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The NK cell-cancer cycle - advances and new challenges in NK cell-based immunotherapies

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Abstract

Natural Killer (NK) cells belong to the innate immune system and in part protect the host through killing of infected, foreign, stressed or transformed cells. Additionally, via cellular cross-talk, NK cells orchestrate anti-tumor immune responses. Hence, significant efforts have been undertaken to exploit the therapeutic properties of NK cells in cancer. Current strategies in preclinical and clinical development include adoptive transfer therapies, direct stimulation, recruitment of NK cells into the tumor microenvironment (TME), the blockade of inhibitory receptors that limit NK cell functions and therapeutic modulation of the TME to enhance anti-tumor NK cell function. In this review, we introduce the NK cell-cancer cycle to highlight recent advances in NK cell biology and discuss the progress and problems of NK cell-based cancer immunotherapies.

Keywords

NK cells; Cancer; Immunotherapy; Tumor microenvironment; Adoptive transfer; CAR NK cells

Natural Killer (NK) cells are effector cells of the innate immune system and belong to the family of innate lymphoid cells (ILCs). By analogy to the classification of T cells, three groups of ILCs have been defined based on cytokine production and expression of transcription factors. Group 1 ILCs include IFN-γ-producing NK cells and ILC1s, while

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Competing interest statement

T. Bald has research agreements with ENA Therapeutics and Bristol Myers Squibb and is on the scientific advisory board of Oncomyx. M.F. Krummel is a founder and shareholder of Pionyr Immunotherapeutics and has research agreements with Bristol Myers Squib, Eli Lilly, Pfizer, Amgen, Abbvie and Genentech. M.J. Smyth has research agreements with Bristol Myers Squibb and Tizona Therapeutics and is on the scientific advisory board of Tizona Therapeutics and Compass Therapeutics. K.C. Barry declares no conflict of interest.

ILC2 produce classical T_H2-cytokines and ILC3 produce T_H17-cytokines[1,2]. NK cells are professional killer cells that recognize and rapidly destroy cells dangerous to the host (e.g. stressed, foreign, infected or transformed cells) and as such contribute to transplantation rejection, viral immunity and cancer immune surveillance (particularly cancer metastasis)[3,4]. However, besides their ability to kill cells, it is now well-established that NK cells also play a critical role in sculpting innate and adaptive immune responses via cellular cross-talk in various disease settings[5]. When compared with T cells, either in a natural tumor immunity or adoptive cellular therapy (ACT) setting, NK cells display certain advantages and disadvantages (Table 1). In particular, NK cells have a more important role in elimination of early tumors and metastasis (minimal disease) and are generally found in fewer numbers in established tumors. NK cells have a broader reactivity with tumor (less specificity without TCR), equivalent effector functions, and reduced proliferative capacity and recall response. In an adoptive transfer therapeutic setting, NK cells have greater off-the-shelf utility and are safer with respect to causing fewer immune related adverse events, but they are more difficult to genetically manipulate.

Similar to myeloid cells, NK cells are a heterogenous and plastic population, which allows them to acquire different phenotypes dependent on the tissue context or signaling cues to which they are exposed[31,32]. Simply, NK cells are defined as $CD3^-CD56^+$ cells in humans and $CD3^-NK1.1^+NKp46^+$ cells in mice. Further, highly cytotoxic human NK cells are defined as $CD56^{dim}CD16^{hi}$ (further referred to as $CD56^{dim}$) and are predominantly found in the blood, while immunomodulatory and cytokine producing NK cells are defined as $CD56^{bright}CD16^{lo}$ (further referred to as $CD56^{bright}$) and preferentially reside in secondary lymphoid organs e.g. lymph nodes (Figure 1)[33,34,35]. A large suite of additional markers can be utilized to further stratify NK cell subsets e.g. CD94/NKG2A, NKp46, CD226 and many more. Functionally similar NK cell subsets have been identified in mice, but with some different markers. For NK cell differentiation in mice NK cells are marked by tumor necrosis factor receptor superfamily member CD27 and the integrin CD11b/Mac-1. The most cytotoxic NK cells are terminally differentiated and express CD11b but little or no CD27 (i.e. are $CD27^-CD11b^+$ or “CD11b SP”), while regulatory NK cells comprise mature NK cells expressing both $CD27^{hi}$ and CD11b, and immature NK cells lacking CD11b (i.e. $CD11b^-$ $CD27^{hi}$ or “CD27 SP”) (Figure 1)[36]. In addition, various subsets of tissue-resident NK cells have been described, differing from conventional NK cells in terms of their origin, development, and/or function (reviewed in ref.[37]).

The NK cell – Cancer Cycle

By analogy to the Cancer-Immunity Cycle, we here introduce the NK cell-Cancer Cycle to discuss recent advances in NK cell biology and their importance for cancer immunotherapy (Figure 2). For productive anti-tumor immune responses, the human body needs to initiate and orchestrate the activation of multiple immune cell subsets. As discussed by Chen and Mellman, the success of immune checkpoint blockade (ICB) in some cancers shows that targeting single molecules or pathways is promising, but is ultimately insufficient across the majority of cancer patients[38]. Thus, our current challenge is to define additional targets in the tumor microenvironment (TME) and secondary lymphatic organs to meet this need.

Step 1. Recruitment of NK cells to the TME.

NK cells are commonly found in human tumors, however at low frequency compared to myeloid and lymphoid cells[39]. Increased abundance of NK cells in the TME has been associated with increased overall survival in patients with hepatocellular carcinoma[40], melanoma[41,42,43,44,45], pulmonary adenocarcinoma[46], gastric cancer[47], squamous cell lung cancer[48], non-small cell lung cancer[49], breast cancer[50], and renal cell carcinoma[51]. Recently, NK cells have been further linked to patient responsiveness to anti-PD-1 immunotherapy in metastatic melanoma[43]. Here, we will discuss the first step in the NK cell – cancer cycle: NK cell recruitment into the TME (Figure 2 and 3). The mechanisms controlling NK cell recruitment to the tumor can be broken down into three broad categories; chemoattractants/receptors; immunomodulation of chemokine axes, and physical barriers.

The two main subsets of NK cells, CD56^{bright} and CD56^{dim}, express unique repertoires of chemoattractant receptors, explaining the differential recruitment of NK cell subsets to various tissues (reviewed in ref.[52]). Peripheral blood CD56^{bright} NK cells typically express and respond to ligands for CCR2, CCR5, CCR7, CXCR3, CXCR4 and CD62L while CD56^{dim} NK cells express and respond to ligands to CXCR1, CXCR2, CXCR4, CX3CR1, S1P5, and ChemR23[53,39,54,55,56,57,58,59,60,61] (Figure 1). As described above, mouse CD11b SP and CD27 SP NK cells are the functional equivalent to the human NK cell subsets (Figure 1). Consistent with this relationship, mouse CD11b SP and human CD56^{dim} NK cells express similar chemokine receptors while CD27 SP NK cells have conserved expression of chemokine receptors with human CD56^{bright} NK cells[62,36] (Figure 1).

Recent single cell RNA sequencing of metastatic melanoma samples has found transcriptional heterogeneity within NK cells in the TME[63]. Even though there may be some transcriptional heterogeneity, CD56^{bright} NK cells have been found to be the dominant NK cells in the TME of a number of cancers, including non-small cell lung cancer (NSCLC) and breast cancer[39,64]. The increased abundance of CD56^{bright} NK cells in the TME of NSCLC and breast cancer is linked to the downregulation of the chemokine CXCL2, which signals through CXCR2, and concomitant upregulation of the chemokines CXCL9, CXCL10, and CCL19, which signal through CCR7 or CXCR3, in the TME[39]. Similarly, in pre-clinical mouse lymphoma models, tumor cell expression of CXCL9 and CXCL10, which signal through the chemokine receptor CXCR3 on NK cells, is important for the recruitment of NK cells into the TME[65,66].

