Natural killer (NK) cells, a subgroup of innate lymphoid cells, act as the first line of defense against cancer. Although some evidence shows that NK cells can develop in secondary lymphoid tissues, NK cells develop mainly in the bone marrow (BM) and egress into the blood circulation when they mature. They then migrate to and settle down in peripheral tissues, though some special subsets home back into the BM or secondary lymphoid organs. Owing to its success in allogeneic adoptive transfer for cancer treatment and its "off-the-shelf" potential, NK cell-based immunotherapy is attracting increasing attention in the treatment of various cancers. However, insufficient infiltration of adaptively transferred NK cells limits clinical utility, especially for solid tumors. Expansion of NK cells or engineered chimeric antigen receptor (CAR) NK cells ex vivo prior to adoptive transfer by using various cytokines alters the profiles of chemokine receptors, which affects the infiltration of transferred NK cells into tumor tissue. Several factors control NK cell trafficking and homing, including cell-intrinsic factors (e.g., transcriptional factors), cell-extrinsic factors (e.g., integrins, selectins, chemokines and their corresponding receptors, signals induced by cytokines, sphingosine-1-phosphate (S1P), etc.), and the cellular microenvironment. Here, we summarize the profiles and mechanisms of NK cell homing and trafficking at steady state and during tumor development, aiming to improve NK cell-based cancer immunotherapy.

INTRODUCTION
Natural killer (NK) cells are an important part of the innate immune system. Unlike T and B cells, they rapidly attack target cells without prior sensitization. These group I innate lymphoid cells (ILCs) express T-bet and produce T helper cell type 1 (Th1)-associated cytokines, such as interferon-γ (IFN-γ). As they mature, NK cells are able to migrate from the bone marrow (BM) into the blood and settle down in peripheral tissues. Their ability to circulate between lymphatic and non-lymphoid organs results in their presence in most tissues. NK cells also acquire effector functions as they mature, including natural cytotoxicity that target cells such as tumor cells or cells infected with virus. NK cells also produce cytokines, growth factors, and chemokines that help shape adaptive immune responses by interacting with other immune cells. These powerful effector functions and extensive tissue distribution enable NK cells to play important roles in a variety of diseases, including cancer, infectious diseases, autoimmunity, and chronic inflammation. NK cells are considered to be an effective innate immune cell subset involved in immune surveillance of hematological malignancies and solid tumors as well as metastatic spreading. Indeed, infiltration of cytotoxic NK cells into tumors is a positive prognostic marker for a variety of cancers, including melanoma, renal cell carcinoma (RCC), liver cancer, lung, and breast cancer. 

NK cell-based immunotherapy has been explored in clinical trials since the 1980s through adoptive transfer of autologous lymphokine-activated killer cells into patients with advanced cancers, who have shown marked tumor regression. The CD4 zeta chimeric receptor was the first chimeric antigen receptor (CAR) introduced to NK cells, and it was highly effective at killing target cells expressing HIV gp120 in vitro. In 2002, Ruggeri et al. reported that outcomes of patients with acute myeloid leukemia (AML) transplanted with hematopoietic stem cells (HSCs) correlated positively with the anti-leukemic effect of allogeneic NK cells. In a pioneering study in 2005, Miller et al. adoptively transferred allogeneic activated NK cells into patients with AML, inducing major anti-tumor responses. The first human clinical trial, reported in 2018, tested the safety of transferring CAR NK-92 cells into patients with relapsed and refractory AML, and it targeted CD33. In the same year, the Kaufman group first derived CAR NK cells from induced pluripotent stem cells (iPSCs) expressing a CAR. In animal models, those cells appeared to have better cytotoxic activity against solid tumors than peripheral blood-derived CAR NK cells or CAR T cells. In 2020, a clinical trial (NCT03056339) derived HLA-mismatched NK cells from umbilical cord blood and armed them with CD19-CAR showed significant benefits in relapsed or refractory CD19-positive lymphoma and leukemia, without substantial toxicity. This suggests that CAR NK cells have the potential to become effective allogeneic anti-cancer immunotherapeutic products, which have sparked great interests in the field of cancer immunotherapy. However, the therapeutic efficacy of nonengineered NK cells is much less than desired, especially in solid tumors. The development of NK cell immunotherapy is summarized in Fig. 1.
Fig. 1 The development of immunotherapy based on NK cells. Applications of various NK cell strategies in preclinical and clinical trials are listed. Since the discovery of NK cells in 1970s, NK cells have been used in clinical treatment. The combination of IL-2 and killer cells in the treatment of non-Hodgkin’s lymphoma (NHL) and metastatic RCC (mRCC), starting from 1987. Antibodies enhancing NK cell effector function were produced starting from 1992, including NK bi-specific monoclonal antibody targeting ovarian cancer cells in 1992, Trastuzumab (anti-HER2) in the treatment of breast cancer and gastric cancer, in 1998, anti-KIR antibody preclinical test in 2014, and tri-specific NK cell engagers preclinical test in 2016. Clinical trials using ex vivo expanded peripheral blood mononuclear cell (PBMC)-derived NK cells for non-small cell lung cancer (NSCLC) were started in 2010, while iPSC-derived NK (iPSC-NK) cells were used for AML treatment in 2017, and for multiple myeloma (MM) in 2019. CD4 zeta chimeric receptors were firstly introduced into NK cells and showed highly effective at killing target cells expressing HIV gp120 in vitro in 1995. Recently, a variety of clinical phase I or II trials were started, including Phase1/2 CD19-chimeric antigen NK cells for CD19-positive lymphoid malignancies in 2017 (NCT03056339), Phase1/2 CD33+CAR NK cells for AML treatment in 2021 and 2022, and CAR iPSC-NK cells for ovarian cancer were started in 2018, while clinical trials of CAR iPSC-NK cells for treating NHL or chronic lymphoblastic leukemia (CLL) in 2020. Created with BioRender.com

The tumor microenvironment (TME) contains a multitude of immunosuppressive factors for NK cells. The density of endogenous NK cells is extremely low in various human solid tumors, with no more than 100 cells per mm² in contrast to several hundred CD8⁺ T cells per mm². This suggests that understanding the trafficking of NK cells into the TME may spawn better strategies for improving the effectiveness of NK cell-based immunotherapy for cancer patients. Here, we review the latest data on the trafficking and homing of NK cells into normal tissues and tumors, focusing mainly on the BM, the blood circulation, tissue specificity, intra-tumor movement, and factors controlling NK cell trafficking.

NK CELL DEVELOPMENT, MATURATION, AND ACTIVATION

NK cells, especially in mice, were thought to differentiate from HSCs exclusively in the BM and then migrate to peripheral tissues. Recently, however, NK progenitors and immature cells were reported to also mature in secondary lymphoid tissues, such as lymph nodes (LNs), tonsils, and spleen. HSCs differentiate gradually from lymphoid-induced pluripotent progenitor cells and then become common lymphoid progenitor cells (CLPs). CLPs produce the precursors of all lymphocyte populations, including T cells, B cells, NK cells, and recently defined ILC subsets. Upon differentiation, NK cells show dramatic phenotypic changes, including expression of cytokine receptors (e.g., CD122, CD217), adhesion molecules (e.g., integrins), chemotactant receptors, and activating or inhibitory receptors that control their maturation, migration, and effector functions.

In mice, HSC populations are defined as lineage negative (Lin⁻) stem cell antigen (Sca)⁻ CD117 (c-Kit)⁻, which differentiate into CLP populations—Lin⁻Sca⁺CD117⁺CD135⁻CD127 (IL7Rα)⁻ CLPs then differentiate into NK progenitors (NKP, Lin CD112⁻NK1.1⁺ DX5⁻) through an intermediate stage, defined as pre-NKPs (Lin CD117⁺CD135⁻CD27⁺CD244⁺CD117⁺CD127⁺CD122⁻) that lack the expression of CD122. The acquisition of CD122, the common β chain of IL-2 and IL-15, is a critical step in the differentiation of NK cells. IL-15 can promote NK cell differentiation, maturation, and survival, and it is constitutively produced by BM stromal cells, activated monocytes, and dendritic cells (DCs). Expression of the activation receptor complex NKGD2/DNAX-activating protein of 10 kDa (DAP10) defines the initial stage of NKPs' progression to immature NK (iNK) cells. Previous studies defined iNK cells and mature NK (mNK) cells as Lin CD122⁻NK1.1⁺ DX5⁻ and Lin CD122⁺NK1.1⁺ DX5⁺, respectively, and showed that expression of DX5 (also called CD49b or integrin α2) initiates the terminal stage of NK cell maturation. However, this scheme neglects a subpopulation of NK cells—NK1.1⁺ DX5⁻ which account for 10% of Lin⁺ CD122⁺ cells. Our recent study refined this developmental process, defining CD122⁺NK1.1⁺ DX5⁻ Nkp46⁻ and CD122⁺NK1.1⁺ DX5⁺ Nkp46⁺ cells as iNK-a and iNK-b populations, respectively, and CD122⁺NK1.1⁺ DX5⁻ Nkp46⁺ as a mNK population. Expression of CD43 and CD11b (also called Itgam or Mac-1) defines terminally mature NK cell maturation. Depending on the expression of CD11b and CD27, NK cell maturation from iNK to mNK can also be divided into four continuous stages: from CD11b⁺CD27⁻ (DN) to CD11b⁺CD27⁺ to CD11b⁻CD27⁺ (DP) and to CD11b⁻CD27⁻. In mNK cells, acquisition of inhibitory Ly49...
receptors, including Ly49A, Ly49C/I, Ly49G, and NK2A, can define functional license (Fig. 2a).46

Human NK cells may be able to develop not only in the BM but also in extramedullary tissues, such as tonsils and lymph nodes.47 The developmental tree of human NK cells is still challenging, especially in relation to ILC subsets, however.6,48 The prevailing linear model posits that human NK cells develop along a continuum in which CLPs (Lin−CD34+CD10−CD127−) gradually downregulate CD34 and upregulate CD56.6,48 HSCs (Lin−CD34+CD120+CD45RA−CD10+) differentiate into multipotent progenitors (MPP, Lin−CD34+CD38−CD90+CD45RA+) and then transition into CLPs (Lin−CD34+CD38−CD45RA−CD10+) with the potential to commit to Pro-B, Pre-T, NKPs (Lin−IL-1R1+CD122−CD38−CD90−CD45RA+) and then other ILC progenitors.6,48 Acqisition of IL-1R1 marks the earlier stages of committed NKPs, while the appearance of CD122 indicates irreversible lineage specification of NK cells from CLPs. NKPs differentiate into iNK cells, characterized by higher expression of IL-1R1 and the appearance of CD314 (NKG2D), CD335 (NKp46), CD337 (NKp30), and CD161 (the human homolog of the mouse NK1.1). The next transitional stage is the appearance of CD56bright NK cells, marked by high expression of CD56 along with maximal expression of NKG2D, CD56bright NK cells have different ontogenies and that NKPs can directly generate CD56 bright and CD56dim NK cells (Fig. 2b).

