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Harnessing Natural Killer Cell Function for Genitourinary Cancers

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BACKGROUND

Natural killer (NK cells) are cytotoxic lymphocytes that are members of the innate immune system. As such, they lack the antigen specificity of T and B cells but recognize cells that have downregulated human leukocyte antigen (HLA) class I and upregulated markers of cell stress, such as MICA, MICB, ULBP-1, and the polio virus receptor. Because loss of class I and expression of these stress ligands often occurs during viral infection and cancer, NK cells are important components of both antiviral defense and tumor immunosurveillance. NK-cell recognition of potential target cells relies on the ability to detect missing self, as initially proposed by Klause Kärre.[1] During surveillance in the peripheral blood, secondary lymphoid organs, and tissue, killer immunoglobulinlike receptors (KIRs) on NK cells recognize HLA-A, HLA-B, and HLA-C expressed by putative targets. Binding of the KIR to its cognate class I HLA molecule delivers an inhibitory signal to the NK cell via phosphorylation of ITIM (immunoreceptor tyrosine-based inhibitory motif) motifs in the KIR's cytosolic domain (Fig. 1, top). Subsequent recruitment of the SHP-1 tyrosine phosphatase results in suppression of NK-cell effector function and prevents the NK cell from killing the target. However, when an NK cell encounters a virally infected cell or tumor cell that has downregulated class I HLA, the inhibitory KIR-HLA signal is not delivered. If

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there is a concurrent, activating signal delivered through interaction of a cell stress ligand with its cognate, activating receptor on the NK cell, then NK effector functions can proceed (see Fig. 1, bottom).

NK cells perform 4 major effector functions after successful recognition of target cells (see Fig. 1, bottom). The first is direct cytolytic activity resulting in specific killing of the target through the induction of apoptosis. NK cells, unlike naive $CD8^+$ T cell, contain preformed, cytolytic granules that are released into the synapse between the NK cell and target cell as the NK cell degranulates. These granules contain perforin, a protein that creates holes in the target cell membrane, and various granzymes: serine proteases that cleave caspases in the target cell, thus initiating an apoptotic cascade. The second NK-cell effector function is the release of cytokines with both tumoricidal and chemoattractant properties. Two of the best-characterized NK-cell cytokines are interferon (IFN-γ) and tumor necrosis factor (TNF-α) IFN-γ promotes upregulation of class I HLA by target cells. Although this generally renders the targets less susceptible to NK cell–mediated killing, it is important for the concurrent or subsequent $CD8^+$ T cell response by facilitating presentation of peptides loaded on class I HLA to specific $CD8^+$ T cell clones. TNF-α has direct tumoricidal effects on binding TNF receptor 1 (TNFR1), and also plays important roles in monocyte/macrophage function, and destabilization of regulatory T cells.[2] More recently, a third NK-cell effector function has been identified pertaining to the recruitment of dendritic cells (DCs). NK cells have been shown to produce the DC chemoattractant cytokines XCL1, XCL2, and CCL5, as well as the DC growth factor FLT3 ligand.[3] These cytokines can recruit DC to tumor-draining lymph nodes and tumor tissue itself, thereby improving antigen presentation to tumor-reactive T cells. In addition, a fourth NK-cell effector function is antibody-dependent cellular cytotoxicity (ADCC). During ADCC, CD16, a receptor that binds the Fc region of immunoglobulin G1, is cross-linked by antibodies bound to extracellular proteins on tumor cells. This cross-linking induces a conformational change in the signaling domains of CD16 leading to phosphorylation of its associated ITAM (immunoreceptor tyrosine-based activating motif), and a subsequent signaling cascade that results in NK-cell degranulation, and lysis of the antibody-coated target cell.[4]

Two major subpopulations of NK cells have been identified in humans: $CD56^{bright}$ and $CD56^{dim}CD16^+$. $CD56^{bright}$ NK cells are considered developmentally less mature but secrete most of the cytokines discussed earlier. $CD56^{dim}CD16^+$ NK cells represent more mature NK cells, and possess more potent cytolytic capability compared with the $CD56^{bright}$ subset. Approximately 10% of healthy peripheral blood mononuclear cells (PBMCs) are NK cells, and, of these ~5% to 10% are $CD56^{bright}$ with the remaining cells belonging to the $CD56^{dim}CD16^+$ subpopulation. However, patients with prostate cancer, and non–muscle-invasive (NMI) bladder cancer (BlCa), have increased frequencies of circulating NK cells,[5,6] suggesting that genitourinary tumors can elicit an immune response in the periphery. Furthermore, infiltration of $CD56^{bright}$ NK cells has been associated with improved survival in both BlCa and renal cell carcinoma (RCC).[7-9]

