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FOUND IN TRANSLATION

Bringing natural killer cells to the clinic

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Cancer is a leading cause of mortality worldwide, with around 10 million deaths every year. Despite huge advances due to immunotherapy, the majority of cancer patients present primary or secondary resistance to these treatments. In this Found in Translation, we focus on the approaches developed to harness the anti-tumor function of NK cells, suggesting promising strategies to complete the therapeutic arsenal of cancer immunotherapies.

Natural born killers

NK cells are innate lymphoid cells that differentiate into the bone marrow and, once reaching maturation, circulate in the peripheral blood, patrol the body, and may lead to tissue-resident natural killer (NK) cells. NK cells are cytotoxic effectors that can recognize and lyse stressed cells including tumor cells. NK cells are tightly regulated by a repertoire of inhibitory and activating receptors, enabling them to kill tumor cells while sparing normal cells. In particular, tumor cells may present a decrease in HLA class I molecule expression, which identifies them as preferential targets for NK cells. Indeed, NK cells express HLA class I–specific inhibitory receptors, such as killer cell Ig-like receptors (KIRs) and NKG2A, that block their effector function against HLA class I$^+$ cells (Chiossone et al., 2018). The triggering of NK cell effector functions also requires the recognition of ligands expressed on tumor cell membrane by activating NK cell receptors including NKp46, NKp30, NKp44, NKG2D, and DNAM-1 (Bottino et al., 2006). Most mature NK cells also express CD16a (FcγRIIIA), a low-affinity receptor for the Fc region of IgG responsible for antibody-dependent cell-mediated cytotoxicity (ADCC). NK cells may therefore contribute to therapies with anti-tumor antigen IgG1 mAbs.

Some important features of NK cell biology encourage their manipulation in cancer treatment: (i) they are able to kill tumor cells that lack HLA class I molecules and would be neglected by T cells, (ii) they do not need prior antigen-specific sensitization for harboring effector functions, (iii) they do not cause graft-versus-host disease (GvHD), and (iv) upon target recognition, NK cells can secrete pro-inflammatory cytokines, such as IFN-γ and TNF-α, which have direct antitumor effects, but also chemokines, including CCL2, CCL3, CCL4, CCL5, XCL1, and IL-8, and growth factors, such as FLT3 ligand and GM-CSF, which contribute to the onset, the orientation, and the maintenance of the adaptive immune response (Chiossone et al., 2018; Vivier et al., 2011). Harnessing NK cells in cancer patients presents the dual advantage of inducing the killing of tumor cells, but also of participating in a multicellular immune response against tumor cells.

NK cell anti-tumoral function has been demonstrated in vitro using human cells and in vivo in the mouse against tumor cells of all histiotypes. Correlations have been observed between patient clinical outcome and NK cell infiltration at the tumor bed or cytotoxicity of peripheral NK cells (Table 1). In the past two decades various approaches have been developed to exploit the capacity of NK cells to control tumor growth (Fig. 1). Clinical studies demonstrated the safety of NK cell infusions for immunotherapy with encouraging results from preclinical or clinical studies in hematopoietic malignancies (Myers and Miller, 2021; Laskowski et al., 2022).

Adoptive NK cell therapies

NK cell infusions were the first NK cell therapy approach developed in cancer patients. The first clinical trials were based on cytokine-activated autologous NK cells, but their clinical efficacy was not satisfactory. HLA-haploidentical hematopoietic stem cell transplantation (HSCT) revealed the antitumor effect of allogeneic NK cells, based on mismatched between donor KIRs and patient HLA class I molecules (Ruggeri et al., 2002). Subsequently, allogeneic NK cells from HLA-related or unrelated healthy donors have been administered after HSCT for therapy of hematological malignancies with positive results in the control of relapse and GvHD. In a non-transplantation setting, infusion of HLA-haploidentical NK cells and IL-2 led to tumor regression in AML (acute myeloid leukemia) patients (Miller et al., 2005). Afterward, clinical trials in other hematopoietic diseases and solid tumors in combination with chemotherapy showed that allogenic NK cell infusions were safe and, in some cases, also effective (Myers and Miller, 2021; Laskowski, 2022).

