monoclonal antibodies + NK), three of which displayed decreased tumor burden. In a separate study by Lee *et al*., breast cancer patients showed encouraging responses after receiving NK-cell therapy and trastuzumab [95]. This treatment combination has proven to be well-tolerated and improved overall outcomes in 25% of the cohort. On the other hand, combined NK-cell therapy with chemotherapy had considerably improved clinical outcomes for gastric cancer and colon cancer patients and was well tolerated [96–98]. Patients receiving combination therapy showed better quality of life and notably higher progression-free survival compared to chemotherapy alone. Another study conducted by Kim *et al*. also showed the therapeutic effects of combination therapy in a triple-negative breast cancer xenograft model [99]. Reduction of tumor growth was evident in the combination group (radiotherapy + NK) compared to the group receiving only NK cells. Given the great potential to prevent relapse and prolong survival in patients with advanced cancers using combination therapy, the same could be applied to ML-NK cells to yield more favorable outcomes.

Table 2. *In vitro* and *in vivo* therapeutic application of ML-NK

Type of cancer	Source of ML-NK	Study design	Therapeutic outcome	References
Acute lymphoblastic leukemia (ALL)	Mouse	Mouse	Enhanced IFNγ production Prolonged survival	[59]
Acute myeloid leukemia (AML)	Human	Cell lines, primary cells Mouse Human	Enhanced IFNγ production Reduced tumor burden, improved overall survival Four out of nine complete remissions	[77]
Melanoma	Human	Cell lines Rodents	Significant reduction in tumor cells HD and patient NK cells show potent cytotoxic action Enhanced IFNγ production, CD107a expression Decrease tumor burden after 21 days	[92]
Ovarian cancer	Human	Cell lines Mouse	Significant reduction in tumor cells Enhanced IFNγ production Reduction in viable tumor cell count Significant reduction in tumor burden in treated mice	[83]
Hepatocellular carcinoma	Mouse, Human	Cell lines Mouse	Varying efficacy against different cell lines, overall enhanced efficacy Reduced tumor burden in treated mice	[84]
AML	Human (allogeneic)	Human	Well tolerated and safe with little toxicities 50% achieve complete remission, overall survival is 42%	[86]
AML	Human (allogeneic)	Human	No CRS, GvDH, and neurotoxicity reported Induced remission in 47% of patients Increased IFNγ and MIP-1α	[93]

There is potential in developing allogeneic ML-NK cells as "off the shelf" products. This therapeutic modality offers the advantage of being non-patient-specific and may appeal to a wider population including the aging population. Recently, a unique combination of chimeric antigen receptors (CARs) and memory-like features on NK cells is proposed, CAR-ML-NK model is designed to achieve a greater on-target off-tumour effect [100]. It is important that ML-NK cells expand, persist, and maintains memory-like phenotype after infusion to generate longer-lasting antitumor effects. In a preliminary study on the efficacy of CAR-ML-NK against lymphoma cell lines using a xenograft model, the CAR-ML-NK cells demonstrated effective expansion, persistence, and enhanced antitumor response against lymphoma targets and controlled tumor burden in a mouse model [100]. Nevertheless, the current progress of this novel technique is limited and still in a very early stage of development. This could be the next venture in cancer immunotherapy given that further studies are warranted.

Conclusion

Overall, accumulating evidence shows that ML-NK-cell therapy holds promising results in improving adoptive NK-cell therapy. Here, we explored the features of ML-NK cells and opportunities to translate this therapeutic approach into a clinical setting. There are areas of ML-NK cells that are not well elucidated, particularly the molecular mechanisms associated with the development of ML-NK cells. In terms of clinical progress, notable improvements in early blood cancer trials have paved way for other cancer types and encouraged further investigation [86, 93]. Other aspects such as persistency and expansion capacity in patients, as well as their therapeutic efficacy in different cancer types should be further evaluated. With the advances and breakthroughs in cellular immunotherapy, ML-NK-cell therapy stands as a remarkable candidate with the potential at improving clinical outcomes in the long run.

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Conflict of Interests

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References

1. Zhang Y, Zhang Z. The history and advances in cancer immunotherapy: understanding the characteristics of tumor-infiltrating immune cells and their therapeutic implications. *Cell Mol Immunol* 2020, 17, 807–21. doi:10.1038/s41423-020-0488-6.