As mentioned earlier, unlike T cells, NK cells do not recognize specific peptide antigens, and instead detect the presence or absence of class I HLA. However, loss of HLA in the presence of additional inhibitory signals elicits a suboptimal effector response, whereas

HLA expression in the context of strong activating signals can elicit a strong response.[10] The magnitude of NK-cell functional responses is calibrated by integrating stimulatory and inhibitory signals delivered through a diverse array of accessory receptors. Three such inhibitory receptors, NKG2A, Tim-3, and TIGIT (T-cell immunoreceptor with immunoglobulin and ITIM domains), are expressed by NK cells in solid tumors (Fig. 2), with Tim-3 expression reported as anticorrelated with survival in patients with BlCa.[11-14] When NKG2A binds its ligand, the noncanonical class I molecule HLA-E expressed on tumor cells, phosphorylation of its cytosolic ITIM occurs, followed by recruitment of the SHP-1 phosphatase. SHP-1 can then prevent phosphorylation of the stimulatory motifs associated with neighboring activating receptors.[15] This process ultimately results in suppression of NK-cell effector functions. Tim-3 binds multiple ligands expressed on both tumor cells and immune cells, including soluble galectin-9 and HMGB1, phosphatidylserine on apoptotic cells, and CEACAM-1.[16] Unlike many other inhibitory receptors, Tim-3 does not contain an ITIM motif but uses the adaptor protein Bat3 to mediate suppression of NK-cell function. In addition, TIGIT mediates NK-cell inhibition via canonical ITIM signaling like NKG2A; however, it shares the ligands CD112 and CD155 with the activating receptor DNAM-1 (CD226). This competition to bind shared ligands provides another layer of control in tuning the NK-cell response. The inhibitory receptor programmed cell death protein 1 (PD-1), although highly relevant in the context of immune checkpoint blockade (ICB) therapy, is predominantly expressed by tumor-resident T cells, with minimal expression by NK cells.

NATURAL KILLER CELLS IN BLADDER CANCER

Natural Killer Cells Infiltrate Bladder Tumors

Most studies of tumor-infiltrating lymphocytes (TILs) in BlCa have focused on T cells, with much recent attention appropriately focused on the role of the PD-1–programmed death-ligand 1 inhibitory axis, and ICB approaches to ameliorate it.[17-19] However, a recent study profiling 50 NMI and muscle-invasive (MI) bladder tumors suggests that ~25% of the immune infiltrate is made up of NK cells, making them the most frequent lineage examined.[7] In addition, despite this small cohort, a statistically significant correlation was found between the frequency of $CD56^{bright}$ NK cells and overall survival (OS).[7] Importantly, in healthy bladder,[20,21] and in noninvolved bladder tissue from cystectomy specimens (Farkas, 2020; unpublished observations), NK cells represent a much smaller component of the resident immune cells. This finding suggests that NK cells can specifically infiltrate and conduct immunosurveillance of bladder tumors, making them rational targets for immunotherapeutic modulation.

Natural Killer Cell Function in the Tumor and Peripheral Blood of Patients with Bladder Cancer

Immune exhaustion occurs during chronic infection as well as cancer, and refers to defects in effector functions that result from prolonged stimulation and suppressive factors in the tumor microenvironment (TME).[22] Few studies have examined the functional potential of tumor-resident NK cells, in part because of challenges associated with establishing a pipeline in which a sufficient quantity of freshly resected tumor tissue is processed and

analyzed in the research laboratory. Similarly, there are few data comparing differences in NK-cell function between patient-matched peripheral blood and tumor. An early study found no defect in the ability of peripheral blood NK cells from patients with BlCa to degranulate in response to HLA-deficient target cells compared with healthy donors. However, there was a substantial defect in degranulation observed in NK cells isolated from tumor and lymph node.[5] In contrast, subsequent work showed that the peripheral blood NK cells from patients with NMI BlCa had no cytolytic defect, whereas those from patients with MI disease did.[23] In addition, a recent study found that tumor-resident $CD56^{bright}$ NK cells produced more IFN-γ than $CD56^{dim}CD16^{+}$ cells, but did not compare these cells with NK cells in healthy or patient blood.[7]