CCL5, the ligand for CCR5, which is uniquely expressed on human CD56^{bright} and mouse CD27 SP NK cells (Figure 1), has also been implicated in NK cell recruitment to the TME. Other atypical pathways may also control NK recruitment, likely through the CCL5 axis. In one mouse model, tumor-derived progranulin (PGRN) serves to inhibit CCL5 production in an autocrine fashion, leading to reduced NK cell infiltration into the TME, loss of tumor control, and increased metastasis[67]. Consistent with a role for CCL5 in recruiting NK cells to the TME, in an experimental model of melanoma lung metastasis, IL-33 in the lung TME induces CCL5 production by CD8+ T cells and eosinophils, which leads to increased recruitment of NK cells and significantly reduced numbers of lung metastasis[68]. Other studies found that ectopic treatment or overexpression of IL-33 in transplantable

melanoma models leads to increased recruitment and activation of NK cells to the TME[69,68], possibly through a mechanism reliant on CCL5. Another atypical pathway shown to regulate recruitment of NK cells to the TME is controlled by the cytokine IL-17D and chemokine CCL2. In this pathway, tumor cell production of IL-17D signals to endothelial cells to induce production of CCL2 and subsequent recruitment of NK cells to the TME[70].

CCL27, which signals through CCR10, is another chemokine linked to regulating NK cell recruitment to the TME. Intratumor injection of adenovirus encoding CCL27 increases recruitment of NK cells to the TME in mouse models as well as inhibit tumor growth[71,72]. However, one study found NK cells to be dispensable for tumor growth control making the importance of CCL27-dependent recruitment of NK cells to the TME less clear[71]. It is interesting to note that endometrial cancer shows a paucity of NK cells in the TME and this correlates with a reduction in CCL27, CXCL12, and CCL21 production as compared to adjacent normal tissue[73]. The role of CCL27 in cancer is complicated by the fact that CCR10 was also shown to have tumor cell intrinsic functions as it may enhance the growth and metastasis of melanoma and breast cancer cells[74,75]. Clearly, more work is needed to fully determine the relative role of CCL27 in protective immune responses and progression of cancer.

CX3CL1, also known as fractalkine, is the ligand for CX3CR1, the chemokine receptor uniquely expressed on cytotoxic $CD56^{dim}$/CD11b SP NK cells. High expression of CX3CL1 is positively prognostic for patient outcome and NK cell infiltration in breast cancer [76], gastric adenocarcinoma[77], colorectal cancer[78], hepatocellular carcinoma[79], and lung adenocarcinoma[80]. Furthermore, CX3CL1 is downregulated in human breast cancer tissue compared to adjacent normal, consistent with the finding that there is a large skewing of $CD56^{bright}$ NK cells in the TME of breast cancer[39]. It has also been well demonstrated that the CX3CL1/CX3CR1 is subverted by the tumor through the production of TGF-β[81,82]. Subsequent work showed that TGF-β1 signaling in NK cells induces the expression of microRNA miR-27a-5p which downregulates the expression of CX3CR1[83]. It was also recently shown that CX3CL1/CX3CR1 signaling plays a role in controlling HCC metastases to the lung[79]. There, it was shown that tumor cells upregulate miR-561-5p which in turn inhibits the production of CX3CL1 and subsequently reduces NK cell recruitment to the tumor[79].

Taken together these results suggest an important role for chemokine signaling in regulating the recruitment of NK cells into the TME and provide the rational for targeting these pathways to increase the number of NK cells in the tumor.

Disruption or modulation of chemokine signaling by two immunomodulatory molecules, HLA-G and CD47, is linked to changes in NK cell recruitment to the TME. HLA-G is a member of the nonclassical HLA-class Ib genes and has been well established to have strong immune-inhibitory functions. HLA-G is expressed in the TME but not in surrounding normal tissue and studies have demonstrated that higher HLA-G expression correlates with increased cancer stage and/or worse patient outcomes (reviewed in ref.[84]). HLA-G can inhibit NK cell activation, cytokine production, and cytotoxicity through the down regulation of STAT3[85,86] while soluble HLA-G can reduce the expression of chemokine receptors in

human NK cells including, CCR2, CXCR3 and CX3CR1[55]. These findings suggest that soluble HLA-G found in the serum of cancer patients could impair the recruitment of NK cells to the TME.

The mechanisms by which CD47 regulates NK cell recruitment to the TME remain less clear. In the TME, CD47 has an important role in inhibiting phagocytosis of cancer cells (reviewed in ref.[87]). However, CD47 is also expressed on NK cells where, upon binding its ligand thrombospondin-1 (TSP-1), it can inhibit NK cell activation and proliferation[41]. Using an anti-CD47 antibody to block TSP-1 binding to CD47 reversed TSP-1/CD47 mediated inhibition in a human NK cell line, inhibited tumor growth in B16 melanoma bearing mice, increased NK cell recruitment to the TME, and enhanced expression of granzyme B and IFN-γ in NK cells[41]. Further studies are needed to explore the mechanisms recruiting NK cells to the TME following anti-CD47 antibody treatment, but these findings suggest CD47 acts as a NK cell checkpoint and highlight this pathway as a potential therapeutic target to modulate NK cell numbers in the TME.

Stromal barriers may also play a role in regulating NK cell recruitment to tumors (reviewed in ref.[82]). In tumor regions where extracellular matrix proteins collagen type IV and laminin were high, NK cells were not seen entering the tumor, suggesting these structures around the tumor could prevent NK cell invasion into the tumor[88]. Consistent with this finding, in human NSCLC tissue NK cells are most commonly found in stromal regions in the tumor, not in direct contact with tumor cells[64]. Furthermore, it has been suggested that even in tumors where there is high expression of NK cell attracting chemokines, there is not always a concomitant recruitment of NK cells (reviewed in ref.[82]). These findings reinforce that more research into the physical barriers that limit intratumor NK cells are needed and suggest that emphasis should be placed on studying NK cell localization and its effect on a beneficial immune response.

Step 2. NK cell recognition of tumor and activation

In contrast to T and B lymphocytes, NK cells utilize an array of activating and inhibitory, germ-line encoded receptors, to identify foreign, stressed, infected or cancer cells and to exert destruction of the target cell after full activation. Thus, complex signals arising from multiple ligand - receptor interactions need to be integrated and form the basis of NK cell recognition and activation. In the following, we will discuss the second step in the NK cell – cancer cycle: NK cell recognition of tumor and activation by means of cell contact-dependent and cell contact independent mechanisms (Figure 2 and 3).

One, if not the most important, signal for NK cells to identify potential target cells is the loss or aberrant expression of class I Major Histocompatibility Complex (MHC-I) molecules. The recognition and elimination of MHC-I lacking cells is called “missing self-recognition”.

NK cells constitutively express a variety of inhibitory receptors of the Ly49-family in mouse and Killer Immunoglobulin-like Receptors (KIRs) in human as well as the CD94-NKG2A heterodimer in both species[89,90]. Inhibitory KIR and Ly49 receptors are critical for the education of NK cells during development as these receptors recognize classical

polymorphic self-MHC-I molecules and thus allow NK cells to distinguish between healthy self-tissue and stressed, infected, foreign or transformed cells[91] (reviewed in ref.[92]). To evade adaptive immunity, cancer cells frequently downregulate classical MHC-I molecules which in turn renders them susceptible to NK cell-mediated control. In addition, CD94-NKG2A recognizes less polymorphic non-classical MHC-I molecules e.g. HLA-E in human and Q-1 in mice[93,94]. But beside inhibitory receptors, NK cells need to receive activating signals to exert their effector function. Here, below we will discuss contact-dependent NK cell activation in the TME (Figure 2 and 3).

NK cells are equipped with an armory of activating receptors, which are thought to recognize stress-induced ligands on cancer cells. Natural cytotoxicity receptors (NCRs), namely NKp46 (*NCR1/CD335*), NKp44 (*NCR2/CD336*) and NKp30 (*NCR3/CD337*) belong to the immunoglobulin (Ig) superfamily and are associated with various ITAM-containing adaptor proteins to recruit and activate downstream kinases (e.g. Lck, Fyn, Syk and ZAP-70) to fully activate NK cells (reviewed in ref.[95]). The identification of cancer cell ligands for NCRs is still a matter of ongoing research. While some ligands for NKp30 have been identified, e.g. B7-H6 and BCL-2-associated athanogene 6 (Bag-6), the ligands for NKp46 remain unknown[96, 97]. NKp80 which has activating properties in NK cells binds to activation-induced C-type lectin (AICL, encoded by *CLEC2B*), which is upregulated by TLR stimulation on myeloid cells[98]. Recently, Barrow et al. identified the platelet-derived growth factor (PDGF)-DD as a ligand for NKp44 using a secretome library screen[99]. NK cells activated with PDGF-DD secreted IFNγ and TNF, leading to cell cycle arrest of melanoma, ovarian and breast cancer cells *in vitro*. Importantly, increased PDGF-DD gene expression correlated with *NCR2* and effector cytokine expression and was associated with a favourable survival in glioblastoma patients[99]. In line with the idea that NCRs can sense soluble mediators Nidogen-1, an extracellular matrix protein, was recently described to bind NKp44[100]. Together these data suggest that NK cells can be activated or inhibited by secreted molecules engaging with NCRs. This novel concept opens up a new avenue of research with potential therapeutic value. Beside NCRs, the lectin-like type 2 transmembrane receptor NKG2D plays a crucial role in NK cell-mediated tumor cell killing. NKG2D is expressed on the majority of NK cells in humans and mice and recognizes a variety of MHC-related ligands that are poorly expressed in healthy tissues but strongly expressed in cancer cells[101, 102]. In mice retinoic acid early inducible-1 (RAE-1), murine UL16-binding protein like transcript-1 (MULT-1) and H60 proteins are ligands for NKG2D, while the ligands for human NKG2D are UL16-binding proteins and MHC class I-chain-related proteins (MICA/MICB)[103, 104].

