Trends in Cancer Research
Vol. 8, 2012

Review

Cancer immunotherapy using natural killer (NK) cells

Maite Alvarez[1], Erik Ames[1], Can M. Sungur[1], Jessica Perez[1], Gail Sckisel[1], and William J. Murphy[1,2]

[1]Department of Dermatology, [2]Department of Internal Medicine, School of Medicine, University of California, Davis, Sacramento, CA, 95817, USA

ABSTRACT

Natural killer (NK) cells have been shown to play an important role in cancer, particularly hematologic malignancies. In contrast to T cells, NK cells are able to kill cancer cells without prior sensitization. They recognize the lack or alteration of self-MHC class I molecules as well the presence of stress ligands, both of which have been shown to be differentially altered in several cancers, making NK-based immunotherapy a plausible therapy against cancer as a single therapy or in combination with current cancer therapies. Some NK-based immunotherapies such as the adoptive transfer of activated allogeneic NK cells after hematopoietic stem cell transplantation (HSCT) in hematological malignancies have demonstrated promising outcomes. Moreover, differences in NK subsets are just starting to be elucidated which are leading to the optimization of therapies which selectively expand those NK cells that would elicit the greatest anti-tumor efficacy. In this review, we will summarize our current understanding of NK cell biology particularly as it relates to use in cancer therapy as well as assess the different mechanisms that tumor cells have evolved to evade NK cells. We will also summarize the NK-based therapies that have previously been applied in cancer, those that are currently under investigation, and possible future directions.

KEYWORDS: natural killer cells, cancer, immunotherapy, bone marrow transplantation

1. INTRODUCTION

In recent decades, there have been significant advances in the field of cancer immunotherapy. Although current cancer treatments using cytoreductive chemotherapeutic drugs and radiation have significantly lengthened patient survival after diagnosis, relapse remains a significant issue in outcome. This failure in preventing relapse has been linked to selective pressure aggravated by the actual treatments which result in the escape of a relatively small cancer population that become resistant. The use of immunotherapy offers a means to further attack these resistant tumor cells. However, traditional cytoreductive cancer treatments are also known to negatively impact immune cells due to their high proliferation rate which probably also accounts for the frequent appearance of a more aggressive secondary cancer after relapse. Therefore, treatments that promote immune responses against cancer have become a promising treatment strategy but must overcome these hurdles. Due to the ability of natural killer (NK) cells to initiate tumor killing without prior sensitization, NK cell-based immunotherapies are currently under consideration for the treatment of a variety of cancers, particularly hematologic malignancies. In this article, we will review the current understanding of NK cell biology, the different mechanisms that tumor cells have acquired to escape from NK recognition, and NK-mediated killing as well as the latest NK-based therapies.

1.1. Definition of NK cells

NK cells are classically considered an innate arm of immune responses against transformed and virally infected cells. Human NK cells were first described by Kiessling and Herberman in the 1970s due to their ability to naturally kill tumor

cells *in vitro* without prior sensitization [1-4]. This *in vitro* activity was MHC-unrestricted, unlike that seen with cytotoxic T cells (CTL) [5]. Interestingly, the first description of NK cell activity was observed years earlier in mice in which lethally irradiated hybrid F1 mice were able to reject parental and allogeneic bone marrow allografts. This was at variance with *in vivo* CTL activity as well as the laws of transplantation in which major histocompatibility complex (MHC)-encoded transplantation antigens are expressed co-dominantly [6].