The Role of Natural Killer Cells in Bladder Cancer Treatments

Bacillus Calmette-Guérin (BCG) was the first immunotherapy widely used for cancer treatment, but its precise mechanism of efficacy is not defined. Several studies have pointed toward a role for NK cells in BCG responders. A study of healthy volunteers receiving BCG vaccination showed that production of interleukin (IL)-1b, IL-6, and TNF-α by NK cells was enhanced during ex vivo restimulation after vaccination, suggesting that NK cells from BCG-experienced individuals develop a form of memory.[24] In addition, NK cells cultured in the presence of BCG for 1 week gained improved cytolytic function against bladder tumor cell lines.[25]

Novel, preclinical studies have also focused on improving NK-cell surveillance against bladder tumors. Combination chemotherapy–epigenetic therapy using cisplatin and an EZH2 inhibitor showed efficacy against MI BlCa, both in direct tumor killing and in improving the NK-cell response against surviving tumor clones.[26] NK cells can be differentiated in vitro from cord blood–derived progenitors, and it is possible to activate NK cells isolated from adult peripheral blood with cytokines such as IL-2, IL-15, IL-12, IL-18, and IL-21, all of which improve effector functionality, induce proliferation, and improve NK-cell survival[27] (Fig. 3). Both of these approaches can be used as sources of NK cells for adoptive cell transfer therapy. For example, adoptive transfer of IL-2/IL-15-activated NK cells from healthy donors into immunodeficient mice bearing orthotopic, chemoresistant bladder tumors resulted in tumor regression. However, transfer of activated NK cells isolated from patients with high-grade NMI BlCa was less effective, suggesting that peripheral blood NK cells from patients with BlCa are exhausted.[28] In addition, preclinical ICB to enhance NK-cell tumor surveillance in murine models and primary human cells ex vivo, although not exclusively in BlCa, have shown that blockade of Tim-3,[29,30] TIGIT,[13] and NKG2A[11] all improve NK function.

Clinical Trials Targeting Natural Killer Cells in Bladder Cancer

Many ongoing and recently completed trials of NK cell–centric immunotherapy involve adoptive transfer of in vitro differentiated NK cells, infusion of preactivated adult NK cells, or transfer of NK cells stably transduced with chimeric antigen receptor (CAR) T cell-–like receptors that confer tumor-antigen specificity (see Fig. 3). Most of these studies use haploidentical donors for source material, and are infused into patients with hematologic malignancies such as chronic myeloid leukemia, acute myeloid leukemia, chronic

lymphocytic leukemia, and non-Hodgkin lymphoma.[27,31] However, there are also NK cell–centric trials for patients with metastatic, locally advanced, and/or cisplatin-ineligible BlCa. For example, there is a phase I/II dose-escalation study of DF1001, a novel biologic that simultaneously targets $Her2^+$ tumors and activates NK cells (NCT04143711). This study will enroll 220 patients with solid tumors, including those with metastatic and locally advanced BlCa, and includes a DF1001+X-PD-1 arm. Several trials will also determine the effects of cytokine activation on NK-cell efficacy in BlCa, such as a phase II study of 205 patients with cisplatin-ineligible MI BlCa who will receive bempegaldesleukin, a PEGylated IL-2, alone or in combination with X-PD-1 (NCT03785925). Two trials being conducted in China are testing the efficacy of so-called cytokine-induced killer (CIK) cells; autologous PBMCs expanded and activated to enhance NK and T cell function. One of these will examine infusion of CIK cells or CIK cells plus chemotherapy in individuals with MI BlCa (NCT02489890). The second will test infusions of autologous PBMC after culture conditions intended to increase the number and activity of NK and T cells, as well as dendritic cells (D-CIK) using IL-2, IFN-γ, IL-1α, X-CD3, and X-PD-1 (NCT02886897), In addition, a phase Ib neoadjuvant trial in patients with MI BlCa before scheduled, radical cystectomy will compare X-PD-1 treatment with a combination of X-PD-1 and lirilumab (NCT03532451). Lirilumab is monoclonal antibody that blocks the interaction of 3 inhibitory KIRs (KIR2DL1/2/3) with various HLA-C alleles, with the goal of decreasing the inhibitory interactions that might preclude optimal NK-cell function.