The activation of NK cells and their cytotoxic attack of target cells is immediate, as it does not require prior antigen presentation or major histocompatibility complex (MHC)-restriction. When NK cells encounter tumor cells, their activation depends on the balance between activating and inhibitory signals that are produced as the various NK cell receptors interact with their ligands from on target cells.46,58 The main activating receptors include natural cytotoxicity triggering receptors (NCRs; NKp30, NKp44, NKp46), killer cell immunoglobulin-like receptors (KIRs: KIR-2DS and KIR-3DS), and C-type lectin receptors (NKGD2, CD94/NKG2C, NKG2E/H, and NKG2F). Inhibitory receptors include mainly KIRs (KIR-2DL and KIR-3DL) and C-type lectin receptors (CD94/NKG2A/B).46,58 MHC class I (MHC-I) molecules, which are present in most host healthy cells, can bind to KIR-2DL, KIR-3DL or CD94/NKG2A/B on NK cells to avoid NK cell-mediated killing. In mice, NK cells use the Ly49 family of lectin-like receptors instead of the KIRs used by humans.46,58

NK cells become activated when they lose their inhibitory signals upon encountering target cells that downregulate MHC-I expression. Activated NK cells eliminate target cells mainly by directly releasing perforin and granzymes, which lyse tumor cells. NK cells can also exert antibody-dependent cellular cytotoxicity (ADCC) via the membrane receptor CD16, or use Fas ligand (FasL) or TNF-related apoptosis-inducing ligand (TRAIL) to induce target cell apoptotic pathways.29

**NK CELL TRAFFICKING AND HOMING AT STEADY STATE**

NK cells develop from HSCs in a continuous process and in a specialized niche, the BM parenchyma, which is localized in
Natural killer cell homing and trafficking in tissues and tumors: from... Ran et al.

Overview of molecules responsible for NK cell homing and trafficking
Factors that control NK cell trafficking and homing include integrins, selectins, and chemokine receptors as well as their corresponding receptors or ligand, signals induced by cytokines, spondigosine-1-phosphate (SIP), and cellular microenvironments (summarized in Fig. 3). Integrins and chemokine receptors are commonly used as biomarkers for describing circulating and tissue-resident subsets.61

Integrins are a group of transmembrane receptors, including 18 reported α subunits and 8 reported β subunits. Each a subunit can bind with multiple β subunits to form various integrin heterodimers.61 To communicate with the extracellular matrix (ECM) and other cells, each integrin heterodimer can bind to multiple extracellular binding partner, including over 20 different ECM components, selectins, and cell adhesion molecules (CAMs).61 The structures and functions of integrins are highly modular and adaptable, enabling cells to change their size and therefore their trafficking speed.61 A variety of integrins expressed on NK cells direct the cells’ migration and tissue residency.61 and are usually used as markers for distinguishing tissue resident or non-resident subsets of NK cells. β2 integrins are lymphocyte specific, and they enable NK cells to migrate in and out of the circulation and also through tissues. β2 integrins also mediate adhesion and immune synapse formation during the lysis of target cells.62 For example, αL (LFA-1)/β2, αM (Mac-1)/β2, αX (CR4)/β2, and αDβ2 expressed on NK cells in blood and lymph nodes can bind CAM family proteins (including vascular cell adhesion molecule-1 (VCAM), ICAM, MadCAM, etc.) in ECM or on non-lymphocytes. As a result, NK cell recruitment from the circulation to enter underlying tissues.61 β1 integrins are highly expressed on—but are not specific to—leukocytes. They mediate both leukocytes navigation to tissue microenvironments and also mediate target cell cytotoxicity. For example, αL (LFA-1)/β2, αM (Mac-1)/β2, α1 (VLA-1)/β1, α2 (VLA-2)/β1, α4 (VLA-4)/β1, and α5 (VLA-5)/β1 are critical signatures of tissue-resident cells, and are commonly expressed on NK cells. They can bind collagen, laminin, E-cadherin, fibronectin, VCAM-1, MadCAM-1, and vitronectin.61,63,64 In addition, the binding of α1/β1 to its partner collagen IV or laminin, or the binding of αE(CD103)/β7 to its partner E-cadherin, or the binding of α4/β7 to its partner MadCAM-1. VCAM-1 and fibronectin is responsible for the tissue residency of NK cells, including in liver, lung, tonsil, uterus, skin, kidney, bone, and spleen. This illustrates that integrins adapt to specific tissue microenvironments and to the development and functions of NK cells.61

Chemokine receptors (G-protein-coupled seven-transmembrane receptors), in concert with selectin, are key regulators of the integrin activation and switch modes of cell migration. After binding to chemokines, chemokine receptors rapidly modulate integrins’ affinity and clustering as well as actin remodeling, thus directing cell migration.61 The four types of chemokine receptors are grouped by structure: C-X-C chemokine receptor (CXCR), C-C chemokine receptor (CCR), C-X3-C chemokine receptor (CX3CR), and XCR. Each has its corresponding chemokine ligands.61 Several chemokine receptors play key roles in NK cell trafficking and function, including CXCR1, CXCR3, CXCR4, CXCR6, CCR7, C-C motif chemokine ligand (CCL) 3/4 (also called as macrophage inflammatory protein-1 alpha/beta, MIP-1α/b), CCL5 (also called as regulated activation, normal T-cell expressed, and secreted, RANTES), and C-C-X-C motif chemokine ligand (CXCL) 1 (also called as activation induced, T-cell derived, and chemokine-related cytokine, ATAC).61 Other molecules, such as CD69 and spondigosine-1-pentaphosphate 5 receptor (ST1PS), are also reported to regulate NK cell homing and trafficking.65 In the following section, we consider how the above molecules direct NK cell homing and trafficking in various tissues or organs.

NK cell trafficking and homing within the BM
NK cells can be found in both the parenchyma and sinusoids of the BM. As they egress, they move first from the parenchyma to the sinusoids and then from the sinusoids into blood. They can remain in the parenchyma only if they express CXCR4, a specific receptor for CXCL12. CXCR4 is highly expressed in immature NK cells, including NKP and iNKs, and it decreases as NK cells mature.65,66

When CXCR4 is antagonized in mice, NK cells are rapidly mobilized from the parenchyma to the sinusoids, and a large number egress from the BM to the periphery.65 This suggests that, during maturation of NK cells, decreased CXCR4 expression promotes egress from the BM. In NOD/SCID mice with a...
reconstructed human immune system, CXCR4 also helps retain human NK cells in the BM, increased expression of CXCR4 in human CAR NK cells enhances their homing to the BM in NOD-SCID IL2Rnull (NSG) mice after adoptive transfer.

In mice, CX3CR1 is expressed prevalently on terminally mature killer cell lectin-like receptor G1 (KLRG1)+ NK cells,71 KLGL1+/CX3CR1+ NK cells originate from KLGL1+/CX3CR1− NK cells.71 In the BM, KLGL1−CX3CR1− NK cells show higher CXCR4 expression and are located mainly in the parenchyma.71 In contrast, KLGL1+CX3CR1+ NK cells show reduced CXCR4 expression and are positioned mainly in the sinusoids.71 In the absence of CX3CR1, KLGL1+ NK cells accumulate in the parenchyma at steady state.71 In humans, single-cell RNA sequencing reveals that functionally mature NK cells highly express CXCR3 and HAVCR2 (TIM-3).72,73 These results suggest that CX3CR1 regulates the withdrawal of NK cells from the BM and guides them into the circulation at steady state.

The interaction between VCAM-1 and VLA-4, a heterodimer of integrin α4 (VLA-4/Cd49d) and β1 (CD29), is important for maintaining HSCs and B cells in the BM sinusoids75,76 and also for retaining NK cells in the BM.71 In mice, neutralizing integrin α4 with monoclonal antibodies selectively reduces NK cell numbers in the BM’s sinusoid cavity. Integrin α4 blockage also dramatically reduces the number of KLGL1+CX3CR1+ NK cells inside the sinusoidal compartment.71 One-year treatment of multiple sclerosis patients with the humanized monoclonal antibody natalizumab, which targets the α4 chain of α4β1 and α4β7 integrins, almost doubled the number of NK cells in peripheral blood, and the number decreased when treatment was withdrawn.72,73 These results show that VLA-4 is needed to retain NK cells in the sinusoids.

In mice, CXCR3 is more highly expressed in the CD11b+CD27− NK cell subset than in other NK cell subsets, and it drives NK cell-specific chemotaxis toward the CXCR3 ligands CXCL10 (IP-10) and CXCL11 (I-TAC).45 At steady state in mice, CXCR3 deficiency results in a dearth of NK cells in peripheral tissues, including lung, liver, and peripheral blood, suggesting that CXCR3 affects NK cells in the BM.79 In a recent study with a multiple myeloma mouse model involving adoptive transfer, CXCR3+ NK cells showed more homing to the BM than CXCR3− NK cells, whereas blocking CXCR3 promoted homing to the BM.80 However, CXCR3-deficient NK cells could still egress from the BM—but not from the spleen—into peripheral blood if they were stimulated with IFN-γ or IL-18.81 Owing to the lack of direct evidence that NK cells accumulate in the BM parenchyma or sinusoids in CXCR3−/− mice, the role of CXCR3 in the trafficking and homing of NK cells into the BM requires further exploration.

