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FOCUSSED RESEARCH REVIEW

Advantages and clinical applications of natural killer cells in cancer immunotherapy

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Abstract The past decade has witnessed a burgeoning of research and further insight into the biology and clinical applications of natural killer (NK) cells. Once thought to be simple innate cells important only as cytotoxic effector cells, our understanding of NK cells has grown to include memory-like responses, the guidance of adaptive responses, tissue repair, and a delicate paradigm for how NK cells become activated now termed "licensing" or "arming." Although these cells were initially discovered and named for their spontaneous ability to kill tumor cells, manipulating NK cells in therapeutic settings has proved difficult and complex in part due to our emerging understanding of their biology. Therapies involving NK cells may either activate endogenous NK cells or involve transfers of exogenous cells by hematopoietic stem cell transplantation or adoptive cell therapy. Here, we review the basic biology of NK cells, highlighting characteristics which make NK cells particularly useful in cancer therapies. We also explore current treatment strategies that have been used for cancer as well as discuss potential future directions for the field.

Keywords NK cells · HSCT · Adoptive transfer · Cytokines · CITIM 2013

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Introduction

Discovery and definition

NK cells are able to recognize and kill virally infected and malignant cells, mostly due to modulations in MHC-I and MHC-Ib molecules on target cells in these states [1]. NK cells are also associated with their ability to spontaneously reject bone marrow allografts in lethally irradiated mice, even from parental strains by F1 hybrids in an observation termed "hybrid resistance" [2]. This observation, when discovered, seemed to oppose the laws of transplantation in which transplantation antigens were thought to be co-dominantly expressed. Bennett et al., however, showed in 1973 that the use of strontium-89, a marrow-seeking isotope, prevented host mice from rejecting bone marrow allografts [3]. However, the identity of this bone marrow-derived effector cell was not known until 1975 when Herberman and Kiessling independently identified background or "natural" cytotoxicity when performing ^{51}Cr-release cytotoxicity assays against syngeneic and allogeneic tumors, even when using effector cells from athymic mice [4, 5]. These studies led to the discovery of a cytotoxic cell, discrete from a T cell, which required no prior sensitization or immunization in order to lyse a target cell and was non-MHC restricted. Thus, the name "natural killer cell" was born. While NK cells are certainly potent in their cytotoxic ability, the name "natural killer cell" is likely an oversimplification of the effector functions of these cells. NK cell production of cytokines may be paramount to cytotoxicity during the early phases of an immune response. For example, the large amounts of IFN-γ produced by NK cells upon encountering a viral infection play an important role in shaping a Th1 response and overall viral resistance [6].

Phenotype

Human NK cells are positively identified by their expression of CD56 and lack of T cell markers, such as CD3 or TCR. NK subsets can be further delineated into $CD56^{bright}$ and $CD56^{dim}$ populations based on the relative intensity of the CD56 stain when analyzed by flow cytometry [1]. $CD56^{dim}$ cells comprise the majority of circulating NK cells in the blood. These cells are highly cytotoxic but produce less cytokines when compared with $CD56^{bright}$ cells. Furthermore, nearly all $CD56^{dim}$ NK cells express the killer cell immunoglobulin-like receptors (KIR) as well as the Fcγ-receptor CD16. These receptors contribute to the high cytotoxicity of $CD56^{dim}$ NK cells by making them sensitive to targets with low-MHC expression or cells bound with antibody, respectively. Conversely, $CD56^{bright}$ NK cells make up a small proportion of circulating NK cells in the blood but are found in greater abundance in the lymph nodes. When stimulated, $CD56^{bright}$ cells are poorly cytotoxic but produce high amounts of cytokines, namely IFN-γ. These cells also lack expression of CD16 and KIR, which may contribute to their lower cytotoxic potential. Due to their presence in the lymph nodes and high cytokine production, $CD56^{bright}$ cells are believed to play an important role in shaping immune responses by regulating DCs [7] and T cell priming toward Th1 [8].

Biology

NK cells share close homology with T lymphocytes, especially CTLs, in their effector functions. Microscopically, NK cells are indistinct from other lymphocytes, though when activated they are known to grow in size and display increased granularity, thus stemming their original name: large granular lymphocytes (LGLs). NK cells share a common hematopoietic precursor with T cells and share many common features with these cells including the release of perforin/granzyme, the types of cytokines produced, and many of the cell surface markers. NK cells, however, mature in the bone marrow and periphery rather than the thymus and lack antigen specificity. Their actions are largely governed by the expression of an array of germline-encoded surface receptors, which allow the NK cell to simultaneously bind many ligands on the target cell [1].