NATURAL KILLER CELLS IN KIDNEY CANCER

In RCC, NK cells represent a significant portion of TILs.[32] In a rat model of RCC, injection of an NK cell–depleting antibody significantly increased the tumor growth rate, suggesting that NK cells are important in antitumor defense.[33] Eckl and colleagues[8] showed that, after stratifying patients into 2 groups based on the percentage of their TILs that were NK cells, patients with more NK cells had significantly longer cancer-specific survival. Another study found that, as the tumor T stage increased, the percentage of infiltrating NK cells decreased significantly.[34]

Natural Killer Cell Function in the Tumor and Peripheral Blood of Patients with Renal Cell Carcinoma

Studies have identified pathways by which the TME inhibits NK-cell function. Prinz and colleagues[35] found that a significantly lower number of NK cells in TIL expressed perforin or granzyme B than NK cells from the nontumor kidney. In addition, TIL NK cells had low levels of phosphorylated ERK1/2 (extracellular signal-regulated kinase) and JNK (Jun kinase), which are required to initiate lytic granule exocytosis. ERK activation depends on diacylglycerol, which is metabolized by diacylglycerol kinase (DGK). DGK levels were higher in TIL NK cells than in normal renal tissue, leading to less ERK activation and poor NK-cell degranulation. In addition, DGK inhibition led to improved NK-cell cytotoxicity. These findings suggest that DGK and suppression of the ERK pathway may be a way for RCC cells to escape NK cell–mediated destruction.[35] Xia and colleagues[36] showed that exosomes from RCC tumor cells inhibited NK-cell activity in a dose-dependent manner. They then showed that the exosomes of patients with RCC expressed increased levels of

transforming growth factor (TGF) β-1 and that inhibiting TGFβ-1 improved NK-cell cytotoxic acitivity.[36]

The Role of Natural Killer Cells in Renal Cell Carcinoma Treatments

There are several medications approved for the treatment of RCC, many of which affect NK-cell activity. IL-2 was the first widely used treatment of advanced RCC and is the only medical treatment that has resulted in a cure. In the 1980s, scientists showed that IL-2 increases NK-cell cytotoxicity.[37] In addition, in patients with metastatic RCC treated with IL-2 plus or minus IFNα and histamine, low intratumoral $CD57^+$ NK-cell count was an independent poor prognostic factor (<50 cells/mm^2 tumor tissue; hazard ratio, 2.1; $P = .01$). These findings show that at least some of the antitumor activity of IL-2 is through NK-cell cytotoxicity. Sunitinib and sorafenib are multikinase inhibitors with antiangiogenic effects. Studies have shown that sorafenib but not sunitinib significantly reduced NK-cell activity, possibly through suppressing the ERK pathway.[38,39] Axinitib has been shown to exert its antitumor effects at least partially through increasing RCC tumor susceptibility to NK cell–mediated degranulation.[40]

Clinical Trials Targeting Natural Killer Cells in Renal Cell Carcinoma

Although NK cells do not express PD-1 to the extent that T cells do, there is an active clinical trial to determine the effects of nivolumab on NK-cell function and cytotoxicity in both the blood and tumor tissue in patients with metastatic RCC (NCT03891485). Most other clinical trials, as in BlCa, involve adoptive transfer of in vitro differentiated NK cells or infusion of preactivated adult NK cells. For example, there is currently a trial underway to determine whether there are any differences in progression-free survival (PFS) between patients treated with the PD-1 inhibitor camrelizumab alone or in combination with CIK in patients with metastatic RCC who have progressed on tyrosine kinase inhibitors (NCT03987698). There are several trials that include incubating CIK cells with DCs. Coculture of DCs and CIKs (D-CIKs) improves CIK cell antitumor activity through cell-to-cell contact by increasing NK-cell proliferation and cytotoxicity. One phase II trial is assessing the effect of a PD-1 inhibitor and D-CIK on PFS (NCT02886897) and another is assessing the effect of axitinib in combination with D-CIKs and the PD-1 inhibitor pembrolizumab on PFS (NCT03736330). Alternatively, DCs can be pulsed with tumor lysates or tumor-associated antigens to create a DC vaccine. A study is underway to compare outcomes of DC vaccines and CIKs compared with IL-2/IFNα in patients with RCC (NCT00862303).