2. Hemminki O, Dos Santos JM, Hemminki A. Oncolytic viruses for cancer immunotherapy. *J Hematol Oncol* 2020, 13, 84. doi:10.1186/s13045-020-00922-1.
3. Lin MJ, Svensson-Arvelund J, Lubitz GS, Marabelle A, Melero I, Brown BD, et al. Cancer vaccines: the next immunotherapy frontier. *Nat Cancer* 2022, 3, 911–26. doi:10.1038/s43018-022-00418-6.
4. Conlon KC, Miljkovic MD, Waldmann TA. Cytokines in the treatment of cancer. *J Interferon Cytokine Res* 2019, 39, 6–21. doi:10.1089/jir.2018.0019.
5. Sharma P, Allison JP. Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential. *Cell* 2015, 161, 205–14. doi:10.1016/j.cell.2015.03.030.
6. Shah NN, Lee DW, Yates B, Yuan CM, Shalabi H, Martin S, et al. Long-term follow-up of CD19-CAR T-cell therapy in children and young adults with B-ALL. *J Clin Oncol* 2021, 39, 1650–9. doi:10.1200/JCO.20.02262.
7. Hucks G, Rheingold SR. The journey to CAR T cell therapy: the pediatric and young adult experience with relapsed or refractory B-ALL. *Blood Cancer J* 2019, 9, 10. doi:10.1038/s41408-018-0164-6.
8. Shah BD, Ghobadi A, Oluwole OO, Logan AC, Boissel N, Cassaday RD, et al. KTE-X19 for relapsed or refractory adult B-cell acute lymphoblastic leukaemia: phase 2 results of the single-arm, open-label, multicentre ZUMA-3 study. *Lancet* 2021, 398, 491–502. doi:10.1016/S0140-6736(21)01222-8.
9. Du N, Guo F, Wang Y, Cui J. NK Cell therapy: a rising star in cancer treatment. *Cancers* 2021, 13, 4129.
10. 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, 3051–7. doi:10.1182/blood-2004-07-2974.
11. Rubnitz JE, Inaba H, Ribeiro RC, Pounds S, Rooney B, Bell T, et al. NKAML: a pilot study to determine the safety and feasibility of haploidentical natural killer cell transplantation in childhood acute myeloid leukemia. *J Clin Oncol* 2010, 28, 955–9. doi:10.1200/JCO.2009.24.4590.
12. Lee DA, Denman CJ, Rondon G, Woodworth G, Chen J, Fisher T, et al. Haploidentical natural killer cells infused before allogeneic stem cell transplantation for myeloid malignancies: a phase I trial. *Biol Blood Marrow Transplant* 2016, 22, 1290–8. doi:10.1016/j.bbmt.2016.04.009.
13. 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, 7.
14. 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, 1233–45. doi:10.1007/s11427-015-4970-9.
15. Khawar MB, Sun H. CAR-NK cells: from natural basis to design for kill. *Front Immunol* 2021, 12, 707542. doi:10.3389/fimmu.2021.707542.
16. Romee R, Schneider SE, Leong JW, Chase JM, Keppel CR, Sullivan RP, et al. Cytokine activation induces human memory-like

NK cells. *Blood* 2012, 120, 4751–60. doi:10.1182/blood-2012-04-419283.
17. Cooper MA, Fehniger TA, Caligiuri MA. The biology of human natural killer-cell subsets. *Trends Immunol* 2001, 22, 633–40. doi:10.1016/s1471-4906(01)02060-9.
18. Poli A, Michel T, Thérésine M, Andrès E, Hentges F, Zimmer J. CD56bright natural killer (NK) cells: an important NK cell subset. *Immunology* 2009, 126, 458–65. doi:10.1111/j.1365-2567.2008.03027.x.
19. Romee R, Foley B, Lenvik T, Wang Y, Zhang B, Ankarlo D, et al. NK cell CD16 surface expression and function is regulated by a disintegrin and metalloprotease-17 (ADAM17). *Blood* 2013, 121, 3599–608. doi:10.1182/blood-2012-04-425397.
20. Bhatnagar N, Ahmad F, Hong HS, Eberhard J, Lu IN, Ballmaier M, et al. FcγRIII (CD16)-mediated ADCC by NK cells is regulated by monocytes and FcγRII (CD32). *Eur J Immunol* 2014, 44, 3368–79. doi:10.1002/eji.201444515.