Each time an NK cell forms an immune synapse with a neighboring cell, it must decide whether to release its cytotoxic granules or move on. The ultimate fate of the NK cell upon each cell encountered is determined by the net signal received from a range of activating or inhibitory receptors. Activation receptors, which often bind stress ligands on the target cell, send an activation signal to the NK cell, while inhibitory receptors send the opposite and suppress responses. Once the activation signals surpass a threshold, the NK cell degranulates resulting in the death of the target cell. The cytotoxic granules released by NK cells upon target cells are largely composed of two proteins, perforin and granzyme. Perforin, a structural relative of the C9 complement protein, creates small transmembrane pores on the surface of target cells. Apoptosis can then be directly triggered by the passage of granzymes into the cell and subsequent cleavage of caspase-3 [9].

Outside of their cytotoxicity, NK activation leads to the release of cytokines which affect both the immune response as well as target cells. IFN-γ is a vital cytokine produced by NK cells which effects naïve CD4 T cells, stimulation of antigen presenting cells (APCs), and upregulation of MHC-I on nearly all cells [1]. Increased MHC-I expression may act through a negative-feedback loop to decrease NK cell activation after an immune response by upregulating inhibitory ligands on target cells as discussed below. GM-CSF production by NK cells promotes the growth of myeloid cell lineages and DC maturation. This cytokine may be particularly important in lymph node interactions but has also been shown to allow NK cells to promote hematopoietic reconstitution after HSCT [10].

Inhibitory receptors

Human NK cells recognize MHC-I (human HLA-ABC) molecules via the killer cell immunoglobulin-like receptor (KIR) family. Receptors in this family of molecules are stochastically expressed on mature NK cells and bind to HLA in a manner independent of the antigen presented by each MHC-I molecule. Viruses or tumors may decrease HLA expression in order to evade CTL detection; however, NK cells co-evolved to become more sensitive to cells in these states. KIR/HLA interactions primarily send an inhibitory signal to the NK cell via intracellular tyrosine-based inhibitory motifs (ITIM) on the cytoplasmic domain of inhibitory receptors or coreceptors. Thereby, cells which display lower levels of HLA will be more likely to be lysed by the NK cell. Structurally distinct from the KIR receptors, the CD94/NKG2A receptor binds the MHC-Ib molecule HLA-E and sends a similar inhibitory signal via an ITIM pathway.

Overall responsiveness to activation signals is linked to the expression pattern of inhibitory receptors on each NK cell. NK cells possessing inhibitory receptors capable of binding self-MHC become fully responsive, while those not expressing these markers remain hyporesponsive [11]. However, this hyporesponsive state can be overridden upon activation, and therefore, unlicensed NK cells can contribute to antitumor or anti-viral responses under the proper conditions [12].

For example, KIR3DL1 binds HLA-A or B molecules with the Bw4 epitope, even though a given person may not possess this epitope [13]. Thereby, KIR3DL1-expressing NK cells in an individual lacking the Bw4 epitope may be prone to autoimmunity, since these cells lack an effective "turn off" signal. However, by never encountering self-antigen during development, these cells remain hyporesponsive and thus prevent autoreactivity. This process, now termed "licensing" [11], "arming" [14], or "NK education" [15], is believed to be the mechanism through which NK cells achieve self-tolerance, much like development of T cells in the thymus or B cells in the bone marrow and has been more extensively characterized in the mouse [14]. The expression patterns of KIRs in donor NK cells have proven to be critical for hematological reconstitution and antitumor responses in the transplant setting as we will describe later.