NATURAL KILLER CELLS IN PROSTATE CANCER

Although, compared with bladder and kidney cancer, prostate cancer is considered less immunogenic, NK cells have been identified in prostate cancer tumors.[41] In both tumor and healthy prostatic tissue, infiltrating NK cells expressed activation markers but had poor degranulation capabilities compared with circulating NK cells. When comparing NK cells found in tumor with those in healthy tissue, expression of the activating receptors NKp46 and NKG2D was significantly decreased and the inhibitory receptor ILT2 was significantly increased. In addition, decreased expression of NKp46 and NKG2D and increased

expression of ILT2 were more pronounced in NK cells from metastatic tumors than from localized or locoregional tumors (ie, tumor with extraprostatic extension, seminal vesicle invasion, or local lymph node invasion).[42]

NK-cell activity has been correlated with prostate cancer outcomes. Increased concentrations of infiltrating NK cells have been associated with a lower risk of cancer progression.[43] When examining circulating NK cells, low levels of NK activity have been associated with an increased likelihood of having a positive prostate biopsy.[41,44,45] Koo and colleagues[46] found that patients with prostate cancer had a significantly higher $CD56^{dim}$/$CD56^{bright}$ cell ratio compared with controls (41.8 vs 30.3; $P<.001$) and that the ratio gradually increased as disease stage progressed (P for trend = .001). They also showed that levels of NK-cell activity were significantly lower in patients with prostate cancer than in controls, and patients with higher-stage disease had a greater reduction of activity.[46] Another study found that, among patients with metastatic prostate cancer, blood levels of the activating receptors NKp30 and NKp46 were predictive of OS and time to castration resistance (TCR) (OS, $P = .0018$ and .0009; TCR, $P = .007$ and $P<.0001$ respectively).[42] There is currently a clinical trial underway to prospectively validate these findings (NCT02963155).

Several studies have also examined how the prostate cancer TME inhibits or evades NK cells. TGFβ has been identified in the prostate cancer microenvironment and is known to inhibit NK-cell function. In addition, in coculture experiments, prostate cancer cells promoted the expression of the inhibitory receptor ILT2 and suppressed the expression of activating receptors NKp46, NKG2D, and CD16, preventing NK-cell activity against tumor cells.[47] As in BlCa, exosomes play a critical role in prostate cancer's ability to invade the immune response. Lundholm and colleagues[48] showed that prostate cancer cells secrete exosomes, which downregulate NKG2D expression, leading to impaired cytotoxicity in vitro. As expected from these results, patients with castration-resistant prostate cancer had a significant decrease in the expression of NKG2D on circulating NK cells compared with controls.[48]

The Role of Natural Killer Cells in Prostate Cancer Treatments

The effects of current prostate cancer therapies on NK cells are not well defined and research on the issue is limited. Studies to determine whether androgen deprivation leads to an increase in NK-cell tumor infiltration have mixed results.[43,49] At present, sipuleucel-T is the only immunotherapy approved to treat prostate cancer. Sipuleucel-T is generated by culturing autologous blood mononuclear cells with a fusion protein composed of prostatic acid phosphatase and granulocyte-macrophage colony-stimulating factor. The final product is composed primary of T cells but also contains NK cells.[50] To better understand the effects of sipuleucel-T on the TME, a trial was performed in which patients with localized prostate cancer were treated with sipuleucel-T as a neoadjuvant. After radical prostatectomy (RP), TILs in the specimen were assessed and compared with the infiltrating immune cells in the pretreatment prostate biopsy specimens. NK-cell levels were not higher in RP specimens, indicating that NK cells do not play a significant role in sipuleucel-T activity.[51]

Clinical Trials Targeting Natural Killer Cells in Prostate Cancer

Immunotherapy as a treatment of prostate cancer has not been as well explored as in renal cancer and BlCa. Therefore, there are currently several studies underway to evaluating the effects of various treatments on NK-cell activity. For example, there is a phase 1 clinical trial assessing the effects of intraprostatic injection of mobilan, which is an adenovirus carrying TLR5 (toll-like receptor 5) and a TLR5 activator, on circulating immune-cell levels in patients with prostate cancer, including NK-cell counts (NCT02654938). At Johns Hopkins, a clinical trial is underway to assess the effect of neoadjuvant enoblituzumab, an antibody directed against cancer stem cells, on the intraprostatic immune response, including mean NK-cell density, after RP (NCT02923180). At Henry Ford, a study of intraprostatic injections of an adenovirus carrying IL-12 in patients with recurrence after brachytherapy is currently underway. Outcomes of interest include the association with disease-specific outcomes, such as prostate-specific antigen response and disease-free survival with serum NK-cell cytolytic activity (NCT02555397). Roswell Park Cancer Institute is currently performing a study to determine whether radiation therapy potentiates the effects of sipuleucel-T in patients with bone metastasis. One of the primary end points will be the quantification of circulating NK cells (NCT01833208). There is also a trial that involves transfer of autologous NK cells and the protease inhibitor bortezomib, which has been shown to increase the sensitivity of cancer cells to NK-cell activity[52] in patients with metastatic prostate cancer (NCT00720785).