21. Li Y, Sun R. Tumor immunotherapy: new aspects of natural killer cells. *Chin J Cancer Res* 2018, 30, 173–96. doi:10.21147/j.issn.1000-9604.2018.02.02.
22. Vivier E, Tomasello E, Baratin M, Walzer T, Ugolini S. Functions of natural killer cells. *Nat Immunol* 2008, 9, 503–10. doi:10.1038/ni1582.
23. 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, 44–9. doi:10.1126/science.1198687.
24. Lo Nigro C, Macagno M, Sangiolo D, Bertolaccini L, Aglietta M, Merlano MC. NK-mediated antibody-dependent cell-mediated cytotoxicity in solid tumors: biological evidence and clinical perspectives. *Ann Transl Med* 2019, 7, 105. doi:10.21037/atm.2019.01.42.
25. Nakajima T, Mizushima N, Kanai K. Relationship between natural killer activity and development of hepatocellular carcinoma in patients with cirrhosis of the liver. *JPN J Clin Oncol* 1987, 17, 327–32.
26. Ishigami S, Natsugoe S, Tokuda K, Nakajo A, Che X, Iwashige H, et al. Prognostic value of intratumoral natural killer cells in gastric carcinoma. *Cancer* 2000, 88, 577–83.
27. Mamessier E, Sylvain A, Thibult 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, 3609–22. doi:10.1172/JCI45816.
28. Bachanova V, Sarhan D, DeFor TE, Cooley S, Panoskaltsis-Mortari A, Blazar BR, et al. Haploidentical natural killer cells induce remissions in non-Hodgkin lymphoma patients with low levels of immune-suppressor cells. *Cancer Immunol Immunother* 2018, 67, 483–94. doi:10.1007/s00262-017-2100-1.
29. Zhang W, Xie X, Mi H, Sun J, Ding S, Li L, et al. Abnormal populations and functions of natural killer cells in patients with myelodysplastic syndromes. *Oncol Lett* 2018, 15, 5497–504. doi:10.3892/ol.2018.8062.
30. Slattery K, Woods E, Zaiatz-Bittencourt V, Marks S, Chew S, Conroy M, et al. TGFβ drives NK cell metabolic dysfunction in human metastatic breast cancer. *J ImmunoTher Cancer* 2021, 9, e002044. doi:10.1136/jitc-2020-002044.
31. Chang CH, Qiu J, O'Sullivan D, Buck MD, Noguchi T, Curtis JD, et al. Metabolic competition in the tumor microenvironment is a driver of cancer progression. *Cell* 2015, 162, 1229–41. doi:10.1016/j.cell.2015.08.016.
32. Rossi GR, Trindade ES, Souza-Fonseca-Guimaraes F. Tumor microenvironment-associated extracellular matrix components regulate NK cell function. *Front Immunol* 2020, 11, 73. doi:10.3389/fimmu.2020.00073.
33. Kaweme NM, Zhou F. Optimizing NK cell-based immunotherapy in myeloid leukemia: abrogating an immunosuppressive microenvironment. *Front Immunol* 2021, 12, 683381. doi:10.3389/fimmu.2021.683381.
34. Petrova V, Annicchiarico-Petruzzelli M, Melino G, Amelio I. The hypoxic tumour microenvironment. *Oncogenesis* 2018, 7, 10. doi:10.1038/s41389-017-0011-9.
35. Lee EHC, Wong DCP, Ding JL. NK cells in a tug-of-war with cancer: the roles of transcription factors and cytoskeleton. *Front Immunol* 2021, 12, 734551. doi:10.3389/fimmu.2021.734551.
36. Melaiu O, Lucarini V, Cifaldi L, Fruci D. Influence of the tumor microenvironment on NK cell function in solid tumors. *Front Immunol* 2019, 10, 3038. doi:10.3389/fimmu.2019.03038.
37. Lazarova M, Steinle A. Impairment of NKG2D-mediated tumor immunity by TGF-β. *Front Immunol* 2019, 10, 2689. doi:10.3389/fimmu.2019.02689.
38. Regis S, Dondero A, Caliendo F, Bottino C, Castriconi R. NK cell function regulation by TGF-β-induced epigenetic mechanisms. *Front Immunol* 2020, 11, 311. doi:10.3389/fimmu.2020.00311.