Due to their morphological phenotype, NK cells were described as large granular lymphocytes (LGL) [7]. They share many characteristics with T lymphocytes including common expression of certain cell surface markers, origin through common lymphoid progenitors (CLP) [8] and some common effector pathways including the release of interferon (IFN)-γ, granzyme and perforin upon activation as well as responding to similar growth factors (Interleukin-2 (IL) and IL-15) [9]. However, it was soon apparent that this lymphocyte population required an alternative classification because the differences with T cells outweighed the similarities. NK cells recognize target cells through the presence of germ line-encoded pattern receptors which are not MHC class I-restricted [10] and do not require somatic gene rearrangement. This was supported by early studies showing NK cells are present in mice deficient in recombination activating genes (RAG1/2) [11] and in humans that were T cell deficient [12]. Other characteristics of NK cells that differentiates them from T cells are that NK cells do not undergo clonal expansion, do not secrete IL-2 upon activation, and pre-synthesize granzyme, perforin and IFNγ which are stored in granules [13]. NK cells also do not traditionally appear to generate long-lived memory responses although there has been recent evidence suggesting this may indeed occur to some extent [13, 14]. The identification of NK cells has been difficult due to the lack of truly cell-specific markers. NK cell characterization is based on the presence of multiple NK-related antigens and the lack of typical T cell markers, such as CD3 or TCR. NK cells can be identified by the presence of the C-type lectin NK-cell receptor protein 1 A (NKR-P1A: NK1.1 in mice, CD161 in human), integrin-α2 (CD49b) commonly recognized by the DX5 antibody clone in mice, the neuronal cell adhesion molecule CD56 in humans and asialo ganglio-N-tetraosylceramide (asialo-GM1) in most species [15]. However, many of these antigens which are thought to be NK-specific can also be found on T cells, mast cells and macrophages. NK cells express low levels of CD11c, a typical marker of dendritic cells (DCs), B220; a B cell marker, and CD2; expressed in T and B cells as well [15]. CD11b, which has been used to further identify NK subsets, is also observed in monocytes, macrophages and DCs [15]. In humans, CD56 expression is used to differentiate between two populations of NK cells with functional and compartmental differences. $CD56^{bright}$ are less cytotoxic and represent the predominant NK subset in the lymph nodes (LN), whereas $CD56^{dim}$ are the predominant population in the peripheral blood and display higher cytotoxic functions. CD56 is not expressed by mouse NK cells. Human and mouse NK cells also use different recognition receptors that recognize MHC class I molecules, the killer immunoglobulin like receptors (KIR) in humans and the C-type lectin Ly49 in mouse, which are critical for differentiation and function. These differences make the direct extrapolation of mouse NK studies to human difficult. The differences between human and mouse NK cells will be discussed later in this section.

It is quite interesting the co-evolution that has existed between mouse NK cells and mouse CMV (MCMV). The presence of NK cells, and particularly those that express the activating NK receptor Ly49H, has shown to be essential for the MCMV resistance of C57BL/6 mice due to the recognition by Ly49H of m157, a viral glycoprotein, which promotes NK expansion and activation [16]. The structure of m157 shares some homology with MHC class I which explains the ability of m157 to bind to Ly49I, an inhibitory NK receptor. This suggests that m157 evolved to inhibit NK activation by binding to Ly49I and therefore evade NK-mediated killing. However, NK cells evolved to resist MCMV as well by expressing the activating NK receptor Ly49H. Ly49H and Ly49I are structurally similar and

mathematical models have suggested the appearance of the activating receptors occurred later than the inhibitory receptors [16]. MCMV infection represents a model for human CMV (HCMV) because both viruses shares similarities regarding viral life cycle, genome structure and host immune responses to the infection [17]. Moreover, half of the described MCMV genes have HMCV homologues [17]. In humans, it has been shown that HCMV proteins are structurally similar to MCH class I and MCH class-I like molecules. A HCMV-encoded protein that binds to KIRs has not been identified yet. However, the HCMV UL18 protein binds to leukocyte immunoglobulin-like receptor-1 (LILR1), an inhibitory receptor, with a higher affinity than MHC class I and inhibits NK cell-mediated cytotoxicity [18]. LIRs are related to KIRs, therefore, it would not be surprising that some proteins encoded by HCMV recognize inhibitory KIRs in order to evade NK cell-mediated anti-viral responses, similar to that of m157 with Ly49I. Activating KIRs might, therefore, have evolved to counteract NK inhibition.