NK cell egress from the BM
After passing through the BM sinusoids, NK cells enter the blood circulation to migrate to secondary lymphoid organs and peripheral tissues. Several studies have demonstrated that S1P5 and CX3CR1 play important roles in these egress.71,72,82,83 During NK cell maturation, expression of S1P5 and CX3CR1 is upregulated in parallel with a progressive decrease in CXCR4 expression.75,76,84 S1P is a lysophospholipid that regulates many biological functions within and outside cells. S1P levels are relatively high in blood and LNs and low in other lymphoid tissues. This concentration gradient between the peripheral circulation and tissues allows cells expressing S1P receptors to flow out of those tissues.85 Among the five S1P receptors, S1P5 is specifically expressed in both human and mouse NK cells.86 Although NK cells are not completely absent from all organs of S1P5-deficient mice, their distribution is significantly different from that in wild-type mice, as their percentages are significantly lower in the spleen, lung, and peripheral blood. In contrast, the percentages of NK cells in the BM and LNs of S1P5-deficient mice are twice as high as in wild-type mice.82,85 Subsequent studies have demonstrated that the role of S1P5 is specific to the egress of NK cells—but not T and B cells—from the BM and LNs. It is also more important for the egress of mature NK cells.85 This migration is driven by a concentration gradient of S1P5, both from the BM and LNs.83 Although S1P1 may be expressed at low levels on NK cells, inhibiting it with FTY720 in vivo produces a mild accumulation of NK cells in the BM but does not block NK cell egress from the BM or LNs.83 In humans, newly expressed to fingolimod, which blocks the S1P receptor, 40% to 50% fewer CD56bright NK cells are detected in peripheral blood 6 h after fingolimod is administered.86

CXCR6 was first thought to cause retention of NK cells in the liver, and recent single-cell RNA sequencing revealed that CXCR6−/− NK cell subsets are preferentially located in liver and are essential for the persistence of memory NK cells.87,88 According to another recent study, however, ILC progenitor cells require CXCR6 to leave the BM.89 CXCR6 is expressed mainly on NK progenitors and immature NK cells, and CXCR6+/− mice have more of those cells in the BM at steady state.90 Also, CXCR6-deficient progenitors are less able to reconstitute the peripheral compartment of NK cells compared with their wild-type counterparts.90 This suggests that NK progenitors and immature NK cells need CXCR6 to exit the BM.

NK cell migration into various normal peripheral tissues
Egress of NK cells into blood from the BM and their eventual entry into peripheral tissues to further mature and create tissue heterogeneity involves the homing of NK cells. Tissue-resident CD56bright NK cells dominate in the gut, tonsils, lymph nodes, and skin, whereas CD56dim NK cells are more frequent in the lungs and liver. In some tissues, tissue-resident NK cells show surface expression of CD69, CD103, and CD49a.93 Molecules such as integrins and chemokine receptors and their corresponding receptors or chemokines, which are commonly responsible for NK cell recruitment into all the described peripheral tissues were discussed in the above section: Overview of molecules responsible for NK cell homing and trafficking.94

In this section, we focus on tissue-specific molecules responsible for NK cell recruitment into peripheral tissues or organs, including liver, lung, tonsil, decidua, and intestine.

In the liver, NK cells account for 30% to 40% of all lymphocytes. Liver ECM is rich in type IV collagen, which shows high binding affinity with α1/β1 integrins that are highly expressed on mouse liver-resident NK cells.61,91,92 Liver NK cells can also be derived from circulating NK cells. VLA-4 mediates the recruitment of NK cells to the liver by binding to VCAM-1, which is expressed on tissue-resident cells such as endothelial cells.94 CXCR6 is involved in the trafficking of NK and T cells to the liver, and 35% to 55% of liver NK cells are CXCR6−/−, while only 3% to 5% of spleen NK cells are positive.88,95,96 CXCR6 can bind CXCL16 in hepatic sinusoids, which may promote the retention of peripheral NK cells. During viral infection, NK cells enter the liver in a CXCR6-dependent manner and remain in there.88 In addition, CXCR6 can activate integrin α4 (VLA-4)/β1, which also helps recruit peripheral NK cells to the liver by binding liver VCAM-1. Once there, the former peripheral NK cells can further upregulate αβ7 and α1/β1 on NK cells that reside in the liver.97,98 CXCR5, which is expressed on immune cells, has been shown to direct lymphocytes toward inflammatory sites.99 Knocking out CCR5 from expanded NK cells (using CRISPR/Cas9 technology) reduces trafficking into liver tissue but increases the number of NK cells in the circulation and lung following adoptive transfer into immunodeficient mice.100 Human Eomes−/− NK cells show high liver residency, whereas circulating Eomeslow NK cells can upregulate Eomes expression if stimulated by cytokines such as IL-15 and transforming growth factor-β (TGF-β) released during inflammation. Upregulated Eomes is believed to increase the expression of CCR5 on NK cells.101 However, with increased understanding of liver-resident NK cells and the emergence of...
the nomenclature ILC1, the previous characterization of liver bulk NK cells needs to be updated with a description that is distinct from ILC1s.

In humans, the major population of lung NK cells is CD56brightCD16− and they show a relatively poor response to target cells but produce more cytokines than peripheral blood NK cells. Tissue-resident NK cells in the lung also highly express CCL5, MIP-1β, and granulocyte-macrophage colony-stimulating factor (GM-CSF), which are chemokines that shape cell migration and recruit other immune cells. In the lung, tissue-resident NK cells expressing αEβ7 are located in the alveolar epithelium, where they bind to E-cadherin. In contrast, α1β1hi NK cells are preferentially located in the basement membrane, which is rich in type IV collagen. VLA-4 mediates the recruitment of NK cells to the lung by binding to VCAM-1, which is expressed on tissue-resident or target cells such as endothelial cells and tumor cells. CCR5 and its ligands CCL3, CCL4, and CCL5 are rapidly upregulated during influenza infection, contributing to extensive recruitment of NK cells, as well as other inflammatory cells to the lung. Single-cell RNA-sequencing data from patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection indicate that the transcription of CXCR3, CXCR6, and CCR5 is greater in NK cells of Coronavirus Disease 2019 (COVID-19) patients with moderate disease than in those with severe disease.

In addition to other secondary lymphoid tissues, the tonsil is also an important site for the development and residence of human NK cells, though their etiology has been debated in the last decade. One early NK cell precursor expressing integrin β7 is considered to seed in tonsil after exiting the BM, as it is also found in peripheral blood expressing L-selectin, which help recruit this precursor to tissues. Tonsil NK cells express high levels of αE, β7, and α1 integrins along with lower levels of αM and αX; these cells are named α1hiαEoxαMlo NK cells. In addition to retaining NK cells in the lung, CXCR6 also helps confer specificity to epithelial tissue.

In the intestine, NK cells locate in both epithelium and lamina propria. NK cells from intestinal epithelial cells produce large amounts of IFN-γ, vascular endothelial growth factor (VEGF), and IL-8. NK cells induce trophoblast invasion, decidual transformation, vascularization, and placentation and also fight against placental infection by expressing cytokines or chemokines or recruiting other cells. Aberrant dNK cell activation induces breakdown of tolerance of the maternal–fetal interface, which can lead to preeclampsia, recurrent spontaneous abortion, endometriosis, recurrent implantation failure, and preterm birth.

Although the origin of dNK cells is still under debate—recruitment from peripheral NK cells vs. differentiation in situ from progenitor cells—some evidence supports the idea that NK cells from peripheral blood are recruited into the uterus. dNK cells show high expression levels of CXCR3, a relatively lower level of CXCR4, and very low levels of CXCR1, CXCR2, CXCR1, or CXCR1, 2, 3. Endometrial or trophoblast cells express chemokines, including CXCL10, CXCL12, CCL3, and CCL3, which induce the recruitment of NK cells from peripheral blood toward decidua mainly via interaction with CXCR3 and CXCR4. Chemerin induces NK cell migration toward decidual stromal cells via the chemerin receptor (ChemR23). Estradiol and progesterone treatments promote the expression of CXCL10 and CXCL11 (ligands for CXCR3) in endometrial samples in an in vitro organ culture system. In a mouse model of IFN-γ-induced abortion, IFN-γ significantly increases the expression of CX3CL1 in the uterus, which recruits CD49b+ NK cells via CX3CR1 to the uterus and eventually provokes fetal loss.

In the intestine, NK cells locate in both epithelium and lamina propria. In epithelium, tissue-resident NK cell expressing high αEβ7 bind to E-cadherin at cell junctions, whereas NK cells upregulate α1/β1 binding to fibronectin, collagen, and laminin in the lamina propria. αE (also called as CD103) is a marker of intraepithelial localization in the gut. TGF-β induces the expression of CD103 in ILCs, indicating that gut NK cells are imprinted by TGF-β, which could explain their reduced expression of cytotoxic molecules compared with peripheral blood NK cells.

A recent integrating high-dimensional analysis of NK cells reveals that human intestinal NK cells contains of ~40% of the CD56dimCD16− subset. This subset does not express the CD16 high-affinity IgG receptor, but highly expresses CD69 and CD103. Expression of thymus-expressed chemokine (TECK, also called as CCL25) is highly restricted to the epithelium of the small intestine, where it mediates the recruitment of CD4+ and CD8+ T cells via its ligand CCR9.22–23 A previous study revealed that peripheral blood NK cells showed surface expression of CCR9 in healthy donors, suggesting that CCL25-CCR9 might possibly regulate the chemotaxis of NK cells in the small intestine.

NK cell homing and egress in secondary lymphoid tissues (SLT)

In addition to extravasation from peripheral blood to various solid tissues, NK cells may eventually egress from peripheral tissues and home to SLT. NK cells traffic to LNs through high endothelial venules in the medulla and paracortical areas, where they interact with DCs. CD62L mediates the initial interaction between leukocytes and vascular endothelium, the first step in the extravasation of leukocytes into tissues. In mice, CD62L is required for NK cell homing and re-recruitment of both resting and activated regional LNs; it binds to CD62L ligand expressed on endothelial cells. CD62L-mediated NK cell recruitment to activated regional LNs is critical for restricting tumor metastasis into secondary lymphoid organs. A previous study revealed that CD62L is also expressed on precursors to all ILC subsets in both humans and mice and that it is required for their entry into LNs. In humans, CD56bright NK cells exhibit relatively higher expression of CD62L than do CD56dim NK cells, T cells, B cells, neutrophils, and monocytes. An ex vivo study found that human CD56bright NK cells show highly efficient adhesion to HEV in BALB/c lymph node tissue sections in a CD62L-dependent manner.