Activation receptors

While the expression patterns of inhibitory receptors are stochastic, activation receptors are more ubiquitously expressed, especially on $CD56^{dim}$ NK cells. NKG2D and the natural cytotoxicity receptors (NCRs; NKp30, NKp44, NKp46) are thought of as the key receptors involved in sending an activation signal when NK cells encounter a target cell in an immune synapse. However, other activation receptors such as DNAM-1, NKG2C/CD94, 2B4, and a class of activating KIR receptors also play a role in the activation of NK cells. In contrast to inhibitory receptors, coreceptors associated with the above proteins express immunoreceptor tyrosine-based activation motifs (ITAMs) to convey an activation signal. The ligands for many NK activation receptors are MHC-Ib molecules which are upregulated during times of cellular stress. For example, the NKG2D ligands MICA/B as well as the UL16-binding protein family (ULBP) can be upregulated during times of rapid proliferation [16]. The ligands for NKp30, BAT-3, and B7-H6 are expressed on stressed or transformed cells, respectively. NKp30 also recognizes the CMV pp65 protein. NKp44 has been shown to bind West Nile and dengue virus envelope glycoproteins [17], and NKp46 has been found to bind vimentin on the surface of mycobacterium tuberculosis-infected cells [18]. Both NKp44 and NKp46 have been shown to bind influenza hemagglutinin (HA); however, further ligands have not been identified. Experiments in which these NCRs are blocked result in a decrease in cytotoxicity against tumor cells not expressing influenza HA, suggesting tumors express yet unidentified NCR ligands [19].

Perhaps the most potent stimulator of NK cells is the CD16 FcγRIIIA. CD16 signals through ITAMs present on the accessory CD3ζ cytoplasmic accessory protein which transmits a powerful activation signal [1]. Recognition of IgG antibodies bound to target cells allows NK cells to lyse these antibody-coated cells through a process called antibody-dependent cellular cytotoxicity (ADCC). NK cells, along with the complement system, thereby act as the final mediators to eliminate pathogenic cells recognized by the humoral immune response.

Death ligands

In addition to active killing mechanisms, NK cells can passively kill stressed cells using apoptotic processes inherent to the target cell. Under times of cellular stress including hypoxia or proliferation, death receptors such as the TNF family members Fas and death receptor 5 (DR5) are upregulated. These death receptors, upon encountering their ligands, initiate a signaling cascade resulting in apoptosis. NK cell expresses the ligands for Fas and DR5: FasL and TNF receptor apoptosis-inducing ligand (TRAIL), respectively. FasL and TRAIL are more highly expressed on activated $CD56^{dim}$ NK cells and add to the repertoire of killing mechanisms available to these cells. In contrast to active killing mechanisms utilizing perforin/granzyme, apoptosis via Fas and DR5 occur more slowly and often cannot be detected in 4-hour cytotoxicity assays which are the gold standard for assessing NK lytic capacities [20].

Cytokine stimulation

The survival, proliferation, and function of NK cells are heavily regulated by the local cytokine milieu—especially those produced by activated DCs and CD4 T cells. Type I IFN, IL-2, IL-12, IL-15, and IL-18 can result in the activation of NK cells. IL-15 is considered the essential cytokine for NK cell development and survival; $IL\text{-}15^{-/-}$ mice lack NK cells and are unable to sustain adoptive transfers of wild-type NK cells [21]. Interestingly, NK cells are responsive to IL-15 without requiring expression of the high-affinity IL-15Rα chain. IL-15 may be presented in *trans* by utilizing IL-15Rα expression on DCs or monocyte/macrophages binding to CD122/CD132 (IL-2/IL-15 receptors β/γ).

Similarly, IL-2 binds to the low-affinity IL-2 receptor (IL-2Rβ/γ) which is expressed by $CD56^{dim}$ NK cells. However, activation induces the upregulation of CD25 on both subsets of NK cells thereby allowing cells to express the high-affinity heterotrimeric receptor and increase responsiveness to IL-2. IL-2 has been widely used to activate NK cells ex vivo into lymphokine-activated killer (LAK) cells. Morphologically, these cells show a characteristic increase in granularity and acquire an irregular shape. The lytic ability of these cells is at least an order of

magnitude above that of resting NK cells; however, LAK cells become highly dependent upon IL-2. Upon transferring these cells in vivo, a lack of stimulation causes these cells to undergo apoptosis unless high amounts of intravenous IL-2 are administered [22].

NK immunotherapies for cancer

Due to their natural ability to recognize and lyse tumor cells using a variety of recognition receptors, NK cells have been examined clinically in several immunotherapeutic strategies for cancer. Therapies utilizing NK cells can be classified as either harnessing endogenous responses by administering NK stimulants or targeting agents, or using exogenous NK cells via hematopoietic stem cell transplant (HSCT) or adoptive cell transfer (ACT) models. As NK cells are found primarily in the blood and rarely infiltrate solid tissue tumors, NK immunotherapies have been most successful in hematopoietic malignancies, although they have also been examined in many non-hematopoietic and metastatic cancers.