39. Gao Y, Souza-Fonseca-Guimaraes F, Bald T, Ng SS, Young A, Ngiow SF, et al. Tumor immunoevasion by the conversion of effector NK cells into type 1 innate lymphoid cells. *Nat Immunol* 2017, 18, 1004–15. doi:10.1038/ni.3800.
40. Kiekens L, Van Loocke W, Taveirne S, Wahlen S, Persyn E, Van Ammel E, et al. T-BET and EOMES accelerate and enhance functional differentiation of human natural killer cells. *Front Immunol* 2021, 12, 732511. doi:10.3389/fimmu.2021.732511.
41. Viel S, Marçais A, Guimaraes FS, Loftus R, Rabilloud J, Grau M, et al. TGF-β inhibits the activation and functions of NK cells by repressing the mTOR pathway. *Sci Signal* 2016, 9, ra19.
42. Stefano R, Fabio C, Alessandra D, Francesca B, Beatrice C, Cristina B, et al. Main NK cell receptors and their ligands: regulation by microRNAs. *AIMS Allergy and Immunology* 2018, 2, 98–112.
43. Espinoza JL, Akiyoshi T, Katsuji Y, Katsuya N, Tokiharu S, Yoshihito K, et al. Human microRNA-1245 down-regulates the NKG2D receptor in natural killer cells and impairs NKG2D-mediated functions. *Haematologica* 2012, 97, 1295–303.
44. Donatelli SS, Zhou JM, Gilvary DL, Eksioglu EA, Chen X, Cress WD, et al. TGF-β-inducible microRNA-183 silences tumor-associated natural killer cells. *Proc Natl Acad Sci U S A* 2014, 111, 4203–8. doi:10.1073/pnas.1319269111.
45. Xu D, Han Q, Hou Z, Zhang C, Zhang J. miR-146a negatively regulates NK cell functions via STAT1 signaling. *Cell Mol Immunol* 2017, 14, 712–20.
46. Castriconi R, Cantoni 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, 4120–5. doi:10.1073/pnas.0730640100.
47. Shaver KA, Croom-Perez TJ, Copik AJ. Natural killer cells: the linchpin for successful cancer immunotherapy. *Front Immunol* 2021, 12, 679117. doi:10.3389/fimmu.2021.679117.
48. Miller JS, Tessmer-Tuck J, Pierson BA, Weisdorf D, McGlave P, Blazar BR, et al. Low dose subcutaneous interleukin-2 after autologous transplantation generates sustained in vivo natural killer cell activity. *Biol Blood Marrow Transplant* 1997, 3, 34–44.
49. Burns LJ, Weisdorf DJ, DeFor TE, Vesole DH, Repka TL, Blazar BR, et al. IL-2-based immunotherapy after autologous transplantation for lymphoma and breast cancer induces immune activation and cytokine release: a phase I/II trial. *Bone Marrow Transplant* 2003, 32, 177–86. doi:10.1038/sj.bmt.1704086.
50. Ciurea SO, Schafer JR, Bassett R, Denman CJ, Cao K, Willis D, et al. Phase 1 clinical trial using mbIL21 ex vivo-expanded donor-derived NK cells after haploidentical transplantation. *Blood* 2017, 130, 1857–68. doi:10.1182/blood-2017-05-785659.
51. Nguyen R, Wu H, Pounds S, Inaba H, Ribeiro RC, Cullins D, et al. A phase II clinical trial of adoptive transfer of haploidentical natural killer cells for consolidation therapy of pediatric acute myeloid leukemia. *J ImmunoTher Cancer* 2019, 7, 81. doi:10.1186/s40425-019-0564-6.
52. Terrén I, Orrantia A, Astarloa-Pando G, Amarilla-Irusta A, Zenarruzabeitia O, Borrego F. Cytokine-induced memory-like NK cells: from the basics to clinical applications. *Front Immunol* 2022, 13, 884648. doi:10.3389/fimmu.2022.884648.
53. Gang M, Wong P, Berrien-Elliott MM, Fehniger TA. Memory-like natural killer cells for cancer immunotherapy. *Semin Hematol* 2020, 57, 185–93. doi:10.1053/j.seminhematol.2020.11.003.