CCR7 is a well-known chemotactic receptor that directs adaptive and innate immune cells to secondary lymphoid tissue. At steady state, CCR7 is expressed almost on all ILCs subsets in both humans and mice and that it is required for their entry into LNs. In humans, CD56bright NK cells exhibit relatively higher expression of CD62L than do CD56dim NK cells, T cells, B cells, neutrophils, and monocytes. An ex vivo study found that human CD56bright NK cells show highly efficient adhesion to HEV in BALB/c lymph node tissue sections in a CD62L-dependent manner.

Lymph node homing of NK cells that had acquired CCR7 via trogocytosis increased by 144% in athymic nude mice. This demonstrates that CCR7 is also important for the homing of human NK cells to lymph nodes. A recent study also showed that all ILCs, especially ILC1s, are recruited to LNs in a CCR7- and CD62L-dependent manner. However, there is still no direct evidence for how CCR7 contributes to the homing and trafficking of murine NK cells.

NK cells in SLT and effenter lymph fluid display slightly different phenotypes. However, there is very little information regarding the mechanisms underlying NK cells’ egression from SLTs, though S1P5 is shown to be more important for egression from the SLT than from the BM. Expression of S1PR2 (an S1P transporter required in lymphatic endothelial cells) and S1P5 is required to localize NK cells at the medullary cords of LNs, and deficiency of either causes NK cells to appear in the T-cell zone of LNs.
**TRAFFICKING OF NK CELLS INTO TUMORS**

General overview of NK cells in the TME

Several recent reviews have detailed interactions between NK cells and tumors.\(^{31,32,137}\) NK cells are commonly found in the TME of human tumors, including primary tumors, metastases, and tumor-infiltrated lymph nodes. NK cells can easily reach hematopoietic tumors in peripheral blood. However, it is challenging to reach and infiltrate solid tumors.\(^{31,32,137}\) The density of endogenous NK cells is extremely low, with no >100 cells per mm\(^2\), in contrast to several hundred CD\(^8\) T cells per mm\(^2\) in various human solid tumors.\(^{33,138}\) CD\(^{56}\) bright NK cells, which are less cytotoxic, are more enriched in various tumors than in most normal peripheral tissues.\(^{53}\) Nonetheless, clinical statistics suggest that the abundance of NK cells in the TME predicts better outcomes in patients with several types of cancer, including hepatocellular carcinoma (HCC), melanoma, breast cancer, non-small cell lung cancer (NSCLC), squamous cell lung cancer, pulmonary adenocarcinoma, and gastric cancer.\(^{146}\) Such infiltration not only enhances direct killing of target cells but also provides immunomodulatory cytokines, which in turn shape adaptive immune responses.\(^{140,147-149}\)

To reach a solid tumor bed, NK cells must first extravasate from the blood, and then traverse the ECM and tumor stroma by degrading ECM with matrix metalloproteinases, urokinase plasminogen activator, and serine dipeptidyl peptidase IV.\(^{150}\) Several chemokine–chemokine receptor axes have been reported to be responsible for recruiting both human and murine NK cells to the TME, including CCL5–CCR5, CCL27–CCR10, and CX3CL1–CX3CR1. We discuss these in detail for various types of cancer in the following sections (Summarized in Fig. 4).

After NK cells reach the tumor bed, integration of complex signals from multiple ligand–receptor interactions lead to NK cell recognition and activation, either after cell–cell contact or when the cells act independently.\(^{31,32,137}\) The loss or aberrant expression of MHC-I molecules on tumor cells are the most important signal for NK cell recognition.\(^{11,12,137}\) Activated NK cells can lyse tumor cells by directly releasing perforin and granzymes and inducing apoptosis with ADCC, FasL, or TRAIL.\(^{13,29}\) When NK cells kill tumor cells, tumor antigens that can prime adaptive immune responses are released.\(^{15,29}\) One piece of direct evidence that NK cells regulate adaptive immune responses is that adoptive transfer of iPSC-NK cells can recruit T cells and cooperate with them to respond to anti-PD-1 antibodies in solid tumor.\(^{151}\) Activated NK cells can also secrete various cytokines, including IFN-\(\gamma\), GM-CSF, G-CSF, M-CSF, TNF, IL-5, IL-10, IL-13, FLT3LG, TGF-\(\beta\), XCL1, CCL3/4/5, and so on, which recruit other immune cells and regulate their anti-tumor responses.\(^{152}\) The ability of IFN-\(\gamma\) to shape the adaptive immune response is the best studied. This cytokine acts on a lot of immune cells, including macrophages, DCs, T cells, B cells, and even NK cells themselves [detailed in ref.\(^{153}\),\(^{153}\)]. NK cells can also directly kill cancer stem cells or undifferentiated tumors and trigger the differentiation of cancer stem cells or undifferentiated tumors by secreting IFN-\(\gamma\).\(^{31,154}\) NK cells also produce an abundance of chemokines, such as CCL5 and XCL1/2, that recruit cDC1s, an anti-tumor immune subset.\(^{155,156}\) Using an AML model, we discovered that ILC1s, which used to be recognized as a subset of NK cells, target leukemia stem cells more potently than NK cells.\(^{157}\)

NK cell responsiveness is often hindered by the immunosuppressive TME. After they infiltrate into the TME, their phenotype and metabolism alter, impairing their cytotoxicity, decreasing their expression of activating receptors (including DNAM1, Nkp80, Nkp30, and CD16), and increasing their expression of molecules related to NK cell exhaustion (including PD-1, CD96, Tim3, and TIGIT).\(^{31,158,159}\) A series of soluble molecules such as TGF-\(\beta\), IL-10, indoleamine 2,3-dioxygenase (IDO), and prostaglandin E2 (PGE2) produced by tumor cells, Treg cells, carcinoma-associated fibroblasts (CAFs), or other cells can also suppress NK cells effector functions.\(^{31,32,137}\) Moreover, immunosuppressive cells including Tregs and myeloid-derived suppressive cells (MDSC), tumor cells, or other immune cells can impair NK cell effector functions through receptor-ligand-mediated interactions (e.g., NKG2A-HLA-E, 2B4-CD48). In addition, tumor cells can express cell-released forms of KIR or NCR ligand, including platelet-derived growth...
factor (PDGF)-DD and nidogen-1 (NID1) glycoprotein (for Nkp44), NKG2D (for NKG2D), and Nkp30L (for Nkp30), which obstruct the direct interaction between NK cells and tumor cells [for details, please refer to ref.169]. CD9 on NK cells acquired from tubo-ovarian high-grade serous carcinoma (HGSC) represses cytotoxicity and anti-tumor cytokine production of NK cells, which might be due to reactivation of ADAM17 cleavage activity that sheds NK ligand or receptors (e.g., cleavage of CD16).160

During tumor progression, NK cells can also be educated by tumor cells, which can switch anti-tumor immunity to pro-tumor immunity, creating an immune-tolerant microenvironment that facilitates tumor cell growth or metastasis.161 For example, murine NK cells isolated from the spleen of mice bearing keratin-14<sup>+</sup> breast cancer cells showed increased expression of inhibitory receptors such as LAG3, LKR6G1, and KLRC1. They also promote the formation of tumor cell colonies and organoid invasion when they are co-cultured with tumor organoid in vitro.162 Most tumor-infiltrated NK cells show decidual-like NK cell phenotypes (CD56<sup>bright</sup>CD16<sup>dimm</sup>). When seeded in the uterus, they secrete angiogenic factors, which support angiogenesis by encouraging NK cells to interact with endothelial cells, recruit monocytes, or polarize macrophages.163 These decidual-like tumor-infiltrated NK cells showed augmented expression of pro-angiogenesis factors such as VEGF, Ang-2, IL-6, CCL3, CXCL1, CCR7, CD1146, CD9, PDGF, and IL-8.163-166 Tumor-derived immune suppressors in the tumor microenvironment, such as TGFβ, soluble HLA-G, PEG2, adenosine, extracellular vesicles, and miRNAs, mainly shift tumor-infiltrated NK cells toward a pro-angiogenic polarization [reviewed in ref.161]. In vitro treatment with TGFβ-1 transforms peripheral blood NK cells from healthy donors into dNK-like phenotypes, promoting the production of VEGF and platelet-derived growth factor.165-168 Cell intrinsic factors, such as STAT5 and hypoxia inducible factor-1α (HIF-1α), have also been reported to regulate pro-angiogenic phenotypes of NK cells.169,170

Here, we review the effects of chemokine receptors and other factors on the infiltration of NK cells in various tumor models, including those for HCC, lung cancer, breast cancer, RCC, and melanoma, as well as in other less-studied models (Table 1 and Fig. 4).