There are several key advantages to harnessing NK cells as part of an immunotherapy. First, NK cells are antigen non-specific and do not require the expression of a specific antigen expressed on a given HLA allotype. Rather, NK cells recognize a broad panel of several dozen ligands which can each induce a cytolytic response. In contrast, therapies utilizing a specific target, such as monoclonal antibodies or vaccine therapies, require the presence of a single antigen. While these therapies may be highly effective and achieve long-term effects in many cases, antigen shedding and escape variants remain a major concern. Second, NK cells can be easily isolated and expanded ex vivo which allows for their use in adoptive or autologous cell therapies. Third, NK cells have a lifespan much shorter than that of T cells; T cell-adoptive therapies utilizing a genetically altered cell often require a suicide vector to prevent the overexpansion of the transferred cells. NK cells, unless genetically altered, have a lifespan of 1 month or less which precludes the necessity for a suicide vector.

The most common therapies for all types of cancers, besides surgical resection, are chemotherapy and radiation therapy. These therapies have proven highly effective at eliminating rapidly proliferating tumor cells. However, recent studies suggest that these therapies are less effective at eliminating cancer stem cells (CSCs; also referred to as tumor-initiating cells). CSCs have been reported to have lower rates of proliferation, higher DNA repair mechanism, and increased drug efflux capacity, all of which may aid in their resistance to these therapies [23]. CSCs have recently been demonstrated to be highly susceptible to NK cell attack, suggesting that NK cells may be useful as part of a multi-pronged approach capable of targeting CSC and non-CSC populations alike [24, 25].

NK cell modulators

As mentioned previously, the use of cytokines alone has a potent effect on the cytolytic ability of NK cells. As such, many NK cell-activating cytokines have been administered clinically with the hopes of improving endogenous NK cell recognition and lysis of malignant cell types. IL-2 has been FDA approved for use in renal cell carcinoma and melanoma and has been shown to increase NK cell numbers in the periphery, NK cell cytotoxicity, and overall survival [26]. Although IL-2 as a single agent has shown encouraging responses, it presents several issues. First, repeated administrations of high-dose IL-2 lead to a number of morbidities including vascular leak syndrome which severely limit the duration of IL-2 administrations and thus the longevity of transferred NK cells [27]. Second, IL-2 selectively expands the less cytotoxic $CD56^{bright}$ population of NK cells since these cells, rather than $CD56^{dim}$ NK cells, more highly express the CD25 high-affinity IL-2 receptor [28]. Third, IL-2 is known to expand and activate T regulatory cells (Tregs) which also constitutively express the high-affinity IL-2Rα and can outcompete NK cells for IL-2 [29]. This Treg expansion can nullify any enhanced antitumor effects from NK cells since NK cells are highly sensitive to Treg-produced inhibitory cytokines, namely TGF-β and IL-10. Thus, a likely therapeutic strategy involves depleting Treg cells prior to the administration of IL-2 to prevent the Treg expansion and thus maximize antitumor effects. In mouse models, we showed that this approach led to a greater expansion of NK cells and greater cytotoxicity when compared with IL-2 administration alone [30].

IL-15 is an attractive candidate for activating NK cells in vivo as it is not known to expand Tregs but can potently activate NK cells and expand memory CD8 T cells. Also unlike IL-2, IL-15 equally activates $CD56^{dim}$ and $CD56^{bright}$ NK cells and leads to the expansion of both populations [31]. Preclinical primate models have not reported severe toxicities with IL-15, and Phase I clinical trials are well underway.

Lastly, several immunomodulators have been shown to increase the sensitivity of tumor cells to NK therapies. Bortezomib, a proteasome inhibitor, is FDA approved for use in multiple myeloma and mantle cell lymphoma. Bortezomib prevents the degradation of IκB from NF-κB and thus the translocation of NF-κB into the nucleus to initiate transcription. This non-specific regulation of NF-κB has direct effects on tumor proliferation and survival. However, bortezomib is also associated with an upregulation in the death receptors Fas and DR5, both of which may

be triggered by an NK cell to initiate an apoptotic cascade [20, 32]. Secondly, disrupting the proteasome limits the availability of peptides which may be presented on MHC-I molecules. This effectively decreases the amount of MHC-I expressed on the tumor cell and further increases its susceptibility to NK cell attack. However, bortezomib is highly toxic to NK cells which necessitates careful timing when administering it along with an NK-based therapy [32].