Impressed by the success of chimeric antigen receptor (CAR)-T cell therapy in acute lymphoblastic leukemia and non-Hodgkin's lymphoma, several groups concentrated

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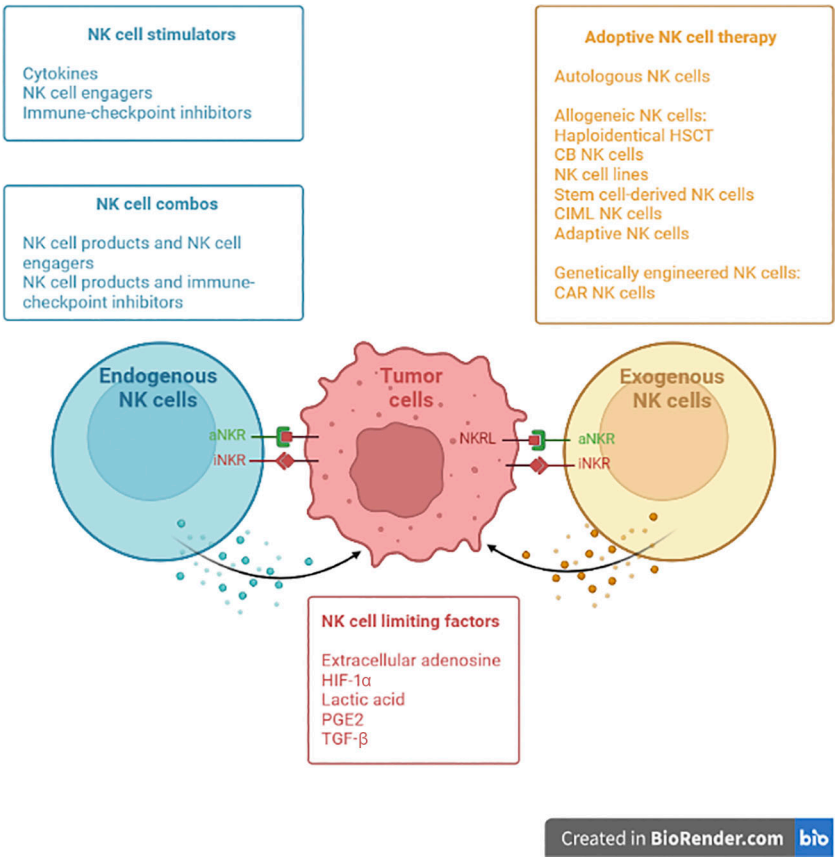

Figure 1. **Therapeutic approaches aimed to enhance anti-tumor NK cell response.** Interactions between activating and inhibitory NK cell receptors and their cognate ligands expressed by tumor cells regulate NK cell anti-tumor activity. aNKR, activating NK cell receptors; iNKR, inhibitory NK cell receptors; NKRL, NK cell receptor ligands; CB, cord blood; CIML, cytokine-induced memory-like NK cells; CAR, chimeric antigen receptor.

their efforts in the engineering of CAR-NK cells. CAR-NK cells have been developed from various sources, such as immortalized cell lines (NK-92), peripheral blood mononuclear cells, cord blood, hematopoietic stem and progenitor cells, and induced pluripotent stem cells (iPSCs). Recently, HLA-mismatched anti-CD19 CAR-NK cells showed remarkable clinical efficacy in a phase 1/2 trial in non-Hodgkin's lymphoma and chronic lymphocytic leukemia (Liu et al., 2020). iPSC-derived anti-CD19 CAR-NK cells also exhibited encouraging results in phase 1/2 clinical trial in relapsed/refractory B cell lymphoma (Bachanova et al., 2021).

Combined cytokine activation with IL-12, IL-15, and IL-18 is used to induce the in vitro expansion of NK cells with potent cytotoxic properties, referred to as cytokine-induced memory-like (CIML) NK cells. After infusion in preclinical models, CIML NK showed superior responses and higher persistence compared with conventional NK cells. Remarkable responses have been reported in AML patients receiving haploidentical cell transplant and CIML NK cells derived from the same donor (Berrien-Elliott et al., 2022). These efforts are being completed by the development of biologicals, such as immune checkpoint inhibitors and NK stimulators, the clinical evaluation of which is being tested at present in various hematological malignancies and solid tumors.

Immune checkpoint inhibitors

NKG2A is an inhibitory cell surface receptor shared by NK and T cells. Its ligand, HLA-E, is a non-classical MHC class I molecule highly expressed in many solid tumors and hematologic malignancies (André et al., 2018). Monalizumab, a humanized mAb specific for NKG2A, showed prolonged progression-free survival (PFS) in combination with durvalumab compared to durvalumab alone in patients with unresectable and chemo-radio-resistant NSCLC (non-small cell lung cancer; Herbst et al., 2022). Following these results, monalizumab is currently being tested in phase 3 in combination with durvalumab in NSCLC.