**Table 1.** Chemokine receptors affect the trafficking of NK cells into tumors

| Diseases                              | Chemokine receptor | References                  |
|---------------------------------------|--------------------|-----------------------------|
| HCC                                   | CXCL9/10, CCR3, CXC3CL↓, CXC3CR1↓ | 174,175,177                 |
| Melanoma                              | CXCL10, CCR3↑, CCL19↑, CMKL1R↑ | 182-185,189,193             |
| Breast cancer                         | CCR3↑, CXC16↑, CCR6↓, CCL2↑, CXCL12-CXCR4↓ | 199,201,202,204,206       |
| Lung cancer                           | CXCL10-CXCR3↑, CXC3CL↑-CX3CR1, CCL5-CCR5↓ | 212,213,215,216             |
| RCC                                   | CXCL9/10/11-CXCR3↑, CXCR2↑ | 218,219                     |
| Colorectal cancer                     | CCL27/28-CCR10↑, CXCL16-CXCR6↑ | 221                         |
| Endometrial carcinoma                 | CXCL12↑, IL-10↑, CCL27↓ | 222                         |
| Pancreatic ductal adenocarcinoma      | CXCL9/10↑-CCR3↓ | 223                         |
| Glioblastoma                          | CCL5↑, CXCL10↑ | 224                         |

**Cytotoxicity of NK cells.**172 In addition to dampening the cytotoxicity of NK cells,172 the expressing in normal liver tissues, it is upregulated in HCC to inhibit the cytotoxicity of NK cells.172 In addition to dampening the functional activities of NK cells, the hepatic TME also affects the chemotactic phenotype of NK cells, including by downregulating CCR1, CCR5, and CXCR3.173 Overexpression of CCL5 in cancer cells by adenovirus increases the chemotaxis of NK-92 cells to the tumor site and enhances their anti-tumor activity.173 This further confirms that NK cells can fight HCC by increasing their infiltration, as shown in other studies. Dipeptidyl peptidase 4 enhances the biological activity of the CXCL10 secreted by HCC cells by inhibiting CD26, thus maintaining the chemotactic activity of the CXCR3-CXCL10 axis.175 Maintenance of that axis increases the trafficking rate of NK cells, which is beneficial for inhibiting HCC development. This observation confirms the role of CXCR3<sup>+</sup> NK cells in HCC.175 Enhancer of zeste homolog 2 (EZH2) is a histone H3 lysine 27 methyltransferase, which impedes the migration of NK cells by inhibiting the transcription of CXCL10 in tumor cells.176 Mir-561-5p controls the infiltration and functions of CX3CR1<sup>+</sup> NK cells through CXC3CL1-dependent regulation. CX3CL1 stimulates the chemotactic migration and cytotoxicity of CX3CR1<sup>+</sup> NK cells through the STAT3 signaling pathway to control the occurrence and metastasis of HCC.177 It is feasible to regulate the migration of NK cells to intratumoral tissues in HCC by targeting CXCR3-CXCL10 and CX3CL1-CX3CR1 through drug stimulation or genetic engineering.

NK cells stimulated by hapten or various viruses return to the liver in a CXCR6-dependent manner and stay there for several months, which may indicate the importance of CXCR6<sup>+</sup> NK cells in HCC.178 Interestingly, in a recent study of diethylnitrosamine-induced liver cancer, CXCR6 deficiency reduced intrahepatic numbers of invariant NKT, CD4<sup>+</sup>, and CD8<sup>+</sup> T cells. Diethylnitrosamine-treated wild-type mice that had received BM from CXCR6-deficient mice developed more liver tumors and fewer invariant NKTs.179 Given that CXCR6 is critical for the homing of both NK cells and T cells to the liver, targeting CXCR6 could be an alternative way to improve the efficiency of HCC immunotherapy.

The degree to which tumor-infiltrating CD49a<sup>+</sup> NK cells accumulate in the liver strongly affects prognosis for patients with HCC.179 A comparison of CD49a<sup>+</sup> and CD49a<sup>-</sup> NK cell transcripts showed that CD49a<sup>+</sup> NK cells highly express CXCR6 and CCR3.179 However, previously defined liver CD49a<sup>+</sup> NK cells are now considered to be liver-resident ILC1s that differentiate from liver-resident Lin<sup>−</sup>Sca-1<sup>−</sup>Mac-1<sup>+</sup> progenitor cells.180 The presumed link between the accumulation of CD49a<sup>+</sup> NK cells (or ILC1s) and prognosis in HCC needs to be reevaluated.

**Traffic of NK cells in melanoma**

Melanoma, one of the most common cancers, is highly metastatic and has high immunogenicity. Melanoma cells escape being killed by NK cells by inhibiting the immune cells’ functional activities.180 At present, using adoptive NK cells to increase the migration and infiltration of NK cells into melanoma seems a promising approach.181 Previous studies reveal that the accumulation of
NK cells in tumors is dependent on the expression of the CXCR3 ligand, CXCL10, in the tumor tissue.\textsuperscript{182,183} CXCR3-deficient murine NK cells showed reduced migration to melanoma cells, decreasing the animals' survival rates.\textsuperscript{182,183} These results convincingly demonstrate that CXCR3 plays a predominant role in the anti-tumor effects of NK cells. Expression of the CXCR3 receptor in human NK cells expanded in vitro with a high dose of IL-2 (1000 IU/ml) was 10 times greater than in resting NK cells, and the expanded cells showed increased migration toward melanoma cells in a CXCL10-dependent manner.\textsuperscript{184} This suggests that strategies for improving CXCR3 expression on NK cells that have been expanded ex vivo may become potential weapons against melanoma.

As described above, CCR7 is one driver of NK cell homing to secondary lymphoid tissue under steady state or pathological conditions.\textsuperscript{133} The frequency of CCR7\textsuperscript{+} CD56\textsuperscript{high} NK cells in peripheral blood and the serum concentration of the CCR7 ligand, CCL19, are significantly higher in patients with phase IV melanoma compared to those with stage III melanoma or healthy people.\textsuperscript{185} Such an increase in serum CCL19 may reduce NK cell migration to melanoma-infiltrated LNs. Melanoma cells also express CCR7 and concurrently express PD-L1 and Gal-9, especially when they are metastatic.\textsuperscript{185} In light of the finding that trogocytosis of tumor cell-derived CCR7 to NK cells results in NK cell trafficking from tumor tissue to LNs,\textsuperscript{186} blockade of the CCR7-CCL19 axis may keep more NK cells in the TME. Thus, inhibiting both the CCR7 and PD-L1 that are expressed on tumor cells can be considered a new approach to treating melanoma.

CCL5 displays chemotactic activity and induces the migration of several immune cell subsets to inflammatory sites because it can bind to three different C-C chemokine receptors—CCR1, CXCR3, and CCR5. CCL5 is released by both tumor cells and immune cells, including NK and T cells, and higher levels of CCL5 in melanoma patients correlate with significantly increased survival.\textsuperscript{186} Expression of CCL5 also correlates strongly and positively with that of NK cell markers in human melanoma tumors.\textsuperscript{186–188} and that association is very significant, as verified in other melanoma studies.\textsuperscript{189,190} Melanoma cells deficient in the growth factor progranulin show increased CCL5 expression and enhanced infiltration of NK cells but not T cells. Silencing CCL5 in progranulin-deficient melanoma cells reverses the increased recruitment of NK cells.\textsuperscript{188} Certain viruses are potent tools for tumor-specific immune activation. Infecting human melanoma cells with lymphocytic choriomeningitis virus (LCMV), a non-oncolytic virus, results in fast tumor regression and enhances NK cell infiltration to the tumor site, and the outcome is dependent on CCL5.\textsuperscript{190}

ChemR23, a receptor for chemerin (also called retinoic acid receptor responder protein 2, RARRES2), is expressed on NK cells. The chemotactic activity of the chemerin-ChemR23 axis was first reported to mediate the migration of plasmacytoid DCs and NK cells involved in autoimmune diseases.\textsuperscript{191,192} Further study revealed that chemerin was downregulated during melanoma growth and that it suppresses melanoma growth by recruiting NK cells in a ChemR23-dependent manner.\textsuperscript{193} Recent studies revealed that all-trans retinoic acid, a potent inducer of chemerin, enhances the recruitment of ChemR23-dependent NK cells and suppresses melanoma growth.\textsuperscript{194} In addition to the interactions between the chemotactic receptors of NK cells and some chemokines secreted by melanoma cells, other internal and external factors associated with the disease also affect the infiltration of NK cells. IL-32, a pro-inflammatory cytokine, is expressed in various cancers and immune cells. A positive correlation between IL-32 expression and infiltration of active NK cells was found in cutaneous melanoma.\textsuperscript{195} In a mouse model geared to understanding interactions between the immune system and the TME, the formyl peptide receptor (FPR) agonist WKYMVm promoted the migration of NK cells toward B16 melanoma cells and repressed tumor growth, whereas the FPR antagonist WRW (4) had the opposite effect.\textsuperscript{196}

**Trafficking of NK cells in breast cancer**

Breast cancer is one of the most common cancers in women worldwide, and about 70% to 80% of patients with early stage (nonmetastatic) disease are curable. In contrast, advanced breast cancer with distant organ metastases is still incurable.\textsuperscript{197} NK cells not only inhibit the occurrence and development of breast cancer but also help control the hematological spread of related cancer stem cells.\textsuperscript{198} The number of tumor-infiltrating NK cells correlates positively with overall survival rates of breast cancer patients and correlates negatively with tumor-draining lymph node metastasis.\textsuperscript{199}

In an analysis of tumor-infiltrating NK cell subsets in patients with breast cancer, CXCR3\textsuperscript{+} NK cells accounted for 60% of total NK cells. The proportion of tumor-infiltrating CXCR3\textsuperscript{+} NK cells was lower in patients with LN metastasis than in those without, indicating the importance of CXCR3\textsuperscript{+} in tumor development.\textsuperscript{199} In the early study, irradiation enhanced the expression of CXCL16 in breast cancer cells and increased the migration of activated NK cells expressing CXCR6 into breast cancer tissue, thus enhancing the NK cells' anti-tumor function.\textsuperscript{200}

Triple-negative breast cancer is the most aggressive form of the disease. Evaluating a retrospective cohort and publicly available data sets of 72 patients with triple-negative breast cancer revealed that high expression of CCL5 related positively to recruitment of NK cells, reflecting the importance of CCL5 in regulating NK cells in breast cancer.\textsuperscript{201} In an animal model of triple-negative breast cancer, NK cells activated by cationic nanoparticles effectively inhibited tumor growth by upregulating the expression of CXCR4 and CCR4.\textsuperscript{202}

Under normal physiological conditions, the presence or absence of miR-155 has no effect on the number of NK cells and cytotoxic receptors in mice. However, the absence of miR-155 weakens the response of NK cells to CCL2 chemokines in vitro. When miR-155\textsuperscript{−/−} mice were attacked by mammary carcinomas, the increased tumor burden was related directly to the decrease in tumor-infiltrating NK cells. It is suggested that miR-155 may promote chemotaxis by regulating the receptors of CCL2 on NK cells.\textsuperscript{203} In mesenchymal stem cells, Sir1t, the closest mammalian homolog of yeast Sir2, promoted the expression of CXCL10, which then recruited NK cells to effectively inhibit tumor growth.\textsuperscript{204} CXCL12, a chemokine secreted by hepatic stellate cells, induced NK cell quiescence through its receptor, CXCR4, promoting breast cancer outgrowth.\textsuperscript{205} Another recent study revealed that a ruthenium polypyridyl complex increased the production of NKG2D ligands by breast cancer cells, in turn promoting the infiltration of adoptively transferred NK cells and enhancing their therapeutic effect on breast tumors in vivo.\textsuperscript{206}