54. O'Leary JG, Goodarzi M, Drayton DL, von Andrian UH. T cell- and B cell-independent adaptive immunity mediated by natural killer cells. *Nat Immunol* 2006, 7, 507–16. doi:10.1038/ni1332.
55. Sun JC, Beilke JN, Lanier LL. Adaptive immune features of natural killer cells. *Nature* 2009, 457, 557–61. doi:10.1038/nature07665.
56. 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.
57. Tarannum M, Romee R. Cytokine-induced memory-like natural killer cells for cancer immunotherapy. *Stem Cell Res Ther* 2021, 12, 592. doi:10.1186/s13287-021-02655-5.
58. Cooper MA, Elliott JM, Keyel PA, Yang L, Carrero JA, Yokoyama WM. Cytokine-induced memory-like natural killer cells. *Proc Natl Acad Sci U S A* 2009, 106, 1915–9. doi:10.1073/pnas.0813192106.
59. Jin F, Lin H, Gao S, Hu Z, Zuo S, Sun L, et al. The anti-tumor role of NK cells in vivo pre-activated and re-stimulated by interleukins in acute lymphoblastic leukemia. *Oncotarget* 2016, 7, 79187–202. doi:10.18632/oncotarget.13007.
60. Ni J, Miller M, Stojanovic A, Garbi N, Cerwenka A. Sustained effector function of IL-12/15/18-preactivated NK cells against established tumors. *J Exp Med* 2012, 209, 2351–65. doi:10.1084/jem.20120944.
61. Suárez-Álvarez B, Baragaño Raneros A, Ortega F, López-Larrea C. Epigenetic modulation of the immune function: a potential target for tolerance. *Epigenetics* 2013, 8, 694–702. doi:10.4161/epi.25201.
62. Chen Y, Zander R, Khatun A, Schauder DM, Cui W. Transcriptional and epigenetic regulation of effector and memory CD8 T cell differentiation. *Front Immunol* 2018, 9, 2826. doi:10.3389/fimmu.2018.02826.
63. Cichocki F, Miller JS, Anderson SK, Bryceson YT. Epigenetic regulation of NK cell differentiation and effector functions. *Front Immunol 2013*, 4, 55. doi:10.3389/fimmu.2013.00055.
64. Ni J, Hölsken O, Miller M, Hammer Q, Luetke-Eversloh M, Romagnani C, et al. Adoptively transferred natural killer cells maintain long-term antitumor activity by epigenetic imprinting and CD4(+) T cell help. *Oncoimmunology* 2016, 5, e1219009. doi:10.1080/2162402X.2016.1219009.
65. Lau CM, Adams NM, Geary CD, Weizman OE, Rapp M, Pritykin Y, et al. Epigenetic control of innate and adaptive immune memory. *Nat Immunol* 2018, 19, 963–72. doi:10.1038/s41590-018-0176-1.
66. Luetke-Eversloh M, Hammer Q, Durek P, Nordström K, Gasparoni G, Pink M, et al. Human cytomegalovirus drives epigenetic imprinting of the IFNG locus in NKG2Chi natural killer cells. *PLoS Pathog* 2014, 10, e1004441e1004441. doi:10.1371/journal.ppat.1004441.
67. Xia M, Wang B, Wang Z, Zhang X, Wang X. Epigenetic regulation of NK cell-mediated antitumor immunity. *Front Immunol* 2021, 12, 672328. doi:10.3389/fimmu.2021.672328.
68. Beaulieu AM. Transcriptional and epigenetic regulation of memory NK cell responses. *Immunol Rev* 2021, 300, 125–33. doi:10.1111/imr.12947.
69. Adams NM, Lau CM, Fan X, Rapp M, Geary CD, Weizman OE, et al. Transcription factor IRF8 orchestrates the adaptive natural killer cell response. *Immunity* 2018, 48, 1172–82.e6. doi:10.1016/j.immuni.2018.04.018.
70. Sciumè G, Mikami Y, Jankovic D, Nagashima H, Villarino AV, Morrison T, et al. Rapid enhancer remodeling and transcription factor repurposing enable high magnitude gene induction upon acute activation of NK cells. *Immunity* 2020, 53, 745–58.e4. doi:10.1016/j.immuni.2020.09.008.
71. Rasid O, Chevalier C, Camarasa TM, Fitting C, Cavaillon JM, Hamon MA. H3K4me1 supports memory-like NK cells induced by systemic inflammation. *Cell Rep* 2019, 29, 3933–45.e3. doi:10.1016/j.celrep.2019.11.043.