Adhesion molecules have also been shown to promote NK cell activation. Lymphocyte function-associated antigen-1 (LFA-1) is expressed on NK cells and interacts with intercellular adhesion molecules (ICAMs) on target cells. Binding of LFA-1 to ICAM-1 can enhance NK cell-mediated cytotoxicity through enhanced polarization of the cytoskeleton machinery, which is required for effective delivery of cytotoxic granules[105]. DNAX accessory molecule-1 (CD226/DNAM-1) also contributes to NK cell adhesion, migration and function[106]. Upon binding its ligand CD155 or CD112, both frequently expressed on cancer cells, CD226 promotes NK cell activation and cytotoxicity[107].

NK cells are also regulated by many soluble extracellular factors in the TME. Notably, it has become increasingly clear that tumor cells and associated myeloid cells and fibroblasts, secrete a number of environmental factors such as cytokines, growth factors, exosomes, and microRNAs impacting the NK cell response. These have been extensively reviewed elsewhere [108,109,110,111] and to name a few key ones include TGF-β1 and associated family members, IL-10, extracellular adenosine, prostaglandin E2, and nitric oxide. Additionally, hypoxia and metabolic reprogramming impacts NK cell responsiveness [112,113,114]. These represent non-receptor immune checkpoints for NK cells that now shape many of the new therapeutic approaches to maintain and boost NK cell effector functions in tumors (see below).

Another important axis of NK cell activation in the TME is driven by proinflammatory cytokines and danger associated molecular patterns (DAMPs). Cytokines, upon binding their cognate receptor, augment activation, survival, proliferation and maturation of NK cells. Cytokines with NK cell stimulatory capacities are IL-2, IL-12, IL-15, IL-18 and IL-21. While IL-2 and IL-15, either alone or in combination with other cytokines promote survival and proliferation, IL-12 and IL-18 mainly stimulate IFN-γ production and cytotoxicity in NK cells. IL-21 can enhance NK cell-mediated cytotoxicity by upregulating granzymes and perforin, and exhibited synergistic effect with IL-2 for NK cell activation[115]. Additionally, type I and III interferons are important for NK cell homeostasis and activation[116,117]. Soluble ligands also have a strong impact on NK cell activation. One example is soluble HLA-G, which is able to activate human NK cells via KIR2DL4 leading to the production of cytokines and chemokines [118]. Soluble NKG2D ligands can inhibit NK cell function by downregulation of NKG2D[119]. In contrast, Deng et al. showed that soluble forms of high-affinity NKG2D ligands led to NK cell activation[120]. Thus, it remains unclear what role soluble NKG2D ligands play in NK cell activation.

Step 3. NK cell killing of tumor cells

NK cells are able to kill local and disseminated tumor cells (Figure 2 and 3, step 3). Furthermore, an eleven-year follow-up study found that reduced NK cell killing capacity in the peripheral blood is correlated with tumor development[121]. Thus, NK cell killing of local and disseminated tumor cells is an important effector function that can help control tumorigenesis. The mechanisms used by NK cells to kill cancer cells have been extensively discussed and are summarized in Box 1. Paradoxically, NK cell killing and abundance in the tumor correlate with better patient outcomes (Reviewed in [122,40, 41,42,43,44,45, 46, 47, 48, 49, 50, 51], but these cells are found at relatively low levels in tumors[40, 41,42,43,44,45, 46, 47, 48, 49, 50, 51]. This finding has led many to ponder how this rare cell type can be integral for protecting against cancer. While NK cell killing is clearly protective, NK cells have a number of functions, such as cytokine and chemokine production, that shape the immune response to cancer (discussed in Step 5 of the NK cell – Cancer cycle) that could amplify their importance. As such, in addition to the obvious anti-tumor activity of direct cytotoxicity, NK cell-mediated killing of cancer cells also has important impacts on the availability of antigen for presentation, both in the context of normal immune responses as well as in more clinical settings where monoclonal antibodies are used to induce antibody-dependent cellular cytotoxicity (ADCC) (reviewed in ref.[123]).

NK cell induced tumor cell death can lead to increased release of tumor antigen, which, if phagocytosed and processed by DCs, can increase the amount of tumor antigen presented to T cells, thus acting as a possible mechanism to boost T cell responses to cancer. Thus, NK cell killing of tumor cells can occur in the primary or disseminated tumor and can lead to a release of tumor antigens to prime an adaptive immune response, but this likely does not explain all of the protection afforded by the presence of NK cells in solid tumors.

Step 4. NK cell orchestration of adaptive immune responses

The most common ways that NK cells exert their effect on the adaptive immune response to cancer is through the production of cytokines and modulating dendritic cell (DC) responses (Figure 2 and 3, step 4).

Activated NK cells produce a variety of cytokines, including IFN-γ, GM-CSF, G-CSF, M-CSF, TNF, IL-5, IL-10, IL-13, and others[124]. IFN-γ is one of the best studied cytokines in the context of anti-tumor immunity and is a major factor in regulating positive and negative anti-tumor immunity. As the details of the mechanisms by which IFN-γ regulates immune responses have recently been discussed in great detail (reviewed in ref.[125,126]), we will focus our discussion on how NK cell production of IFN-γ is linked to changes in the adaptive immune response to cancer.

IFN-γ acts directly on a variety of immune cells including macrophages, DCs, B cells, T cells, and even NK cells themselves. IFN-γ signaling in macrophages activates these cells leading to increased inflammatory cytokine production, increased phagocytosis and antigen presentation, and enhanced nonspecific cytocidal activity toward microbial pathogens and tumors[127]. Additionally, IFN-γ induces DC maturation, which induces an increase in MHC-I and II expression, upregulation of co-stimulatory molecules, and upregulation of the cellular machinery needed for processing antigens to present to T cells[128]. In addition to upregulating antigen presentation machinery, IFN-γ activation of DCs has been shown to induce expression of the cytokines IL-12 and IL-15 in DCs which can play an important role in inducing anti-tumor Th1 CD4 T cell and CD8 cytotoxic T cell immune responses[129,104,130,131,132,133]. IFN-γ signaling also affects T cell function directly. IFN-γ signaling in CD4+ T cells can push them into an anti-tumor Th1 phenotype and induces an upregulation of granzyme and IL-2 receptor on CD8+ T cells, licensing these cells to their full cytotoxic potential (reviewed in ref.[125,126]). Furthermore, IFN-γ can directly increase antigen presentation in tumor cells, leading to increased tumor immunogenicity[134,135,136,137,138,139].

It is important to note that while IFN-γ is a strong driver of anti-tumor immunity it also contributes to immune evasion through increased expression of immune suppressive molecules such as programmed death-ligand 1 (PD-L1) on tumor and myeloid cells in the TME. Thus, this cytokine can act as a double-edged sword and its function may depend on how it is spatially distributed. More work is required to fully understand the role of IFN-γ in tumor progression and anti-tumor immunity; however, there is clear evidence that IFNγ production by NK cells is a major factor that allows NK cells to be integral players in shaping the adaptive immune responses to cancer and disease.