**Trafficking of NK cells in lung cancer**

Being open to the environment, lungs often develop tumors and are attacked by microscopic pathogens. So not surprisingly, lung cancer is the leading cause of cancer death among both men and women in the U.S.\textsuperscript{207} NK cells are located mainly in the parenchyma of the lungs, accounting for 10% to 20% of lung parenchyma in mice.\textsuperscript{208} NK cells accounted for 60% of total NK cells in tumors.\textsuperscript{209} In an analysis of tumor-infiltrating NK cells in the TME of human NSCLC, these changes included downregulated S1PR1 and CXCR1 and increased expression of CXCR3, CXCR6, and CXCL13 compared with non-tumor NK cells.\textsuperscript{210} In an orthotopic mouse lung tumor model, mature NK cells were attacked by mammary carcinomas, the increased tumor burden was related directly to the decrease in tumor-infiltrating NK cells. It is suggested that miR-155 may promote chemotaxis by regulating the receptors of CCL2 on NK cells.\textsuperscript{203} In mesenchymal stem cells, Sir1t, the closest mammalian homolog of yeast Sir2, promoted the expression of CXCL10, which then recruited NK cells to effectively inhibit tumor growth.\textsuperscript{204} CXCL12, a chemokine secreted by hepatic stellate cells, induced NK cell quiescence through its receptor, CXCR4, promoting breast cancer outgrowth.\textsuperscript{205} Another recent study revealed that a ruthenium polypyridyl complex increased the production of NKG2D ligands by breast cancer cells, in turn promoting the infiltration of adoptively transferred NK cells and enhancing their therapeutic effect on breast tumors in vivo.\textsuperscript{207}
Some recent studies of the mechanisms behind new strategies for controlling lung cancer have also implicated NK cell migration. Jinfukang, a traditional Chinese medicine, not only increased the cytotoxicity of NK cells but also promoted the expression and secretion of the chemokine CX3CL1 in circulating tumor cells, thus recruiting NK cells and inhibiting lung cancer metastasis. Systemic delivery of TUSC2 (a tumor suppressor gene) in nanovesicles (as already performed in clinical trials) combined with anti-PD-1 antibody enhanced the proliferation and infiltration of NK cells in a mouse lung cancer model. In a mouse model of NSCLC, rocaglamide, a natural product, not only inhibited autophagy and restores levels of granzyme B derived from NK cells but also promoted the infiltration of NK cells into tumor tissues. Mechanistically, rocaglamide improves the expression of the chemokines CCL5 and CXCL10 in NSCLC cells. Whether there is a direct relationship between infiltration of NK cells and increased expression of CCL5 and CXCL10 in NSCLC cells remains to be studied.

In summary, the roles and mechanisms relating to NK cell migration in lung cancer are still largely unknown. Nevertheless, it is likely that further in-depth studies could provide opportunities for improving the effectiveness of NK cell-based therapy in lung cancer.

**Factors affecting NK cell trafficking and homing**

**Cytokines**

- IL-15
- IL-12
- IL-2
- IL-18
- IFN-γ
- G-CSF
- TGF-β
- CXCL9/10
- CCL27
- CCL5
- CXCR3
- CCR7
- CD62L
- CD4+ T cell
- Tumor microenvironment

**Transcription factors**

- NCAM1
- Dusp1
- CD44
- CD147
- RORC
- TGF-β
- IL-12/15/18
- ADF5
- CD68
- T cell
- B cell
- DC

**Hypoxia**

**Intercellular interaction**

**Factors affecting NK cell trafficking and homing.** Cell intrinsic factors (e.g., transcription factors), extrinsic factors (e.g., cytokines, hypoxia, low pH), and cell-cell interactions affect NK cell trafficking and homing in both healthy and tumor tissues. During NK cell development, some transcription factors cell-intrinsically regulate the expression of chemokine receptors, selectins, and integrins, etc., thus helping to regulate NK cell homing and trafficking in a cell-intrinsic manner. Cytokines and immune suppressive factors from the TME (e.g., hypoxia, low pH, and adenosine) can also sabotage the recruitment and homing of NK cells in specific tissues by affecting the expression of chemokine receptors, selectins, and integrins on NK cells or their corresponding ligands in the TME. DCs, B cells, and CD4+ cells also affect the migration of NK cells via the chemokine–chemokine receptor axis. Created with BioRender.com

NK cells to tumor tissues by affecting the expression of migration-related receptors and their corresponding ligands should help improve the infiltration of adoptively transferred NK cells into tumor tissue. Here, we summarize some possible factors affecting NK migration, including cell-intrinsic factors (e.g., transcriptional factors), cell-extrinsic factors (e.g., cytokines, hypoxia, low pH), and intercellular interactions (summarized in Fig. 5).
Cell-intrinsic factors governing NK cell homing and trafficking During their differentiation and development, NK cells are regulated by various transcription factors, such as T-bet, NFIL3, RUNX3, ID2, FOXP1,232 Smad4,233 and XBPI,234 etc. Some transcription factors directly or indirectly promote or inhibit the expression of chemokine receptors, selectins, integrins etc, thus helping to regulate NK cell homing and trafficking in a cell-intrinsic manner (Fig. 5). Expression of some transcriptional factors might also be changed in the TME. We discuss some of them in detail below.

Deficiency of T-bet, a critical transcription factor in NK cells, decreased the number of NK cells in blood and spleen but increased their number in the BM and LNs.235 A comparison of the expression of CXCR6, S1P5, and CCR5 between T-bet-deficient and wild-type mice indicated that T-bet promotes the expression of CXCR6 and S1P5, which NK cells need to egress from the BM.236 Several repressive factors in the TME, including TGF-β,236 inhibit T-bet expression, and impaired T-bet expression in NK cells may promote tumor development and progression by downregulating NK cell recruitment, especially in liver cancer.237

The IkARos family member IKZF3 (Aiolos) is required for terminal maturation of NK cells. Although IKZF3 deletion impaired NK cell terminal maturation and did not affect the cytotoxic function of freshly isolated NK cells in vitro, it enhanced in vivo anti-tumor activity in a mouse lung melanoma metastasis model and tended to increase the ratio of NK cells to tumor cells in the TME.238 RNA-seq analysis showed that IKZF3-deficient mice had significantly higher mRNA levels of CXCR2 than wild-type NK cells. Collectively, this evidence indicates that IKZF3 negatively regulates NK cell trafficking.238

GATA3 is dispensable for NK cell development but is required for IFN-γ production and controlling infection.239 GATA3 deficiency causes NK cells to stay in the BM and specifically reduces NK cells in the liver but not the spleen, suggesting impaired trafficking from BM to the liver.239 Accordingly, GATA3-deficient NK cells showed increased CD62L expression but reduced CD11c and VLA-4 expression.239 VLA-4 enhanced the recruitment of cells to the liver by binding VCAM-1 onto endothelial cells.240 However, the mechanistic details of how GATA3 specifically regulates NK cell trafficking to the liver during tumor development are largely unknown.

We previously identified FOXP1 as a negative transcription factor for NK cell development and effector function. NK cell FOXP1 activity was absent from the TME in a mouse model of lung melanoma metastasis. FOXP1 promotes NK cell homing to peripheral LNs, which might depend on its ability to regulate the expression of CXCR6 and CCR5. Whether FOXP1’s ability to regulate NK cell homing helps promote NK cell infiltration into the TME is still unknown.

One recent study suggested that the transcription factor promyelocytic leukemia zinc finger protein (PLZF) may also regulate the homing of NK cells to the liver, as its expression was higher in intrahepatic CD56bright NK cells—and especially in CXCR6+CD69+ liver-resident NK cells—than in peripheral blood CD56bright NK cells.241 A small population of PLZF+CD56bright NK cells in peripheral blood that also express CXCR6 and CD69 might have been an intermediate population that contributed to the renewal of liver-resident NK cells.242 One recent study found that IFN-β treatment reduced the expression of PLZF on peripheral blood NK cells in healthy donors,243 suggesting that PLZF might help recruit NK cells to the liver during an inflammatory response, such as during cytomegalovirus induced hepatitis.244,245

One of our recent studies revealed that a gene encoding a well-known mA reader, YTHDF2, positively controlled NK cell egress from the BM to the periphery. Knockout of YTHDF2 from NK cells significantly reduced NK cell numbers in peripheral blood but not in the BM. Through i.v. injection of anti-CD45 antibodies to label immune cells, we found a significantly lower frequency of CD45+ YTHDF2-deficient NK cells than wild-type NK cells in the sinusoids of the BM.246 However, the detailed mechanisms that allow YTHDF2 to regulate NK cell trafficking are still largely unknown.

Cell-extrinsic factors affecting NK cell homing and trafficking Cytokines. Several cytokines, such as IL-2, IL-12, and IL-15, are critical for NK cell development, survival, and activation, and they are also used to expand NK cells before adoptive transfer. Cytokine treatments have profound and diverse effects on the chemokine receptor profiles of NK cells, depending on dosage and length of treatment (Fig. 5).