72. Lee J, Zhang T, Hwang I, Kim A, Nitschke L, Kim M, et al. Epigenetic modification and antibody-dependent expansion of memory-like NK cells in human cytomegalovirus-infected individuals. *Immunity* 2015, 42, 431–42. doi:10.1016/j.immuni.2015.02.013.
73. Hammer Q, Rückert T, Borst EM, Dunst J, Haubner A, Durek P, et al. Peptide-specific recognition of human cytomegalovirus strains controls adaptive natural killer cells. *Nat Immunol* 2018, 19, 453–63. doi:10.1038/s41590-018-0082-6.
74. Schlums H, Cichocki F, Tesi B, Theorell J, Beziat V, Holmes TD, et al. Cytomegalovirus infection drives adaptive epigenetic diversification of NK cells with altered signaling and effector function. *Immunity* 2015, 42, 443–56. doi:10.1016/j.immuni.2015.02.008.
75. Rapp M, Lau CM, Adams NM, Weizman OE, O'Sullivan TE, Geary CD, et al. Core-binding factor β and Runx transcription factors promote adaptive natural killer cell responses. *Sci Immunol* 2017, 2, eaan3796. doi:10.1126/sciimmunol.aan3796.
76. Villanueva L, Álvarez-Errico D, Esteller M. The contribution of epigenetics to cancer immunotherapy. *Trends Immunol* 2020, 41, 676–91. doi:10.1016/j.it.2020.06.002.
77. Romee R, Rosario M, Berrien-Elliott MM, Wagner JA, Jewell BA, Schappe T, et al. Cytokine-induced memory-like natural killer cells exhibit enhanced responses against myeloid leukemia. *Sci Transl Med* 2016, 8, 357ra123. doi:10.1126/scitranslmed.aaf2341.
78. Wu Y, Tian Z, Wei H. Developmental and functional control of natural killer cells by cytokines. *Front Immunol* 2017, 8, 930. doi:10.3389/fimmu.2017.00930.
79. Romee R, Leong JW, Fehniger TA. Utilizing cytokines to function-enable human NK cells for the immunotherapy of cancer. *Scientifica* 2014, 2014, 205796. doi:10.1155/2014/205796.
80. Carson WE, Giri JG, Lindemann MJ, Linett ML, Ahdieh M, Paxton R, et al. Interleukin (IL) 15 is a novel cytokine that activates human natural killer cells via components of the IL-2 receptor. *J Exp Med* 1994, 180, 1395–403. doi:10.1084/jem.180.4.1395.
81. Li Q, Ye LJ, Ren HL, Huyan T, Li J, Shi JL, et al. Multiple effects of IL-21 on human NK cells in ex vivo expansion. *Immunobiology* 2015, 220, 876–88. doi:10.1016/j.imbio.2015.01.009.
82. Liao W, Lin JX, Leonard WJ. IL-2 family cytokines: new insights into the complex roles of IL-2 as a broad regulator of T helper cell differentiation. *Curr Opin Immunol* 2011, 23, 598–604. doi:10.1016/j.coi.2011.08.003.
83. Uppendahl LD, Felices M, Bendzick L, Ryan C, Kodal B, Hinderlie P, et al. Cytokine-induced memory-like natural killer cells have enhanced function, proliferation, and in vivo expansion against ovarian cancer cells. *Gynecol Oncol* 2019, 153, 149–57. doi:10.1016/j.ygyno.2019.01.006.
84. Zhuang L, Fulton RJ, Rettman P, Sayan AE, Coad J, Al-Shamkhani A, et al. Activity of IL-12/15/18 primed natural killer cells against hepatocellular carcinoma. *Hepatol Int* 2019, 13, 75–83. doi:10.1007/s12072-018-9909-3.
85. Ewen EM, Pahl JHW, Miller M, Watzl C, Cerwenka A. KIR downregulation by IL-12/15/18 unleashes human NK cells from KIR/HLA-I inhibition and enhances killing of tumor cells. *Eur J Immunol* 2018, 48, 355–65. doi:10.1002/eji.201747128.