It is well established that there is cross-talk between NK cells and DCs and that the interaction between these two innate immune cell types leads to profound adaptive immune responses to disease and cancer (reviewed in ref.[140,141]). DCs are key players in the induction of T cell immune responses and, as antigen presenting cells (APCs), bridge the gap between the innate and adaptive immune system, making them an important partner in NK cell regulation of adaptive immune responses. Developmental, phenotypical and functional criteria distinguish DCs into two broad classes, the conventional DCs type 1 (cDC1s) and conventional DCs type 2 (cDC2s) in humans and mice. cDC1s are classically described as mediators of cellular immunity against intracellular pathogens and cancer, at least partially due to their specialization to cross-present antigens to CD8+ T cells, while cDC2s are more heterogenous and thought to be more efficient at inducing CD4+ T cell responses in cancer (reviewed in ref.[142]). There is a rich literature describing the relationship between NK cells and DCs. Initial studies showed that NK cells likely play an important role in shaping DC responses through editing DCs by directly killing immature DCs or by inducing maturation of DCs [143,144,145,146,147,148,131,149]. Thus, as is a common theme in immunology, the NK – DC interaction may act to induce proper and full DC function, but in certain settings can also negatively regulate adaptive immune responses. However, all of these data together clearly provide evidence of a functional link between NK cell activation and anti-tumor adaptive immune responses.

In addition to the important role of NK cells in regulating DC maturation, recent studies have found that NK cells play a role upstream of DC maturation and are also key regulators of DC recruitment, retention, and/or survival in the TME[43,44]. In a transplantable $BRAF^{V600E}$ mouse model of melanoma it was shown that NK cells are key in producing the chemokines CCL5 and XCL1/2 to recruit cDC1 into the tumor[44]. Importantly, the NK cell-dependent recruitment of cDC1 to the tumor is only seen in the absence of tumor-produced prostaglandin E_2 (*Ptgs1*/*Ptgs2*$^{-/-}$), suggesting that NK cell production of CCL5 and XCL1/2 and recruitment of cDC1 into the tumor is acutely sensitive to the immune-suppressive prostaglandin E_2 (PGE_2). NK cells in the TME also make the cytokine FLT3LG, the formative cytokine for cDC1[150,151], and NK cell levels and *FLT3LG* expression in the tumor correlates with increased cDC1 levels in the TME[43]. FLT3LG production by NK cells may be regulating cDC1 levels in the TME by increasing differentiation of precursor DCs (pre-DCs) in the TME or by increasing the survival of cDC1 within the TME[43,152]. Together, these findings suggest that NK cells, in addition to regulating DC maturation and subsequent T cell priming, are integral in recruiting and supporting cDC1 levels in the TME, an important function given the role cDC1 in the TME play in supporting protective immune responses to cancer[43,152,142,153,154,155].

In this step of the NK cell – cancer cycle, it is clear that NK cells directly modulate T cell activity through the production of IFNγ while also shaping the DC response to cancer through induction of DC maturation and the recruitment and maintenance of DCs in the TME. The findings presented here suggest that NK cells play an integral role in coordinating and initiating the adaptive immune response to cancer.

Targeting NK cells in cancer – progress and challenges

In the previous sections, we discussed individual steps required for NK cell recruitment, activation and effector function in the TME. The knowledge gained from basic and preclinical research over the last 40 years lay the foundation for the development of NK cell-based cancer immunotherapies. In the next section, we will discuss encouraging results and limitations of NK cell-based therapeutic approaches currently under preclinical and clinical evaluation (Figure 4).

The ideal therapeutic approach should aim to improve NK cells at every step in the NK cell-cancer cycle. A major problem for targeting NK cells in cancer patients is that many tumors are sparsely infiltrated with NK cells. Thus, a leading approach to boost NK cell-mediated tumor immunity is the adoptive transfer of *ex vivo* activated autologous (from the same patient) or allogeneic (from a healthy donor) NK cells. While adoptively transferred T cells can cause many severe side effects (e.g. cytokine release syndrome, graft-versus-host disease, etc.), the transfer of NK cells is comparatively safe (reviewed in ref.[10]). For example, two clinical trials showed efficacy of adoptively transferred haploidentical NK cells in non-Hodgkin's lymphoma and refractory or relapsed myelodysplastic syndrome as well as secondary AML and de novo AML patients, respectively[156,157]. Although encouraging results have been achieved in patients with liquid cancers, response rates in patients with solid cancers remain unsatisfying. Genetic engineering of NK cell products is a promising approach to improve the efficacy of NK cell transfer therapies (Box 2). One strategy currently under preclinical evaluation is to overexpress activating molecules including NKG2D, CXCR2 or membrane bound IL-15 in NK cells[158,159,160,161], or alternatively, to reduce the expression of inhibitory receptors like NKG2A[162]. Kamiya et al. elegantly showed that expression of a single-chain variable fragment binding NKG2A fused with an endoplasmic reticulum-retention domain in NK cells prevents the shuttling of NKG2A to the cell surface[162]. NKG2A-modified NK cells showed superior killing against HLA-E expressing target cells[162]. The discovery and development of the CRISPR/Cas9 genome editing technology, further opens up a future avenue to enhance NK cell products by modifying the expression levels of activating or inhibitory molecules (reviewed in ref.[163]).

NK cell expression of chimeric antigen receptors (CARs), directed against surface antigens expressed by tumor cells, is another encouraging approach. For example, NK cells expressing anti-CD19 CARs can efficiently kill autologous acute lymphoblastic leukemia (ALL) cells, which are resistant to CAR-negative NK cell-mediated killing[164]. Similarly, Li et al. recently showed that anti-mesothelin CAR expressing NK cells, derived from induced pluripotent stem cells (iPSCs) significantly impaired the growth of ovarian cancer in a xenograft model[165]. There, not only were they able to generate CAR NK cells from iPSCs providing another resource for an "off-the-shelf" NK cell product, but they also designed next-generation NK cell-specific CAR constructs. After screening multiple CAR-variants, NK cells expressing a CAR containing the transmembrane domain of NKG2D, the co-stimulatory domain of 2B4 as well as the signaling domain of CD3ζ showed the strongest efficacy[165]. Importantly, a landmark study by Liu et al. demonstrated that adoptive transfer of allogenic anti-CD19 CAR NK cells was safe and effective in high-risk $CD19^+$ chronic

lymphocytic leukemia and non-Hodgkin lymphoma patients[166]. This clinically relevant study, is encouraging and highlights the potential for future CAR NK-based therapies.

NK cells express or upregulate a variety of inhibitory receptors such as KIRs, NKG2A/CD94, programmed death-1 (PD-1), T cell immunoglobulin- and mucin-domain-containing molecule 3 (TIM-3), cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), lymphocyte activation gene 3 (LAG-3), T cell immunoreceptor with Ig and immunoreceptor tyrosine-based inhibition motif domains (TIGIT), and CD96 (reviewed in ref.[167,168, 169,170]). Recent reviews have thoroughly discussed our understanding of these inhibitory pathways and NK cells in cancer (reviewed in ref.[167,168, 169,170]). In many cases, the underlying biological mechanisms of these inhibitory pathways, as well as the clinical benefit of targeting the majority of these inhibitory receptors, need to be further explored. Here, we will focus our discussion on inhibitory pathways that have recently been studied in a clinical setting.

The first strategy tested to enhance NK cell function aimed to block HLA-inhibitory receptor interactions. The engagement of inhibitory receptors with HLA-molecules is considered a major impediment to NK cell activation. IPH2101 (1-7F9, human IgG4) mAb binds with high affinity to the inhibitory receptors KIR2DL-1,−2 and −3 and thereby prevents inhibitory signalling mediated by HLA-C molecule allotypes[171]. Phase I clinical trials showed that IPH2101 enhanced NK cell activation and *ex vivo* cytotoxicity and is safe and well tolerated by cancer patients[172, 173]. Pre-clinical studies supporting the combination of KIR blocking with lenalidomide or with rituximab for the treatment of multiple myeloma and lymphoma, respectively, have been previously reviewed[174]. Although in vitro studies had suggested IPH2101 induced KIR-ligand mismatched tumor killing by NK cells, a phase II clinical trial in patients with smoldering MM (KIRMONO) was terminated early due to a lack of clinical efficacy[175]. Subsequent investigations revealed that KIR2D molecules were removed from the surface of IPH2101-treated NK cells by trogocytosis, with reductions in NK cell function directly correlating with loss of free KIR2D surface molecules[176]. These data raise concerns that the unexpected biological events could compromise some antibody-based strategies designed at augmenting NK cell tumor killing[177]. In spite of its limited clinical benefits as a single agent, future studies will need to assess the potential of IPH2101 in combination therapies.