The preferred concentration of IL-2 for expanding NK cells in the clinic is ~1000 IU/ml. After NK cells were treated with that dose for 48 h, both CXCR1 and CXCR4 were downregulated, while CXCR3 was upregulated. Consequently, the cells showed reduced homing to the BM and instead trafficked into inflammatory sites, which could limit their alloreactive potential against hematopoietic malignancies.244 A 6-day treatment with 100 IU/ml IL-2 increased CXCR6 expression on human peripheral NK cells.244 After NK-92 cells or human primary NK cells were incubated with IL-2 (100 IU/ml) for 3 to 24 h, CXCR3 mRNA was rapidly downregulated whereas both CCR1 and CXCR4 mRNA were strongly upregulated.246 However, the CXCR4 data differed if the cells were treated with 1000 IU/ml IL-2 for 48 h.244

IL-12 is one of the stimuli that promote the production of IFN-γ, which also cooperates with IL-18 to enhance the cytotoxicity of NK cells.247 After NK cells were stimulated with IL-12 for just 3 h, expression of CCR1, CCR2, and CXCR4 was upregulated while expression of CXCR3 is downregulated.248 Stimulation of NK cells with IL-12 for over 24 h also downregulated the expression of CXCR3 and upregulated the expression of CX3CR1.246 In early studies, stimulation with IL-12 increased the binding of human NK cells to endothelial cells. Mechanistically, IL-12 affects the activity of LFA-1 molecules and enhances the chemotactic response of NK cells.249 These studies suggest that expression of chemokine receptors can be modulated for therapeutic purposes by culturing NK cells ex vivo. To attack tumor cells in the BM, for example, NK cells could first be expanded with 1000 IU/ml IL-2. Then the expanded population can be stimulated for a few hours with IL-12 to upregulate CXCR4 and downregulate CXCR3 before adoptive transfer. This strategy might help NK cells home back into the BM more efficiently.

IL-15, a pivotal cytokine for NK cell development and survival,238,239 strongly represses mRNA and protein levels of CXCR3. In contrast, IL-2 has the opposite effect.240 After BM cells were stimulated with IL-15 (100 ng/ml) for 10 days, CX3CR1 expression was almost undetectable.250 Such low levels reduced the chemotaxis of NK cells to CX3CL1 in mice.251 In cultured CD56+ NK cells, short-term stimulation with IL-15 decreased surface expression of CX3CR1 and diminished the cells’ chemotaxis toward CX3CL1. After long-term co-culture with IL-15 (>5 days), NK cells showed reduced expression of CD62L and CXCR1, undetectable mRNA and protein expression of CX3CR1, and increased expression of CXCR3 and CXCR6.252 However, long-term stimulation with IL-15 did not affect the expression of CXCR4 or the cells’ responsiveness to CXCL12, a CXCR4 ligand, in mice250 and humans.251

Stimulation with combinations of the above interleukins dramatically changes chemokine receptor expression profiles in a complex manner. After NK-92 cells or human primary NK cells were incubated with IL-2 (100 IU/ml) in combination with IL-12 (10 U/ml) or IL-18 (10 ng/ml) for 3–24 h, CXCR3 mRNA was rapidly downregulated. In contrast, both CCR1 and CXCR4 mRNA were strongly upregulated.246 Moreover, treatment with 10 ng/ml IL-12 plus 25 ng/ml IL-15 for 6 days increased the expression of CXCR6 on human peripheral blood NK cells but not on hepatic NK cells. Stimulation with 100 IU/ml IL-2 plus 25 ng/ml IL-15 for 20 h upregulated the expression of CXCR6 on human peripheral blood
NK cells. IL-18 is often used in combination with other cytokines, but by itself it helps upregulate CCR7 in NK cells. Some other cytokines are also reported to regulate NK cell migration. NK cells exert their anti-tumor effects by producing IFN-γ, which is also required to recruit NK cells into the TME. In mice, IFN-γ promoted CXCR3 expression on NK cells and also increased the expression of its ligands, CXCL9 and CXCL10, on virus-infected cells and tumor cells. These ligands are important for NK cells’ anti-infection and anti-tumor properties. In a mouse model of IFN-γ-induced abortion, IFN-γ increased the expression of CX3CL1 in the uterus, prompting CD49b⁺ NK cells to home into the endometrium and eventually cause pregnancy failure. In healthy human donors, in vivo administration of granulocyte colony-stimulating factor (G-CSF) enhanced the expression of CD62L and CXCR4 on the surface of NK cells in peripheral blood. TGF-β, an important immunosuppressive factor in the TME, increased the expression of CXCR4, CX3CR1, and CXCR3 on NK cells in vitro. This enhancement may potentially promote the egress of NK cells from the TME and represents a novel mechanism for tumor escape. Blocking TGF-β signaling increased allogeneic NK cells against glioblastoma stem cells in a glioblastoma stem cell-engrafted mouse model. Whether blocking TGF-β affects the infiltration of NK cells was not addressed in that study. Despite the lack of evidence that cytokines directly affect NK cell homing and trafficking in vivo, cytokines alone or in a cocktail with other cytokines extensively change chemokine receptor profiles in vitro. Thus, it is pivotal to establish a precise protocol for expanding NK cells in vitro, especially by specifying the types and concentrations of cytokines that would be appropriate for a particular type of cancer.

Hypoxia in the TME. Hypoxia, a common feature of tumors, profoundly influences the expression patterns of chemokines, cytokines, and chemokine receptors of NK cells. It also affects the cells’ chemotaxis toward specific chemokines, such as CCL19, CCL21, and CXCL12. When cultured under hypoxic conditions in vitro for 24 to 96 h, peripheral blood NK cells showed increased surface expression of CXCR4 and CCR7; this effect occurred preferentially (CXCR4) or selectively (CCR7) in CD56⁺ NK cells (Fig. 5). Similar phenotypic changes, such as downregulation of CCR1, CCR5, and CXCR3, were observed in the TME in vivo. Low pH in the TME. Owing to lack of oxygen, tumor cells must obtain energy through anaerobic glycolysis, which leads to the accumulation of lactic acid. At the same time, ion exchange proteins on the plasma membrane continue to transport H⁺ from the cell interior to its exterior to avoid tumor cell self-acidosis. These cellular reactions decrease the TME’s pH, leading to various levels of acidity. Studies have shown that tumor-derived lactic acid increases the number of myeloid-derived suppressor cells, which reduces the cytotoxicity of NK cells. Thus, tumor cells indirectly inhibit the functionality of NK cells by producing lactic acid. In an immunogenic mouse model of melanoma, tumors that produced less lactic acid developed at a significantly slower rate than the control group, and a greater number of IFN-γ⁺ NK cells infiltrated the tumor sites. This indicates that lactic acid may also inhibit the invasion of NK cells into the TME.

High levels of adenosine in the TME. Adenosine also promotes the accumulation of tumor-derived metabolites. Adenosine monophosphate inhibits the maturation of NK cells in the TME, mainly via the adenosine A2A receptor. When that receptor was knocked out, CX3CR1 transcription was upregulated in NK cells, suggesting that adenosine monophosphate may inhibit the infiltration of NK cells into the TME by inhibiting their expression of CX3CR1 (Fig. 5). Interstitial interactions. Interactions among cells also help regulate NK cell trafficking (Fig. 5). Therefore, understanding the effects of various cells on the chemotaxis of NK cells is essential for understanding NK cell dynamics in the TME. DCs are a main orchestrator of anti-tumor immune responses, and the effects of their interactions with NK cells in the TME on anti-tumor immunity have been extensively reviewed. DCs can activate NK cells by producing IL-12, IL-15, and IL-18, thus enhancing anti-tumor activity. Generating CXL9 and CXCL10 to recruit effector T cells is also part of DCs’ anti-tumor repertoire. However, whether CXL9 and CXCL10 produced by DCs have the same effect on NK cells during disease states needs to be verified. After intraperitoneal injection of plasmacytoid DC in mice, the number of NK cells in the abdominal cavity increased significantly. This process depended on the expression of CXCR3 and CD62L in NK cells. T cells, the most important contributors to adaptive immunity, are classified as CD4 (CD4⁺ or helper T cells) or CD8 (CD8⁺ or cytotoxic T cells) according to their functional characteristics. In a mouse tumor model, NK cells activated at a distant site by a TLR7/8 agonist released tumor antigens and induced tumor-specific CD4⁺ T cells. However, depleting CD4⁺ T cells significantly reduced the number of NK cells in the TME in that model, indicating that CD4⁺ T cells actively recruit NK cells to the tumor site. Mechanically, CD4⁺ cells in the tumor recruits NK cells to the tumor site by stimulating tumor cells and other immune-infiltrating cells to release CXCL10. NK cells and CD8⁺ T cells are both cytolytic lymphocytes. At present, it is known that CD8⁺ T cells can promote the maturation of liver-resident NK cells, but there is little information regarding the regulation of NK cells by CD8⁺ T cells in tumors.

In African green monkeys infected with simian immunodeficiency virus (SIV), IL-6 produced by B cells induced NK cells to express CXCR5 and migrate to B-cell follicles to combat the virus. This suggests that B cells regulate NK cell trafficking via CXCR5. In an LPS-induced acute lung injury mouse model, T-bet⁺ NK cells were the critical source of pulmonary CXCL1 and CXCL2, contributing to the recruitment of polymorphonuclear neutrophils. This might indicate crosstalk between NK cells and neutrophils.

In a mouse osteoarthritis model, NK cells and neutrophils were among the first cells to accumulate in the synovium of the joint capsule and promote the progression of osteoarthritis. Depletion of neutrophils reduced the number of NK cells in the synovium, revealing that neutrophils are responsible for recruiting NK cells to the synovium, likely by expressing CXCL10.

To sum up, changes in environmental factors can also affect the migration of NK cells into the TME. Thus, improving the microenvironment might be a strategy for enhancing the infiltration of NK cells into diseased tissue.

STRATEGIES TO IMPROVE CAR NK CELL EFFICIENCY TO ENHANCE NK CELL TRAFFICKING

Historical progress with CAR NK cells
CAR is a synthetic cell-surface receptor that redirects T cells, NK cells, NKT cells, yδT cells, and macrophages toward tumor cells carrying the corresponding antigens, and all of those cells show promise for improving tumor immunotherapy. CAR proteins have an extracellular antigen-recognition domain and multiple intracellular signal activation domains that dramatically activate intracellular immune responses after they bind to tumor antigens. CAR T cell therapy has enjoyed unprecedented progress in hematological diseases, and it has already been approved by the US Food and Drug Administration for treating some hematological malignancies. Other CAR immune cell therapies are summarized in Table 2.
| Cell sources | Features | Cytotoxicity mechanisms | Cytokine release | Clinical trials |
|--------------|----------|------------------------|-----------------|----------------|
| CAR NK       | Autologous, iPSC-derived | Phagocytosis signaling domains; macrophage-mediated killing | No clinical data, but expected to be common | No clinical data, but expected to be common |
| CAR T        | Autologous, iPSC-derived | CAR-dependent cell killing | Only one clinical trial | Only one clinical trial |
| CAR NKT cells| Autologous, iPSC-derived | CAR-dependent cell killing, CAR-independent killing of target cells by TNF-α, γδ T cell-mediated cell killing | No clinical data, but expected to be common | No clinical data, but expected to be common |
| CAR γδ T cells| Autologous, iPSC-derived | CAR-dependent cell killing; CAR-independent γδ T cell-mediated cell killing | Only one clinical trial | Only one clinical trial |
| CAR Macrophages| Autologous, iPSC-derived, cell lines | CAR-dependent phagocytosis, macrophage-mediated killing | Only one clinical trial | Only one clinical trial |

**Table 2.** Comparison of different kinds of CAR immune cells

**Table 3.** However, the therapeutic efficacy of CAR NK cells in the TME could play many roles in improving treatment outcomes. Therefore, in this review, we focus mainly on how co-expressing CAR and chemotactic receptor affects NK cell trafficking (Summarized in Fig. 6).