86. Berrien-Elliott MM, Cashen AF, Cubitt CC, Neal CC, Wong P, Wagner JA, et al. Multidimensional analyses of donor memory-like NK cells reveal new associations with response after adoptive immunotherapy for leukemia. *Cancer Discov* 2020, 10, 1854–71. doi:10.1158/2159-8290.CD-20-0312.
87. Jorgovanovic D, Song M, Wang L, Zhang Y. Roles of IFN-γ in tumor progression and regression: a review. *Biomark Res* 2020, 8, 49. doi:10.1186/s40364-020-00228-x.
88. Glasner A, Levi A, Enk J, Isaacson B, Viukov S, Orlanski S, et al. NKp46 receptor-mediated interferon-γ production by natural killer cells increases fibronectin 1 to alter tumor architecture and control metastasis. *Immunity* 2018, 48, 107–19.e4. doi:10.1016/j.immuni.2017.12.007.
89. Hayakawa Y, Takeda K, Yagita H, Smyth MJ, Van Kaer L, Okumura K, et al. IFN-gamma-mediated inhibition of tumor angiogenesis by natural killer T-cell ligand, alpha-galactosylceramide. *Blood* 2002, 100, 1728–33.
90. Liu Y, Liang X, Yin X, Lv J, Tang K, Ma J, et al. Blockade of IDO-kynurenine-AhR metabolic circuitry abrogates IFN-γ-induced

immunologic dormancy of tumor-repopulating cells. *Nat Commun* 2017, 8, 15207. doi:10.1038/ncomms15207.
91. Braumüller H, Wieder T, Brenner E, Aßmann S, Hahn M, Alkhaled M, et al. T-helper-1-cell cytokines drive cancer into senescence. *Nature* 2013, 494, 361–5. doi:10.1038/nature11824.
92. Marin ND, Krasnick BA, Becker-Hapak M, Conant L, Goedegebuure SP, Berrien-Elliott MM, et al. Memory-like differentiation enhances NK cell responses to melanoma. *Clin Cancer Res* 2021, 27, 4859–69. doi:10.1158/1078-0432.CCR-21-0851.
93. Bednarski JJ, Zimmerman C, Berrien-Elliott MM, Foltz JA, Becker-Hapak M, Neal CC, et al. Donor memory-like NK cells persist and induce remissions in pediatric patients with relapsed AML after transplant. *Blood* 2022, 139, 1670–83. doi:10.1182/blood.2021013972.
94. Ishikawa T, Okayama T, Sakamoto N, Ideno M, Oka K, Enoki T, et al. Phase I clinical trial of adoptive transfer of expanded natural killer cells in combination with IgG1 antibody in patients with gastric or colorectal cancer. *Int J Cancer* 2018, 142, 2599–609. doi:10.1002/ijc.31285.
95. Lee SC, Shimasaki N, Lim JSJ, Wong A, Yadav K, Yong WP, et al. Phase I trial of expanded, activated autologous NK-cell infusions with trastuzumab in patients with HER2-positive cancers. *Clin Cancer Res* 2020, 26, 4494–502. doi:10.1158/1078-0432.CCR-20-0768.
96. Cui J, Li L, Wang C, Jin H, Yao C, Wang Y, et al. Combined cellular immunotherapy and chemotherapy improves clinical outcome in patients with gastric carcinoma. *Cytotherapy* 2015, 17, 979–88. doi:10.1016/j.jcyt.2015.03.605.
97. Wang Y, Wang C, Xiao H, Niu C, Wu H, Jin H, et al. Adjuvant treatment combining cellular immunotherapy with chemotherapy improves the clinical outcome of patients with stage II/III gastric cancer. *Cancer Med* 2017, 6, 45–53. doi:10.1002/cam4.942.
98. 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, 134–48. doi:10.1016/j.jcyt.2017.09.009.
99. Kim KW, Jeong JU, Lee KH, Uong TNT, Rhee JH, Ahn SJ, et al. Combined NK cell therapy and radiation therapy exhibit long-term therapeutic and antimetastatic effects in a human triple negative breast cancer model. *Int J Radiat Oncol Biol Phys* 2020, 108, 115–25. doi:10.1016/j.ijrobp.2019.09.041.
100. Gang M, Marin ND, Wong P, Neal CC, Marsala L, Foster M, et al. CAR-modified memory-like NK cells exhibit potent responses to NK-resistant lymphomas. *Blood* 2020, 136, 2308–18. doi:10.1182/blood.2020006619.