In addition to KIRs, the heterodimer NKG2A-CD94 has also received great attention. The heterodimer NKG2A-CD94 binds to the non-classical HLA class I molecule HLA-E (Qa1b in mice), which is often up-regulated on cancer cells. Recently, it has been shown that NKG2A inhibits NK activation and target cell killing[123]. Monalizumab (IPH2201), a blocking humanized anti-NKG2A antibody, has been tested in a number of clinical trials as a single agent or in combination across different cancers, however many open questions remain (Box 3 and extensively reviewed in[178]). Two recent studies have further shed light on NKG2A as a cancer immunotherapeutic target (reviewed in ref.[179]). Van Montfoort et al. demonstrated in preclinical solid tumor models that peptide vaccination combined with antibody blockade of NKG2A on $CD8^+$ T cells improved response rate and survival of mice over peptide vaccination alone[180]. While, André et al. demonstrated activity of NKG2A inhibition in combination with anti-PD-1/PD-L1 blockade in mouse lymphoma models and in human *in vitro* experiments[181]. In addition, the interim results of a clinical

trial in head and neck squamous cell carcinoma patients of combination of monalizumab and cetuximab, a clinically approved anti-EGFR antibody, led to a 30% objective response rate in immunotherapy refractory patients. Although these results are very promising and provide hope for future improvement of immunotherapies for cancer patients, many open questions remain and need to be addressed in preclinical and clinical studies.

As described in step 3 of the NK cell cancer cycle, cytokines play an important role in NK cell activation and function (Figure 2 and 3). Consistent with preclinical studies clinical work has focused on using NK cell stimulatory cytokines to increase NK cell activity and abundance in the TME, with some of these immunotherapies having beneficial effects on patient survival and disease outcome[182]. In this review we will focus on three NK cell stimulatory molecules being explored in the clinic: IL-2, IL-15, and IFN-α.

IL-2 activates NK cells by binding to the heterotrimeric IL-2 receptor (IL-2Rα/CD25, IL-2Rβ/CD122, and IL-2Rγ/CD132). Early studies found that recombinant IL-2 expands effector T cells and NK cells, but also induces a robust T_{reg} cell expansion in patients. Thus, a number of drugs have been developed that lead to preferential activation and expansion of CD8+ T cells and NK cells. The preferential targeting of CD8+ T cells and NK cells has been accomplished by skewing binding of IL-2 away from the IL2Rα subunit, which is more abundant on T_{reg} cells[182,183]. One such drug, NKTR-214 (Bempegaldesleukin), is a modified recombinant IL-2 protein that is well tolerated by patients and is capable of inducing a robust activation and increase of $CD8^+$ T cells and NK cells within the TME without changing T_{reg} cell numbers[184]. Monotherapy with NKTR-214 to heavily pre-treated, non-responsive patients with solid tumors led to 9 of 26 patients showing some level of stable disease, although no objective responses were measured by RECIST criteria[184]. These findings led to the combination of NKTR-214 with anti-PD-1 immunotherapy in patients with advanced solid tumors and preliminary results presented at the 2019 Society for Immunotherapy of Cancer (SITC) Annual Meeting, suggest that response to this combination therapy were durable and increased over time, with evaluable patients showing an objective response rate (ORR) of 53% (20/38) and 34% (13/38) of patients achieving complete responses (CR) at a median time of follow-up of 18.6 months (NCT02983045). Phase three trials are currently underway using NKTR-214 in combination with Nivolumab (anti-PD-1) in melanoma (NCT03635983), muscle invasive bladder cancer (NCT04209114), and renal cell carcinoma (NCT03729245). Other IL-2 combination therapies have been less successful, as bevacizumab, an inhibitor of VEGF and an antibody thought to induce ADCC, showed no benefit above IL-2 therapy alone[185].

IL-15 also has stimulatory capacity for NK cells, but does not induce T_{reg} expansion. Intravenous infusion or subcutaneous injection of recombinant human IL-15 (rhIL-15) led to expansion and activation of NK cells and $CD8^+$ T cells in patients with solid tumors[186,187]. While there were hints to clinical benefit in patients treated with rhIL-15, no responses were detected based on RECIST criteria[186,187]. Larger studies will be needed to fully elucidate the clinical benefit of rhIL-15 monotherapy. rhIL-15 has also been combined with haploidentical NK cell infusions in refractory acute myeloid leukemia (AML) and this treatment led to remission in 35% of patients and better rates of *in vivo* NK cell expansion and remission compared to previous trials with IL-2[188]. While

rhIL-15 holds therapeutic promise, animal studies have found that the IL-15 superagonist, IL-15 pre-associated with its soluble receptor IL-15Rα, can lead to a large increase in biological activity and enhanced activity as a cancer immunotherapeutic[189]. ALT-803 is a pharmacological grade IL-15 superagonist that promotes expansion and activation of NK cells and $CD8^+$ T cells in patients with post-relapse hematologic malignancies or solid tumors[190,165]. ALT-803 has shown some clinical response as a monotherapy in hematologic malignancies and solid tumors and can also induce signs of clinical response in combination with anit-PD-1 immunotherapy in patients with non-small cell lung cancer (NSCLC) that had refractory or relapsed disease from previous anti-PD-1 treatment[190,165,191]. These early clinical studies highlight an important role for NK cell expansion by recombinant IL-15 or IL-15 superagonists and have led to the initiation of a number of active Phase 2 or 3 studies across different cancer indications and in combination with other therapies (including: NCT02989844, NCT03586869, NCT03387098, NCT03329248, NCT03228667, NCT03136406, NCT02523469, NCT02384954, NCT01885897, NCT03022825, NCT02138734).

IFN-α treatment can activate NK cells and T cells to kill cancer cells in acute myeloid leukemia patients[192]. A major issue facing patients receiving allogeneic hematopoietic stem cell transplantation (allo-HSCT) to treat acute leukemia is post-transplant relapse. Patients with minimal residual disease following allo-HSCT were found to have lower relapse rates if they were treated with IFN-α[193,194]. This finding was attributed to immunomodulation of NK cells and T cells, or alternatively, through direct inhibitory effects of IFN-α on blast cells[193,194]. Future multicenter clinical studies will be necessary to confirm the efficacy of IFNα treatment to protect against relapse in leukemia patients with minimal residual disease.

Clearly, the modulation of NK cells through the treatment of patients with cytokines is an important area of clinical research. The potential benefits of cytokine therapies are clear, but these therapies are better understood in hematologic malignancies than in solid tumors. The large number of ongoing clinical trials in hematologic malignancies and solid tumors are going to lead to exciting findings and novel treatments that will undoubtedly target NK cells at every step of the NK cell – cancer cycle.

Bi-specific or tri-specific killer cell engagers (BiKEs or TriKEs) represent an alternative strategy to efficiently engage CD16 and induce ADCC-like responses (reviewed in ref.[174, 195, 196, 197]). Initial BiKE and TriKE constructs fused a single-chain variable fragment (Fv) against CD16 with, in the case of BiKEs, a single-chain Fv against a tumor antigen, or in the case of TrIKEs, two tumor antigens. Strategies for BiKEs and TriKEs are many, but include: single tumor antigen targeting (e.g. CD19, CD20, CD30, CD133, PMSA, BCMA, Her2, CEA, EGFR, etc.), dual tumor antigen targeting (allowing for avidity-tuned binding to two cancer antigens which increases the range of targetable tumors), dual TME targeting (allowing for avidity-tuned binding of two antigens on tumor-promoting cells occurs without affecting cells in healthy tissues), and dual targeting of both tumor and TME antigens (combining the prior two approaches). Some molecules like PD-L1, CD155, CD47, and CD38 may serve the purpose of acting as tumor antigens or immunosuppressive immune cell surface antigens elevated in the TME. The Fc itself when required may be afucosylated

to enhance ADCC or Fc mutated to silence that function. Nanobodies (Nbs) derived from camelid animals are emerging as a new force in antibody therapy.