**Strategies for improving infiltration and effector functions of CAR NK cells**

CAR T cells produce abundant TNF-α, IL-1β, and IL-6, which can cause cytokine release syndrome when tumor cells are targeted with specific antigens. In contrast, CAR NK cells launch anti-tumor activity via direct cytotoxicity, as they release neopterin after lysing target cells, and the abundant cytokines they produce, especially IFN-γ, do not trigger cytokine release syndrome.\(^{278}\) IFN-γ, a strong immune enhancer, plays multifaceted roles in remodeling the TME. As well as directly inhibiting tumor proliferation and targeting cancer stem-like cells, it also increases MHC expression and antigen presentation, thereby augmenting the functions of tumor-infiltrating adaptive immune cells including Th1 cells and cytotoxic T lymphocytes (CTLs). IFN-γ also suppresses Treg cell function and angiogenesis mediated by stromal cells.\(^{127,287}\) Thus, strategies for enhancing the infiltration of CAR NK cells in the TME could play many roles in improving treatment outcomes.

There have been intense efforts to infiltrate enough CAR cells into tumors to overcome the obstacles in the hostile TME. In several preclinical studies, co-expression of a CAR and a chemokine receptor on T cells increased the cells’ invasion into tumors and also boosted their effectiveness against solid tumors.\(^{288}\) CAR T cells equipped with CXCR2\(^{289}\) or CCR2\(^{290}\) or CXCR5\(^{291}\) increased their chemotaxis to tumors, where the anti-tumor effects of T cells are enhanced. Overexpression of CXCR1 did not increase the cytotoxicity of NKG2D-CAR NK cells, but it boosted trafficking into tumors to reduce tumor load after ovarian cancer cells were injected intravenously or peritoneally.
Transgenic expression of CXCR4 on human CD19-CAR NK92 cells or human primary NK cells also increased chemotaxis to BM stromal cells without affecting the cells’ CD19-specific cytotoxic activity. EGFR-CAR NK cells with CXCR4 overexpression showed increased chemotaxis toward U87-MG glioblastoma cells that secreted CXCL12/SDF-1α, improving survival in a mouse model of glioblastoma. Co-expression of CCR7 with high-affinity CD16 improved rituximab-induced ADCC against lymphoma cells, K562 cells, or MM. It also increased NK cell migration toward the lymph node-associated chemokine CCL19 in vitro.

In addition to combining targeted chemokine receptors with CAR NK cells, co-expressing or combining cytokines or chemokines with CAR NK cells may play a role in cancer treatment. Co-expressed IL-15 and CD19-CAR NK cells showed long-term persistence and expansion as well as the stronger anti-tumor activity in vivo. Co-expression of NKG2D-CAR and IL-15 in the PiggyBac system significantly increased the anti-AML activity of human peripheral blood NK cells. In the latest study, PSCA-CAR NK cells expressing soluble IL-15 showed therapeutic efficacy in human metastatic pancreatic cancer models without signs of systematic toxicity, providing a strong rationale for clinical development. Owing to the increased understanding the structure bases of the pleiotropy of cytokine receptors and the great advance of synthetic biology, engineered cytokines or chemokines improve their specificity or affinity to target cells and reduce their side effects. For example, engineered IL-2 “superkine”, IL-15 “superagonist”, and IL-12 partial agonists show enhanced affinity or specificity to target cells, including NK cells. Thus, in the future, the combination of CAR NK cells and cytokines or chemokines should also be more extensively tested in the hope of acquiring a desired therapeutic effect.

To overcome the suppressive TME caused by TGF-β, IL-4, and IDO, a switch receptor strategy was recently developed. It equipped a CAR with an extracellular domain targeting suppressive antigen in the TME and an intracellular domain with activation signals.

### Table 3. Ongoing clinical trials related to CAR-NK cells

| CAR-name | Condition or disease | Intervention/treatment | Phase | Identifier |
|----------|----------------------|------------------------|-------|------------|
| HLA haploidentical CD19-CAR NK cells | B-cell NHL | / | Phase 1 | NCT04887012 |
| CD19-CAR NK with IL-15R fusion | BCL, CLL, R/R NHL | Cyclophosphamide, Fludarabine, Mesna | Phase 1/2 | NCT04639739, NCT03056339 |
| CD19-CAR NK cells expressing IL-15 | BCL | Rituximab, Carmustine, Cytarabine, Etoposide | Phase 1/2 | NCT03579927 |
| BCMA-CAR NK92 cells | MM | Fludarabine, Cytoxan (Cyclophosphamide) | Phase 1/2 | NCT03940833, NCT03506536 |
| CD33/CLL1-CAR NK cells | AML | Fludarabine, Cytoxan (Cyclophosphamide) | Phase 1 | NCT05215015, NCT05008575 |
| CD33-CAR NK cells | AML | / | Phase 1/2 | NCT02944162, NCT03692767 |
| CD22-CAR NK cells | R/R BCL | / | Early Phase 1 | NCT03692767 |
| FT596: iPSC-CAR-19 NK cells, IL-15R fusion | BCL, CLL, NHL | Cyclophosphamide, Fludarabine, Rituximab, Obinutuzumab | Phase 1 | NCT04245722, NCT04555811 |
| FT576: iPSC-BCMA-CAR NK cells, IL-15R fusion | MM | Cyclophosphamide, Fludarabine, Daratumumab | Phase 1 | NCT05182073 |
| CD5-CAR NK cells, IL-15 fusion | Hematological malignancies | Fludarabine phosphate, Cyclophosphamide | Phase 1/2 | NCT05110742 |
| PDL1-CAR NK cells, IL-2 fusion | Various | Nivolumab, Atezolizumab, Avelumab, Pembrolizumab, Durvalumab | Phase 2 | NCT04290299, NCT03228667, NCT04847466 |
| NKG2D-ACE2-CAR NK cells, IL-15 fusion | COVID-19 | IL-15-NK cells | Phase 1/2 | NCT04324996 |
| NKG2D-CAR NK cells | AML, MDS, refractory metastatic colorectal cancer, metastatic solid tumors | / | Phase 1 | NCT04623944, NCT05213195, NCT05247957, NCT03411500 |
| HER2-CAR NK cells | Glioblastoma | / | Phase 1 | NCT03383978 |
| ST4-CAR NK cells | ST4-positive advanced solid tumors | / | Early Phase 1 | NCT05194709 |
| Mesothelin-CAR NK cells | Epithelial ovarian cancer | / | Early Phase 1 | NCT03692637 |
| PSMA-CAR NK cells | Castration-resistant prostate cancer | / | Early Phase 1 | NCT03692663 |
| ROBO1-CAR NK cells | ROBO1-positive tumors/ Pancreatic cancer | / | Phase 1/2 | NCT03940820, NCT03931720, NCT03941457 |

R/R relapsed/refractory, BCL B-cell lymphoma, BCMA B-cell mature antigen, Mesna 2-mercaptoethane sulfonate sodium
In summary, genetically modifying CAR NK cells to enhance their chemotaxis to tumor cells and overcome immunosuppressive obstacles in the TME appears to be a hopeful strategy for using such cells against cancer in the near future. However, most of the above studies were performed in rodent models, especially mice, due to low animal cost, low drug consumption, short growth cycle, and wide availability of transgenic animals. However, the murine models may not recapitulate humans as the two species may have different trafficking molecules. Therefore, therapies that are effective in mice might not necessarily be effective in humans.

CONCLUSION AND FUTURE PERSPECTIVES

NK cell-based immunotherapy, especially with CAR NK cells, has proven safe and effective. It also has the potential to produce allogeneic, “off-the-shelf” products and to benefit even relapsed cancer patients, including some who are resistant to CAR T cell therapies. Increased understanding of the mechanisms that regulate NK cell homing and trafficking in various normal tissues and the TME will provide us with more strategies for improving the infiltration and anti-tumor immunity of both endogenous and exogenous NK cells. Altering the expression levels of chemokines or integrin ligands in the TME will help enhance the infiltration, expansion, and activation of endogenous NK cells in the TME. With improved methods for expanding NK cells in vitro and better understanding of how in vitro expansion affects the chemokine receptor profiles of NK cells, it will become possible to further enhance the therapeutic efficiency of NK cell adoptive transfer (e.g., CAR NK, CAR iPSC-NK cells) by using gene editing, optimizing in vitro expansion protocols, and combining NK cells with other treatment(s) (e.g., cytokines, chemokines, or antibodies) to modulate NK cell trafficking. Via gene editing or in vitro training, we could also endow NK cells with the capacity to immunomodulate the TME from “cold” to “hot” to arouse exhausted adaptive immune cells into a collective fight against cancer. One experimental study demonstrated that NKG2D-CAR NK cells can kill myeloid-derived suppressor cells in the TME, which would help improve the activity of CAR T cells against solid tumors. Also, each type of CAR immune cell therapy has its own advantages and limitations, and different CAR cells can help each other in the TME. In most cases, moreover, combination therapies are usually more effective than monotherapies. Thus, we should combine CAR NK cells with other therapeutics, including another cell therapy such as CAR T cells or CAR NK cells that target a different antigen, to have optimal efficacy in treating cancer, especially solid tumors.
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