SUMMARY

NK cells recognize target cells that have downregulated HLA class I and upregulated markers of cell stress. These changes often occur during viral infections and cancer and therefore NK cells play an important role in the body’s defense against these disease processes. The magnitude of NK cell’s functional response is determined by integrating stimulatory and inhibitory signals delivered though an array of receptors on the NK cell. NK cells infiltrate bladder, kidney, and prostate tumors. In all 3 malignancies, the frequency of NK cells in tumor tissue has been correlated with survival. There are currently several trials designed to increase NK-cell activity to improve cancer outcomes.

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DISCLOSURE

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CLINICAL CARE POINTS

- When an NK cell encounters a potential target cell there are 2 possible outcomes.
  - Inhibition: KIRs on NK cells recognize HLA class 1. Binding of the KIR to its cognate class I HLA molecule delivers an inhibitory signal to the NK cell and prevents the cell from killing its target.
  - Activation: when an NK cell encounters a tumor cell that has downregulated class I HLA, an inhibitory signal is not delivered.
- Magnitude of NK-cell activity depends on the stimulation of several accessory receptors found on NK cells.
- NK cells have been found in bladder, kidney, and prostate tumor tissue.
- In all 3 malignancies, higher NK-cell levels are associated with better outcomes.
- Blockade of inhibitory NK-cell receptors (such as Tim-3) have been shown, in preclinical models and in ex vivo human cells, to improve NK-cell function and may serve as future drug targets.
- NK cells likely play a greater role in cancer immunotherapies than previously realized. For example, IL-2, which was the first immunotherapy for RCC and the only one that has led to durable cures, is a potent NK-cell activator.
- Several trials are underway to determine how to best activate NK cells.
- At present there are many clinical trials that involve adoptive transfer of preactivated NK cells.

KEY POINTS

- Recent studies show that urologic tumors are infiltrated by natural killer (NK) cells and that these NK cells are often dysfunctional.
- Strategies interfering with inhibitory axes have significant potential to alleviate this dysfunction.
- Preclinical studies show that NK-cell antitumor functions can be enhanced.
- Diverse, NK cell-centric clinical trials are ongoing for patients with genitourinary cancers.