Killer engagers are designed by fusing Fv domains that recognize tumor cell antigens with Fv domains binding CD16 and other relatively NK cell-specific surface activation molecules (eg. NKp30, NKp44, NKp46, NKG2D). There are very few of these other NK cell-specific molecules of choice at this stage, but molecules like NKp30 appear to be stably expressed by all NK cells and its ligation can trigger strong degranulation and cytokine release from NK cells. In the absence of cytokines, resting NK cells can be stimulated by combining the activity of a hierarchy of several activating receptors. CD16 is unique in its ability to mediate ADCC and to activate significant cytotoxicity and cytokine secretion when triggered alone. Many of the ligands recognized by NK cell receptors represent the body's method to detect altered or defective cells and support immune activation. The amount of ligand expression on the cell surface can also be modulated by ligand shedding, secretion of ligands, or excretion in macrovesicles. The degree that such ligand regulation affects immune targeting remains to be determined. Recently, Ferrari de Andrade et al. generated an antibody against the human NKG2D ligand MICA/B (Major Histocompatibility Complex Class-I chain related gene A/B), which prevented proteolytic shedding of the extracellular domains of MICA and MICB and stimulated antitumor immunity by activation of NKG2D and CD16 on NK cells. In a metastasis model pre-treated with human NK cells, this antibody effectively inhibited tumor growth in vivo[198].

A number of Important considerations need to be considered in this area are. First, how does the multivalent therapeutic overcome tumor escape from NK cells? Second, is the engager regulated by the TME (e.g. by molecules such as TGF-β)? For TriKE constructs it remains to be seen if the engagement of the NK cell-specific engager (eg. NKp30) is sufficient or if CD16 signalling is also required? It also remains to be seen if all (or a subset of) NK cell effector functions and proliferation are enhanced by these molecular designs. Another important consideration is if the activation by these therapeutics is too effective, will this lead to NK cell exhaustion? Further, more work is needed to determine what combination therapies would make a good partner with multivalent therapeutics to improve NK cell numbers, recruitment to and survival in the TME? Lastly, there is a risk that antigen negative variants of tumors will selectively grow out over time.

Regarding the TME, tailored additional modules (like TGF-β traps) and survival promoting cytokines (like IL-15 and IL-2) remain attractive strategies built into NK cell engagers (e.g. BiKEs, TriKEs, and killer enagers). An interesting strategy of simultaneous blockade of the immune checkpoint ligand PD-L1 and TGF-β, using a bifunctional antibody-ligand trap was recently reported[199]. But overall, these multivalent drugs are currently being investigated in preclinical studies and safety remains a concern with the potential to trigger cytokine cascades. With these future therapeutics different constructs have been engineered and indeed hundreds of possible formats may be tested pre-clinically, while some subset of these will reach the clinic in the future. We believe this whittling process should be guided by the rational provided by the NK cell – cancer cycle presented herein.

Outlook

The therapeutic potential of NK cells is incredibly high and the recent findings that CAR-transduced NK cells have low toxicity and are associated with high response rates drive home the importance of NK cells as potential immunotherapies to treat patients[166]. NK cell-based immunotherapies have been more efficacious in hematologic cancers, but progress is also being made developing these therapies for solid tumors (reviewed in ref. [200]). There is a growing excitement about the use of these cells to target cancer and our understanding of the NK cell – cancer cycle is constantly evolving. While NK cell cytotoxicity is an obvious effector function that plays important roles in controlling cancer, an equally important role of NK cells is to shape the TME and to modulate adaptive immune responses to cancer. Given the diverse roles NK cells play in shaping the immune response to cancer, described here in the NK cell – cancer cycle, as we develop and design novel therapies it will be important to try to target pathways that will hit all, or the majority, of the steps in the NK cell – cancer cycle with a focus not just on inducing NK cell cytotoxicity, but also to harness the immunomodulatory effects of NK cells.

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Box 1:

NK cell-mediated killing

Cytotoxic granules contain a number of cytotoxic proteins, including the pore-forming proteins perforin and/or granulysin and effector proteases called granzymes[201,202,203]. A recent study using mass cytometry to profile the expression of cytotoxic molecules in PBMCs found that human NK cell subsets have differential expression of cytotoxic molecules; with $CD56^{bright}$ NK cells showing low GzmB and perforin expression and high GzmK expression, while $CD56^{dim}$ NK cells showed high GzmB and perforin expression, with low GzmK expression[204]. These findings are consistent with the different cytotoxic activities of both NK cell subsets. In the context of tumor immunity this is also interesting given the clear bias towards recruitment of $CD56^{bright}$ NK cells to the tumor (as described in section: NK cell recruitment). Granule exocytosis normally accounts for antibody-mediated cellular cytotoxicity (ADCC), a mechanism through which Fc receptor-bearing NK cells can recognize and kill antibody-coated target cells expressing tumor-derived antigens on their surface. This is a particularly relevant consideration for antibody-based therapies. ***Death receptor signaling:*** In addition to NK cell-mediated killing by cytotoxic granules, activated NK cells are capable of inducing apoptosis in target cells through the interaction of Fas ligand (FasL/CD95L) and/or TNF-related apoptosis-inducing ligand (TRAIL) with death receptors on the surface of target cells. TRAIL and FasL have been shown to play an important role in controlling immune responses to cancer[205]. In particular these mechanisms appear important in NK cell-mediated control of liver metastasis[206,207], NK cell-mediated killing of immature DC[208], and prenatally in the function of fetal NK cells[206]. In the context of the NK cell cancer cycle, tumor necrosis factor alpha (TNF-α) likely plays an important role in modulating the TME and inducing direct cytotoxicity of tumor cells[209]. However, studies have shown that TNF-α alone is weakly cytotoxic or cytostatic and that TNF-α induces apoptosis only when metabolic inhibitors are present (Reviewed in ref.[210]). Thus, it remains unclear whether NK cell-derived TNF-α controls tumors directly by cytotoxicity of tumor cells, or indirectly on the immune microenvironment, and on the tumor vasculature[209].

Box 2:

CAR NK cells harness the power of innate lymphocytes

Expression of chimeric antigen receptors (CAR) in T cells represents an effective strategy to redirect the specificity of effector cells. Adoptive transfer of CAR T cells induces significant and durable responses averaging at around 60–70 % across multiple cancer types. Thus, two anti-CD19 CAR T cell products are approved by the FDA and currently in clinical use. However, beside logistical challenges (e.g. sophisticated and expensive production) the biggest problem of CAR T cells is the severe toxicities observed in patients including cytokine release syndrome, neurotoxicity and Graft-versus-host-disease[211]. A recent clinical trial using cord blood-derived HLA-mismatched anti-CD19 CAR NK cells showed a 73% response rate in non-Hodgkin's lymphoma and chronic lymphocytic leukemia patients. Most importantly, the treatment was not associated with adverse immune-related events[166]. This very promising study highlights the safety and therapeutic potential of "off-the-shelf" CAR NK cells. There are a number of properties of NK cells that need to be considered with respect to CAR NK cell development. NK cells are more difficult to expand in large numbers than T cells and their persistence in vivo after infusion is limited. NK cells are also educated continually by molecules such as MHC class I[212] and their functions are highly regulated by a balance of inhibition and activation signals, meaning that their action may be short-lived. NK cells cryopreserve quite well like T cells, but NK cells are more difficult to transfect and genetically engineer than T cells. CAR have higher affinity for tumor antigen than either endogenous NK cell receptors or TCR, but synthetic immunology is very effective for NK cells in the context of ADCC. Many questions remain open, such as what is the best target and would additional genetic manipulation of NK cells create superior products? Furthermore, the question of whether CAR NK cells are superior over CAR T cells needs to be addressed in prospective clinical trials.

Box 3:

NKG2A one of the most promising new targets in cancer immunotherapy?

The inhibitory receptor complex NKG24/CD94 is an attractive and promising target[213,214]. Currently, six Phase I and II clinical trials using monalizumab as single agent or in combination are recruiting patients across different solid and hematological malignancies including Head and Neck Squamous Cell Carcinoma and Non-Small Cell Lung Cancer (NSCLC) (NCT02671435, NCT02921685, NCT02643550, NCT03822351, NCT03794544, NCT03088059). Furthermore, two phase II trials in microsatellite stable colorectal cancer and immunotherapy resistant NSCLC patients are about to start (NCT04145193, NCT03833440). Despite these clinical efforts, translational research will need to address pressing questions around the NKG2A-pathway. Little is known about the expression of NKG2A/CD94 in tumor infiltrating $CD8^+$ and $CD4^+$ T cells. Additionally, we need to understand the regulation and expression of HLA-E in healthy and malignant tissue to potentially be able to stratify patients based on the expression of the NKG2A Ligand. The work by André et al. and van Montfoort et al. also raise the question about the relative contribution of NKG2A for the function of NK and T cells[181,180]. Overall, blocking or genetic deletion of NKG2A in effector cells seems to be a rational strategy and future studies will shed further light on the underlying biology and clinical relevance of this molecule.