Hematopoietic stem cell transplantation

HSCT is most often used as a therapy for hematological malignancies and can be separated based on the source of the donor cells as autologous or allogeneic. Allogeneic transplants require an HLA-matched donor from which HSCs are harvested then infused into the patient having undergone high-dose chemotherapy. Allogeneic HSCT adds the benefit of a graft-vs-tumor (GVT) effect which occurs when graft immune cells recognize malignant cells as "foreign" and mount an immune response against them. Conversely, patients undergoing allogeneic HSCT have a risk of developing graft-versus-host disease (GVHD) which occurs when graft immune cells attack normal host tissues, often the skin, liver, and lungs. To reduce the risk of GVHD, donor grafts are often depleted of T cells, which not only eliminates GVHD but also hampers GVT effects [33]. NK cells are the first lymphoid cell type to repopulate after HSCT and have demonstrated an ability to mediate GVT affects while not contributing to GVHD [10].

An important clinical study from Ruggeri et al. [34] first identified a correlation between donor KIR and host KIR ligand expression in tumor relapse occurrence. KIR mismatches, in which donor NK cells expressed KIRs which had no ligand in the patient, showed superior GVT effects in AML, but not ALL, patients when compared with KIR-matched transplants. This study in particular shifted the focus of NK-based therapies to examining NK subsets. Both licensed and unlicensed NK cells which develop early after allogeneic HSCT show hyper-responsiveness early after the transplant, but the unlicensed cells gradually become tolerized to self around day 100 [35]. Interestingly, similar observations with regard to KIR/KIR ligands have been made in autologous settings in which patients lack ligands for at least one of their own inhibitory KIR. Here, a missing KIR ligand correlated with improved clinical outcomes in patients undergoing autologous HSCT for stage IV neuroblastoma [36].

Donor lymphocyte infusion and adoptive cell transfers

The adoptive transfer of mature NK cells has been used either alone or in combination with autologous or allogeneic HSCT. These therapies utilize fully differentiated and often activated NK cells rather than relying on immature NK cells which develop from hematopoietic precursors in HSCT. NK cells activated ex vivo exhibit cytotoxicity far greater than that can be achieved in vivo and cause minimal toxicities when infused into patients in either allogeneic or autologous models [1]. However, as mentioned previously, these therapies are severely limited by the necessity to maintain the NK cells with super-physiological levels of NK-survival cytokines, predominantly IL-2. While IL-2 is effective in prolonging the survival and thus antitumor effects of adoptive NK therapies, it carries the same toxicities and shortcomings as mentioned above when given as a single agent. Nonetheless, adoptive transfers of autologous NK cells have been used in models of glioma, lymphoma, and renal cell carcinoma and have been well tolerated, though clinical responses have not always been observed [37, 38].

Allogeneic adoptive cell transfers present the added benefit of potentially mismatching KIR/KIR ligands between the host and donor NK cells. These therapies, however, pose a greater risk of developing toxicities, especially when used in combination with conditioning regimens that cause a destruction of normal host tissue. However, several studies using allogeneic adoptive NK therapies have shown strong clinical benefits. In a study using haploidentical allogeneic NK cells in patients with AML, complete remissions were observed in 26 % of patients, and nearly all patients saw an expansion in NK cells after IL-2 therapy [39].

While pre-activated NK cells carry the added benefit of higher cytotoxicity, their longevity becomes shortened due to dependence on cytokine signaling [40]. Future therapies must walk a tightrope between using highly activated or naïve NK cells by assessing the advantages and disadvantages of each. Creative genetic therapies may also be effective at prolonging the lifespan of transferred NK cells through the transduction of cytokines which can stimulate NK cells in an autocrine fashion.

With the recent success of a therapy in which T cells were genetically engineered to express a chimeric-antigen receptor (CAR) for CD19, similar strategies have been used for NK cells. For example, NK cells have been transduced with a disialoganglioside GD2-recognizing CAR for use in neuroblastoma and have shown enhanced killing in in vitro assays [41]. Similarly, CARs directed toward CD19 or CD20 have been examined in NK cells for use against leukemias and lymphomas expressing these markers [42, 43].

The use of NK cell lines, such as NK-92, may also find a clinical niche. NK-92 is derived from an NK cell lymphoma and exhibits the phenotype of a $CD56^{bright}$/CD16-/KIR- NK population [44]. However, these cells maintain

both characteristics of an NK cell, i.e., cytotoxicity and cytokine production, and that of a malignant cell, i.e., limitless replicative potential. NK-92, however, is IL-2 dependent and thus must be sustained with infusions of IL-2 in a clinical scenario when using this cell line. Alternatively, an IL-2-independent NK-92 variant has been developed, NK-92MI, which produces IL-2 to stimulate the cells via autocrine mechanisms [45]. While NK-92 cells have shown clinical benefits, recent studies have been obligated to use irradiated NK-92 cells from medical-governing bodies [46, 47]. While this irradiation severely limits the survival and function of the transferred cells, it precludes any concerns associated with transferring live malignant cells into patients.