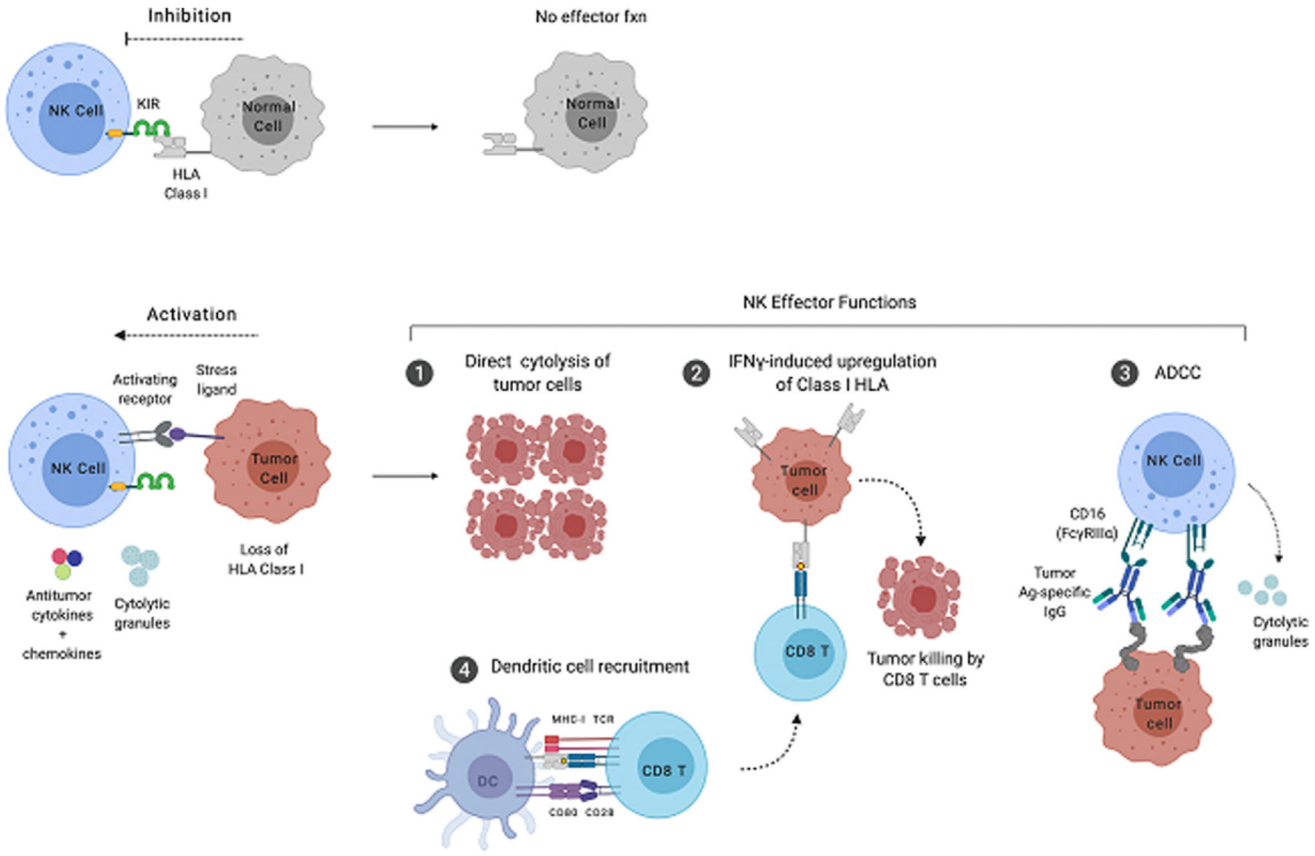

Fig. 1.

Target recognition and effector functions of NK cells. (*Top*) In the absence of viral infection and cancer, NK cells receive inhibitory signals through class I HLA-KIR interactions. (*Bottom*) Cells that have lost class I HLA expression and upregulated molecules associated with cell stress deliver stimulatory signals to NK cells, resulting in the execution of effector function. The major antitumor functions of NK cells include production of tumoricidal cytokines, direct cytolytic activity, recruitment of dendritic cells (DCs), and antibody-dependent cellular cytotoxicity (ADCC). Ag, antigen; CD, cluster of differentiation; fxn, function; IFN, interferon; Ig, immunoglobulin; MHC, major histocompatibility complex; TCR, time to castration resistance.

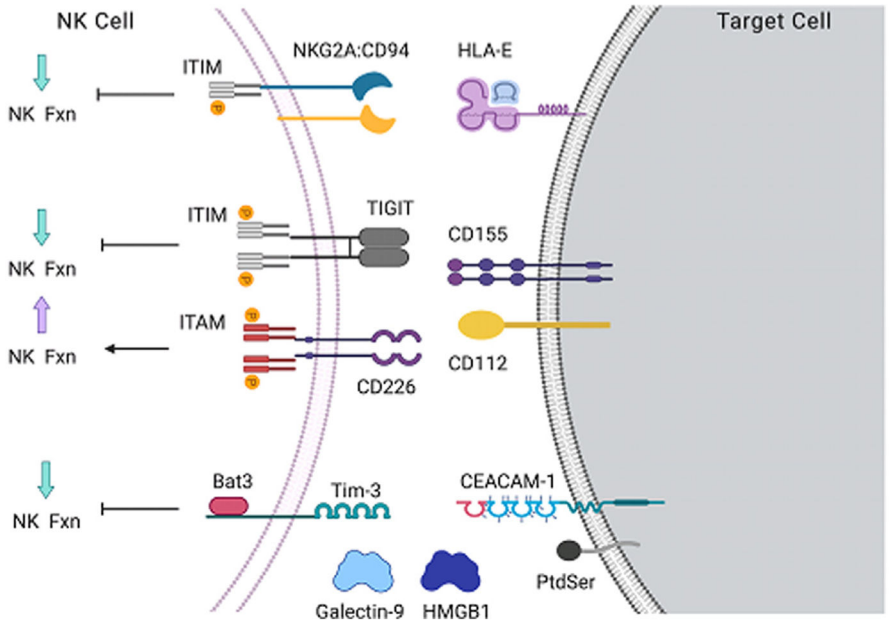

Fig. 2.

Inhibitory receptors tune the magnitude of NK cell effector function. Expression of inhibitory receptors expressed by NK cells in solid tumors. The ligands for each receptor are shown on the right, and the effect on NK cell effector function shown on the left. ITAM, immunoreceptor tyrosine-based activating motif.