Another promising inhibitory receptor to block in cancer therapy is LAG3 (lymphocyte-activation gene 3). It is expressed by tumor-infiltrating CD8⁺ T cells, NK cells, B cells, and plasmacytoid dendritic cells. Several clinical trials are evaluating various LAG-3 targeting molecules in solid tumors, including blocking mAbs, soluble LAG-3–Ig fusion proteins and anti–LAG-3 bispecific drugs coupling LAG-3 to PD-1, PD-L1, or CTLA4 blocking.

TIGIT (T cell Ig and ITIM domain) is a receptor that is expressed on NK cells and on effector, memory, and regulatory T cells, where it functions as a co-inhibitory receptor in synergy with PD-1 and Tim-3. Anti-TIGIT blocking mAbs are being tested in various solid tumors in combination with PD-1, PD-L1, or PD-L2 blocking mAbs. Recently, two phase 3 trials revealed no clinical efficacy of tiragolumab combined to atezolizumab alone or in combo with chemotherapy in NSCLC. Anti-TIGIT/anti–PD-1 bispecific antibodies have also been developed and are now in phase 2 clinical trials in solid tumors.

NK cell stimulators

The NK cell stimulatory cytokine IL-2 was approved for the treatment of several malignancies almost 20 yr ago. Yet, its administration revealed limited efficacy due to its short half-life and severe toxicity, and several modifications have been engineered to address these limitations, including polyethylene glycol conjugation, fusion to tumor-targeting antibodies, and alteration of receptor-binding affinity. IL-15 entered into cancer therapy thanks to its capacity to activate NK and CD8⁺ T cells while sparing regulatory T cells. It displayed a safer profile compared to IL-2 but limited in vivo efficacy. Recently, an IL-2 prodrug masked by the IL2 receptor α chain linked to a tumor-associated protease substrate has been proposed as an innovative strategy to efficiently target tumor-infiltrating lymphocytes, minimizing systemic toxicity (Hsu et al., 2021).

Bi-specific, tri-specific, or tetra-specific molecules targeting one or more activating NK cell receptors and/or cytokine receptors represent a new class of therapeutic

Table 1. **Correlation between patient clinical outcome and NK cell infiltration or fitness**

Indication	Results	Author
Breast cancer	Trastuzumab-responsive tumors present a trend toward increased NK cell infiltration compared to non-responsive tumors	Arnould et al., 2006
	Tumor-infiltrating NK cells were significantly associated with pathologic complete response and prolonged disease-free survival	Muntasell et al., 2019
	NK cell infiltration correlates with improved prognosis	Denkert et al., 2018
Cervical cancer	Downregulation of NKp46, NKp30, and NKG2D on NK cells were correlated with tumor progression	Garcia-Iglesias et al., 2009
Colorectal cancer	Higher NK cell infiltration correlated with better overall survival	Foroutan et al., 2021
	Higher numbers of pre-operative NK cells correlated with reduced risk of recurrence	Tartter, 1987
	NK cell activity is a strong prognostic factor for patients with metastatic colorectal cancer	Liljefors et al., 2003
	Preoperative peripheral NK cell activity has a significant prognostic value in curatively operated colon cancer	Kondo et al., 2003
Gastric cancer	The density of the NK cell infiltration in tumor foci after imatinib mesylate treatment independently predicted PFS	Rusakiewicz et al., 2013
	NK cell abundance is associated with the response to sorafinib, and higher NK cell abundance may prolong overall survival	Wu et al., 2020
	NK cell IFN-γ production after 2 mo of treatment is an independent predictor of long-term survival in advanced gastrointestinal stromal tumors treated with imatinib	Ménard et al., 2009
	Patients with high circulating NK cell counts had a better overall survival	Pernot et al., 2020
Head and neck cancer	$CD8^+$ T cell infiltration and CD56dim NK cell infiltration each correlated with superior survival in head and neck squamous cell carcinoma	Mandal et al., 2016
	Peripheral NK cell cytotoxicity was inversely related to subsequent death with disease progression	Schantz et al., 1991
	Patients with elevated NK activity had an improved disease-free survival	Schantz et al., 1986
	Diminished NK cell function was associated with an increased risk of death from uncontrolled regional and distant metastases	Schantz et al., 1989
Hepatocellular carcinoma	Patients with lower NK cell infiltration had shorter overall survival	Zhu et al., 2009
	Recurrence-free survival of patients with tumors lacking the expression of the NKG2D-ligand ULBP1 was significantly shorter	Kamimura et al., 2012
Lung cancer	High NK cell infiltration correlated with better outcome	Federico et al., 2021
	Increased NK, DC, T $CD8^+$, and B cell infiltration correlated with improved outcome	Soo et al., 2018
	Increased NK cell infiltration correlated with better survival	Villagas et al., 2002
Melanoma	Higher ratio of CD56dim/CD56bright in tumor-infiltrated lymph nodes correlated with improved survival	Ali et al., 2014
	Higher NK cell infiltration in tumors correlated with improved survival	Cursons et al., 2019
	Higher NK cell infiltration in tumors correlated with improved survival and predicted PD-1 responsiveness	Barry et al., 2018
	NKp46 expression predicted melanoma outcome	Messaoudene et al., 2016
Neuroblastoma	Increased level of tumor-infiltrating NK cells was associated with better prognosis	Semeraro et al., 2015a
	Patients with high-risk neuroblastoma in remission after induction chemotherapy had a higher risk of relapse if their circulating and bone marrow NK cells express the immunosuppressive NKp30 C isoform	Semeraro et al., 2015b
Prostate cancer	NKp46 expression by circulating NK cells in prostate metastatic patients inversely correlated with the levels of prostate-specific antigen	Pasero et al., 2016