Antibody-dependent cellular cytotoxicity

Antibody targeting agents for cancer have been used in the clinic for many years and have a well-respected pedigree. Two common examples are trastuzumab for $HER2^+$ breast cancer and rituximab for $CD20^+$ lymphomas and leukemias. These therapies use both compliment-mediated cytotoxicity and ADCC to lyse antibody-coated cells. The contribution of NK cells to the antitumor effects have been clearly exemplified in trials where IL-2 was combined with rituximab in tumors which were previously resistant to rituximab [48]. Therapies combing NK cells and antibody targeting add the advantage of locally activating NK cells at the tumor site via CD16 activation. Interestingly, unlicensed NK cells were shown to be the predominant subset of NK cells responsible for potent ADCC due to their lack of inhibitory receptors for self [13]. Recent studies have further attempted to locally activate NK cells by conjugating NK-activating cytokines to Fc regions of humanized antibodies. For example, conjugations of both IL-2 and IL-12 to an anti-CD30 antibody for Hodgkin's lymphoma has shown efficacy in preclinical mouse models [49]. These therapies may also expand NK cells at the tumor site which may be particularly important in solid tumors where NK infiltrates are rare.

Concluding remarks

While NK-based immunotherapies demonstrated a number of recent success stories, long-term effects from these therapies are often lacking and the type of cancers in which they have been successful are limited. This brings about several questions in NK cell therapies. First, in which types of cancer will NK cell therapies show the best responses? The majority of clinical trials have targeted hematological malignancies most likely due to the long pedigree of NK-based therapies in the blood with regard to the kinetics of the NK cells, their activation status, and known toxicities. Relatively, little is known regarding the migration patterns and lifespan of NK cells, especially after infusions of exogenous cells. Better knowledge of the mechanisms NK cells use to home to various sites will be important in future therapies to allow NK cells to travel to the tumor site(s). Solid tissue tumors show high sensitivity to NK cells in vitro, and mouse models in which NK cells are injected intra- or peritumorally have shown promise [50]. Since many of these tumors arise in an environment largely devoid of NK cells, they may show more sensitivity to NK cell killing when compared with hematological malignancies. In hematological malignancies, immunoediting occurs between the malignant cells and NK cells allowing clones of tumor cells to grow out which are no longer recognized by NK cells [51, 52]. In solid tissue tumors where NK infiltrates are rare, this process most likely does not occur to the same degree and may allow a robust NK response to destroy the entire tumor before the tumor can adapt. Any cancer therapy, if given for a long duration, will most likely allow for the expansion of resistant tumor clones. This may necessitate the use of powerful, short-term therapies, which combine several approaches.

Second, how can NK cells best be activated and should this activation occur in vivo or ex vivo? When NK cells are stimulated ex vivo, they can be activated with extremely high concentrations of cytokines to develop into powerful cytotoxic cells. Furthermore, the activation of NK cells appears to override the effects of licensing [13]. Therefore, the activation status of cells used in therapies may dictate which subset of NK cells is desired. However, highly activated cells have a limited lifespan when infused into a patient due to their high cytokine dependence. It may be that a stronger lytic response trumps the need for a longer durational response, especially when combined with multiple therapies to simultaneously attack tumor cells through different pathways. This is similar to many traditional cancer regimens combining several chemotherapies to attack the tumor from multiple angles.

Finally, what specific mechanisms do tumors use to evade NK cells and how can these be overcome? Tumors have evolved multiple means to evade the immune system and NK cells in particular. While these individual mechanisms are outside the scope of this focused review, in brief, tumors may secrete suppressive cytokines, downregulate or shed activating ligands, express death receptor ligands to kill NK cells, and/or recruit immunosuppressive cell types. Many of these individual immune-suppressive mechanisms have been targeted in preclinical and clinical therapies, but a more holistic approach may be necessary.

NK cell therapies are gradually building a track record in the treatment of cancer. Above all else, most NK-based

therapies have not been associated with adverse effects. NK cells clearly have a role in future immunotherapies for cancer and should continue to be evaluated in clinical trials.

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