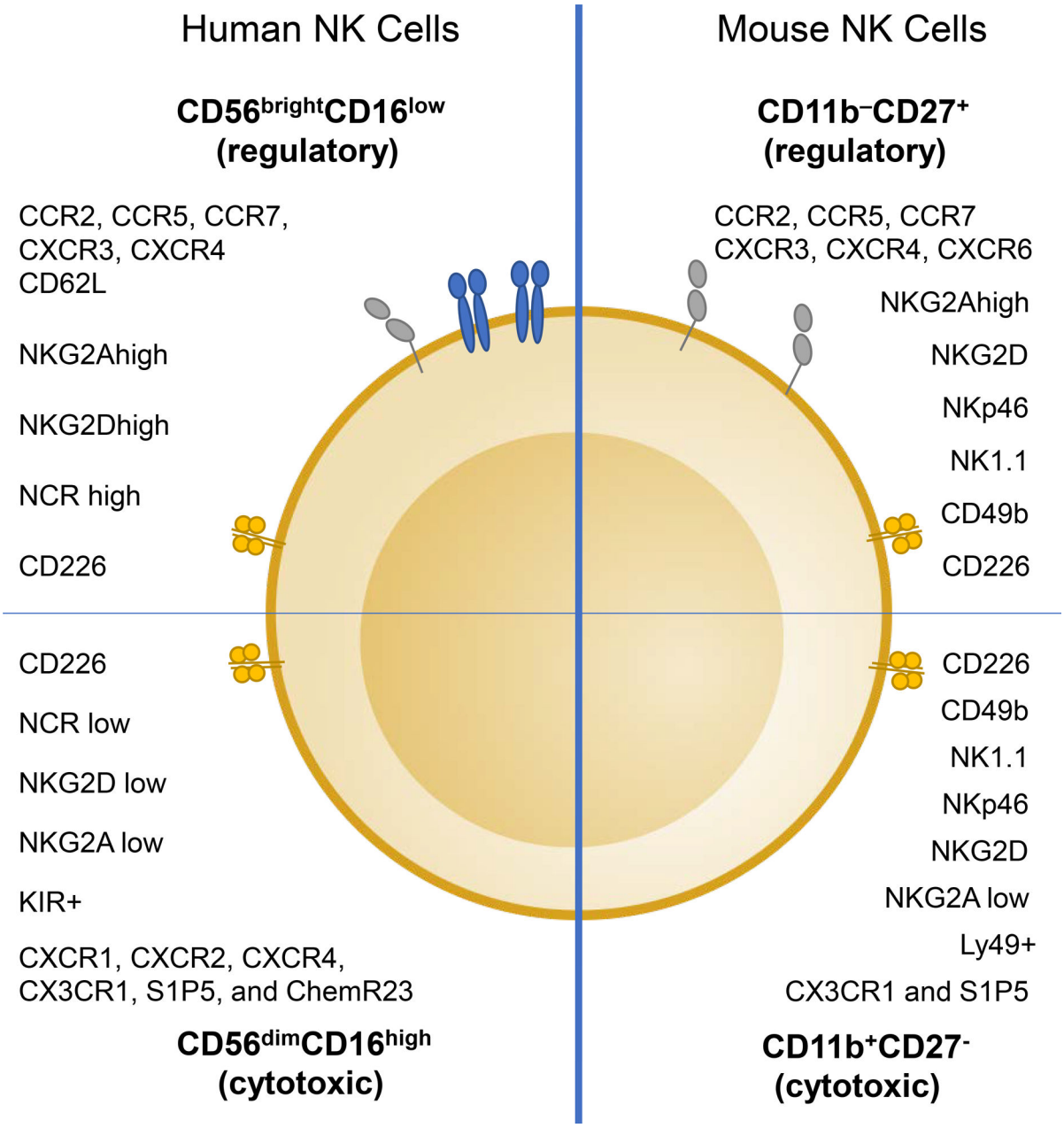

Figure 1.

Human and Mouse NK cells. Simplified human and mouse NK cells can be subdivided into three main subsets based on the expression of CD56 and CD16 in human and CD11b and CD27 in mice. Depicted are important molecules required for NK cell recruitment, activation and effector function in the TME.

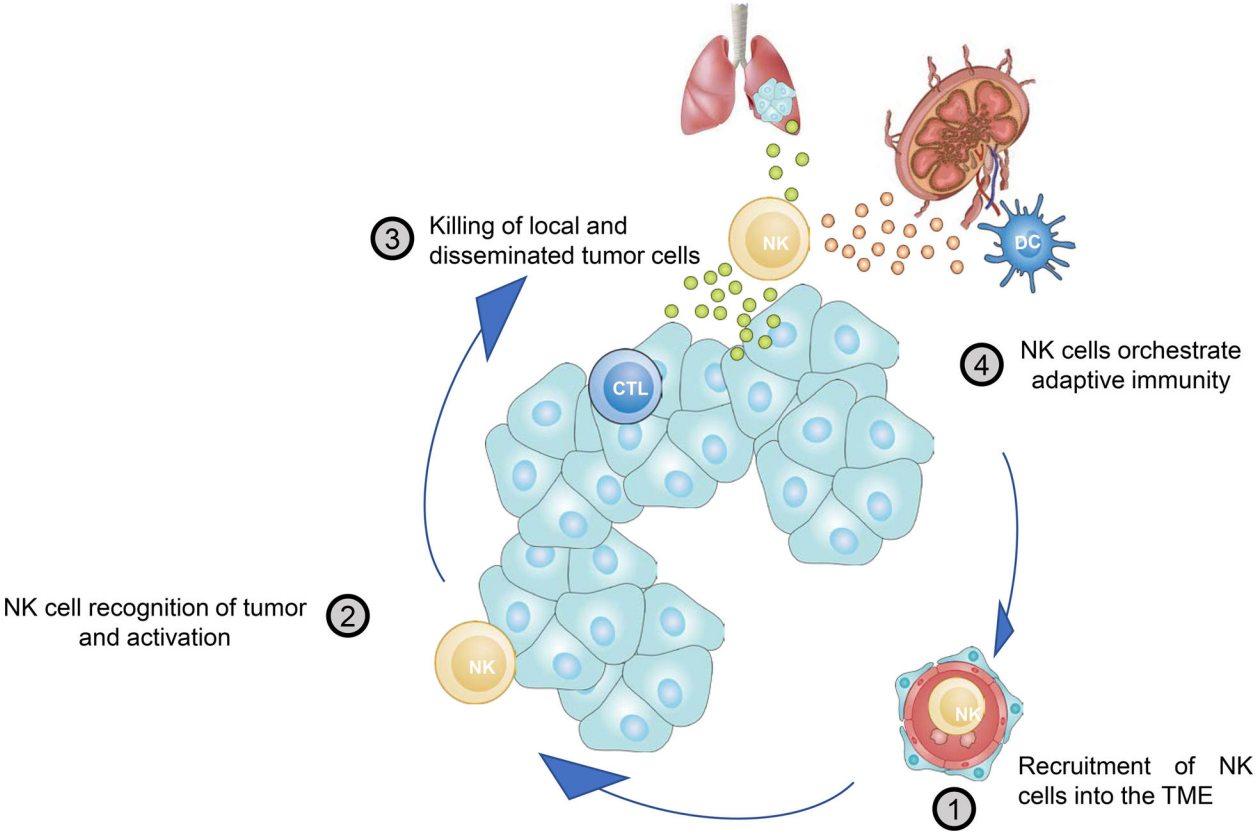

Figure 2.

The NK cell-Cancer Cycle. To effectively eliminate cancer cells, the body needs to initiate a self-propagating anti-tumor immune response. The NK cell-cancer cycle consists of 4 steps important to initiate and maximize the efficacy of this innate response. 1. NK cells need to be recruited into the TME; 2. NK cells recognize cancer cells and undergo full activation; 3. NK cells kill cancer cells locally and systemically; 4. NK cell orchestrate innate and adaptive immunity e.g. by alerting dendritic cells. Finally, due to elimination of cancer cells locally in the TME or at distant sites antigens, chemokines and danger associated molecular patterns are liberated further fueling innate adaptive responses and allowing the cycle to begin again.