Table 1. **Correlation between patient clinical outcome and NK cell infiltration or fitness (*Continued*)**

Indication	Results	Author
Hematologic malignancies	Higher number of NK cells in the bone marrow at diagnosis positively correlated with the response to treatment and the frequency of remission	Mizia-Malarz and Sobol-Milejska, 2019
	The presence of active cytotoxic NK cells had a positive effect on the disease control after chemotherapy	Sullivan et al., 2014
	Higher NK cell numbers correlated with reduced severity	Palmer et al., 2008
	Higher NK cell numbers correlated with increased survival	Gonzalez-Rodriguez et al., 2010
	Higher number of circulating NK cells was associated with better prognosis and overall survival	Plonquet et al., 2007
	Higher NK cell infiltration correlated with favorable prognosis	Álvaro-Naranjo et al., 2005
Multiple cancer types	High NK cell activity in peripheral blood was associated with reduced risk of cancer, whereas low activity was associated with increased risk of cancer	Imai et al., 2000

molecules, referred to as NK cell engagers, that are being developed to favor tumor cell recognition and enhance NK cell activation (Demaria et al., 2021). Some of them are being tested in phase 1/2 clinical trials, such as GTB-3550, a tri-specific killer engager targeting CD16 and CD33 and bearing an IL-15 domain that promotes NK cell proliferation; DF1001, a tri-specific antibody targeting HER2, CD16, and NKG2D; or AFM13 and AFM24, which are trispecific NK cell engagers targeting CD16a on NK and myeloid cells and the tumor-associated antigens CD30 or epidermal growth factor receptor, respectively. We developed a series of NKp46 targeting NK cell engagers that provided encouraging results in preclinical models (Gauthier et al., 2019). IPH6101/SAR′579, an IgG1 antibody coengaging NKp46, CD16, and the tumor antigen CD123 is now in phase 1/2 in acute leukemias and myelodysplastic syndromes. IPH64/SAR′514 is an engineered IgG1 for enhanced ADCC, targeting CD16, NKp46, and the B cell maturation antigen. Finally, we are also developing tetra-specific molecules engaging CD16, NKp46, a tumor antigen, and the IL-2 receptor β and γ chains by an IL-2 variant, leading to activation and proliferation of NK cells, as well as control of invasive and subcutaneous tumors in mice.

Future prospects

The future of cancer immunotherapy lies in combined therapeutic approaches. Along this line, several interesting possibilities include the combination of NK cell infusions with immune checkpoint inhibitors or with NK cell engagers. It is also important to take into consideration the factors present in the tumor microenvironment, which limit the anti-tumor function of NK cells (Fig. 1). Although the targeting of these inhibitory pathways has shown disappointing clinical results so far, the combination of some of these drug candidates with NK cell therapies will be quite interesting to test. Finally, it remains to assess NK cell therapies in solid tumors, where mechanisms leading to recruitment and activation need to be more clearly understood to reach clinical benefits in patients.

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