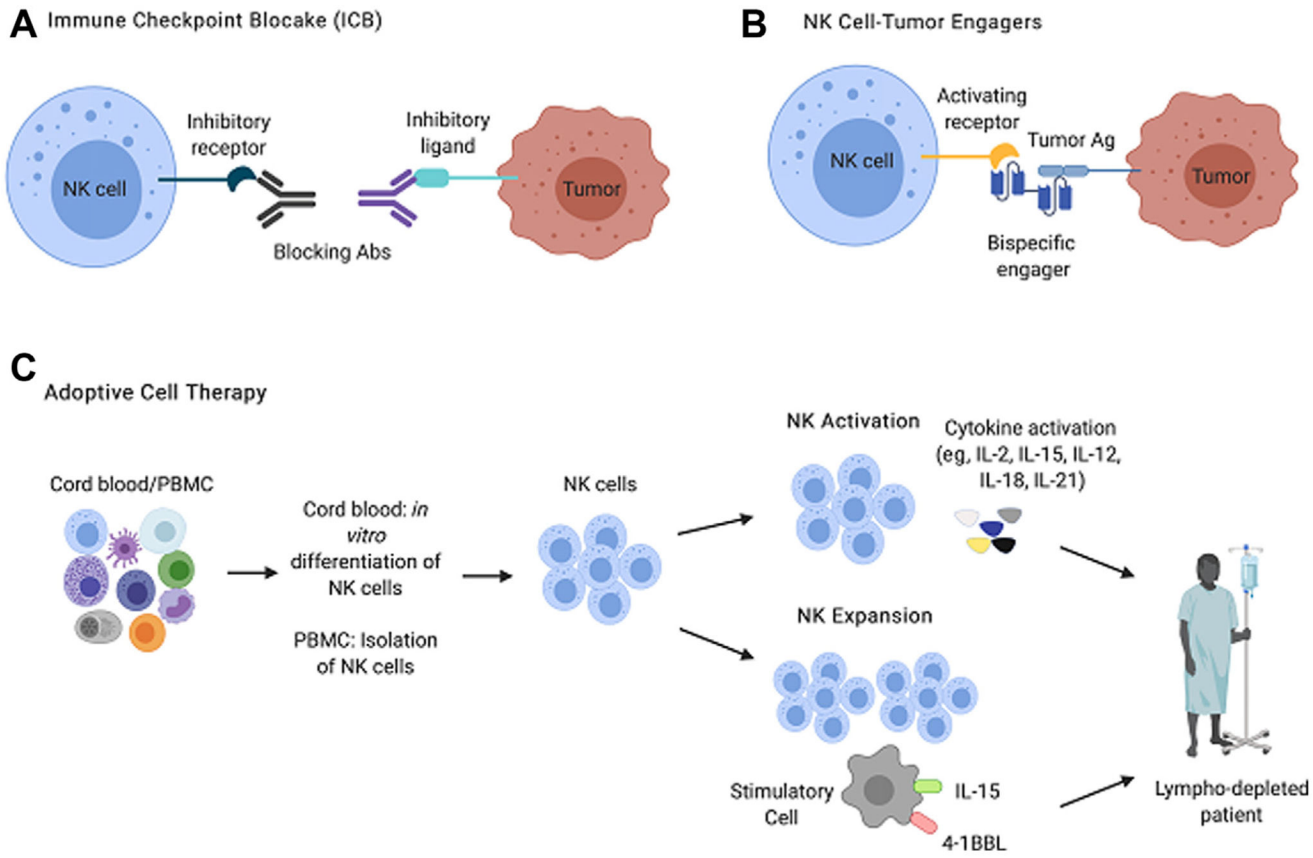

Fig. 3.

Major approaches to NK cell immunotherapy: 3 broad immunotherapeutic strategies to improve NK cell tumor surveillance currently used preclinically and in clinical trials. (*A*) ICB relies on the administration of monoclonal antibodies that prevent signaling through inhibitory receptors. (*B*) NK-tumor engagers are biologics with double or triple specificities. These molecules confer specificity to NK-tumor interactions by binding a protein antigen expressed by tumor cells and simultaneously delivering a stimulatory signal to the NK cell through an activating or cytokine receptor. (*C*) Adoptive cell therapy approaches infuse NK cells isolated from PBMCs, or differentiated in vitro from cord blood progenitors, into autologous, haploidentical, or allogeneic recipients. The transferred NK cells can be preactivated with cytokines to enhance NK cell effector functions or can be expanded using irradiated stimulatory cells engineered to express cytokines and stimulatory ligands. Ab, antibody.