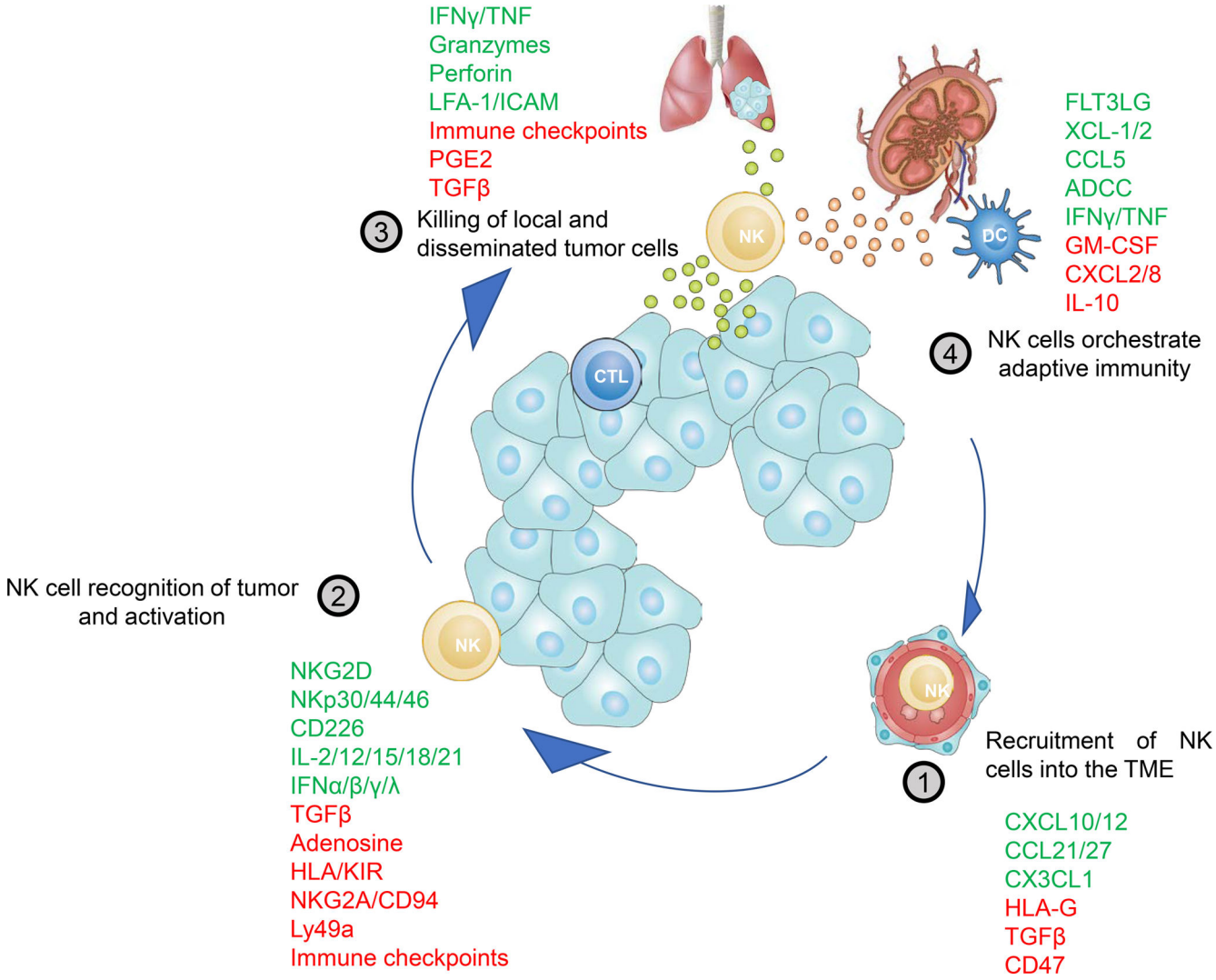

Figure 3.

Positive and negative molecules influencing the NK cell-Cancer Cycle. Each step in the NK cell-Cancer Cycle is affected by a variety of stimulatory or inhibitory signals integrated by NK cells. Positive molecules are depicted in green and promote NK cell activity, while molecules negatively affecting NK cell activity are shown in red. These negative signals are required to prevent overshooting immune responses leading to exacerbated tissue damage. However, in the TME these pathways are hijacked by the cancer cells to escape NK cell mediated immunity and to scotch the initiation of adaptive immunity.

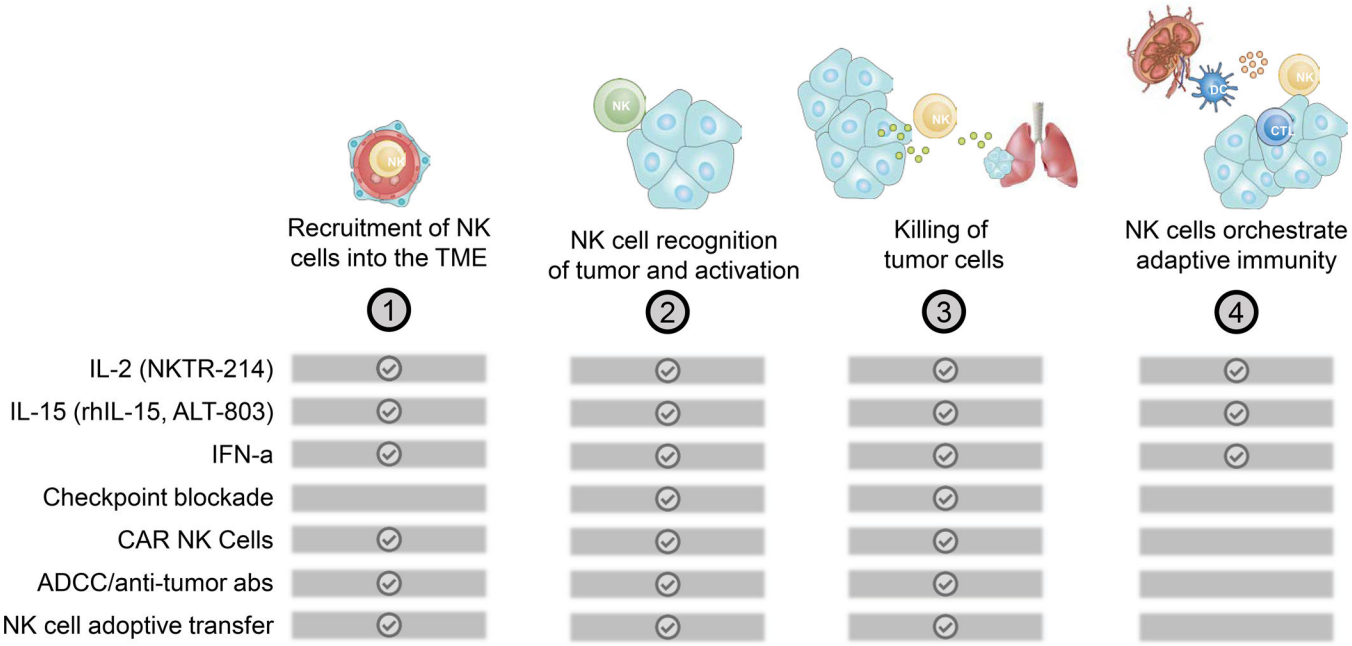

Figure 4.

Therapies affecting individual steps of the NK cell-Cancer cycle.

Table 1.

Advantages and disadvantages of NK cells over T cells in cancer therapy

Feature[1]	NK cells	T cells	Reference
Tumor stage	Elimination and metastasis	Equilibrium and Escape	6, 7
Number in tumor	+	+++	
Tumor cell recognition	+++ (NKR)	+ (TCR)	8, 9
Specificity	+	+++	10, 8
Killing capacity	+++	++	11, 12, 13, 14, 15, 16
Cytokine release	++	+++	13, 8
Proliferative capacity	+	+++	17, 18, 19
Recall response	0	+++	17, 18, 8
Immune related-adverse events	+	+++	20, 21, 22, 23, 24, 25
Genetic engineering	+	++	9, 26
Off-the shelf utility	+++	+	27, 28, 29, 30

[1]Feature on a scale of 0− to +++