1.2. NK development

Several stages have been identified in order to distinguish mature NK cells that display functional properties from immature progenitors. NK development has been separated into three or four stages [15, 19]. NK cells develop from hematopoietic precursors that have lost pluripotency as hematopoietic stem cells (HSC) differentiate to a more committed NK-lineage. Early lymphoid progenitors (ELP) and common lymphoid progenitors (CLP) are hematopoietic-derived precursors that still retain the ability to differentiate into B, T and NK cells. NK precursors are defined as the first stage of NK lineage commitment [15]. NK precursors express IL-2 receptor beta (IL-2Rβ/CD122) and lack other mature NK surface markers ($Lin^-NK1.1^-DX5^-CD122^+$). IL-15 is an essential cytokine that regulates NK maturation and survival and requires IL-2Rβ expression, demonstrated by the lack of mature NK cells in IL-15 deficient mice and the inability of transferred mature NK cells to survive in these mice [20-23]. However, IL-15 seems to play a more important role later during NK maturation rather than in the precursor stages as IL-15Rα deficient mice do not have a defective NK precursor population [24] and $CD34^+$ progenitor cells from human umbilical cords can be differentiated into NK precursors in an IL-15 independent-manner [25]. Stem cell factor (SCF/c-Kit-L), fetal liver kinase 2 ligand (FLK2), fms-like tyrosine kinase 3 ligand (Flt3-L), and IL-7 are responsible for the formation of NK precursors [19, 25]. NKG2D, an NK cell activating receptor, is also present in a small population of NK precursors, although no lytic function has been attributed to NK precursors [26]. NK precursors evolve to an immature stage by NKR-P1A expression in both humans (CD161) and mice (NK1.1) followed by the acquisition of DX5 (mouse) or CD56 (human) and the NKG2/CD94 complex. MHC class I specific receptors are the last to be acquired before becoming mature NK cells [15, 19]. Once mature, mouse NK cells have been further differentiated in function by the expression of CD11b and CD27 to represent the progressive acquisition of NK effector functions within the mature stage [27, 28]. Four maturation stages have been identified: $CD11b^{low}CD27^{low}$, $CD11b^{low}CD27^{high}$, $CD11b^{high}CD27^{high}$ and $CD11b^{high}CD27^{low}$ [28]. $CD11b^{low}CD27^{high}$ cells are found in LN, spleen, liver and BM whereas $CD11b^{high}CD27^{low}$ are located in the spleen and liver, and are the predominant population in the peripheral blood and lung.

Although the BM is still considered the major site for NK development, NK precursors can be found in the blood, spleen, LN and liver [15, 29]. The current model of NK development is that NK cells initiate their maturation in the BM and as precursors, leave the BM through the bloodstream to reach other organs where they mature [15, 29]. Mature human NK cells are located in the blood, spleen, liver, LN, BM, lung and in the uterus during pregnancy [15]. A recent study has suggested that fetal NK progenitor cells are initially generated in the liver as early as day 13 of the embryo, whereas the spleen and BM become hematopoietic organs later during embryogenesis. Therefore, the liver is the main hematopoietic organ before birth; followed by the BM that becomes the primary site for NK development after birth. In mice, NK function is

impaired for the first several weeks despite being present [30].

In humans, NK cells represent approximately 5-10% of total lymphocytes in the blood with increased percentages present upon infection. Two NK subsets have been characterized by varied expression of CD56 and are differentiated by their effector functions [31, 32]. $CD56^{dim}$ NK cells are highly cytotoxic but poor cytokine producers and represent approximately 90% of total NK cells found in the blood. $CD56^{dim}$ NK cells express CD16, a low affinity Fc Receptor (FcRγIII) which binds to the Fc portion of IgG antibodies and is responsible for antibody dependent cellular cytotoxic (ADCC) function [33]. CD16 binding also triggers FcεRI signaling which induces IFNγ and GM-CSF release and granzyme degranulation [10]. In contrast, the $CD56^{bright}$ subset is poorly cytotoxic but a higher cytokine producer and represents the main NK population in the LN [34]. $CD56^{bright}$ NK cells have low expression of CD16. Due to this subset's ability of secreting high amounts of IFNγ and its presence in the LNs, it has been suggested that resting $CD56^{bright}$ NK cells have a role during the early stages of immune responses [35]. However, cytokine production has also been attributed to the $CD56^{dim}$ subset which upon stimulation is able to produce IFNγ within the first 2-4 hours. IFNγ production by $CD56^{dim}$ NK cells is undetectable after 16 hours of stimulation [36]. The lineage separation between $CD56^{bright}$ and $CD56^{dim}$ NK cell subsets is at the present unclear. An *in vitro* study has demonstrated that human $CD56^{bright}$ NK cells can potentially differentiate into $CD56^{dim}$ NK cells [37]. The hypothesis is that $CD56^{bright}$ NK subset is the developmental precursor of $CD56^{dim}$ where $CD56^{bright}$ cells hypothetically leave the LN after maturation and may explain why the presence of $CD56^{dim}$ NK cells is low in LNs [29, 37, 38]. However, it is still unclear whether $CD56^{bright}$ and $CD56^{dim}$ are two distinct populations with different functions, as a recent study has demonstrated that $CD56^{dim}CD16^{-}$ NK cells that are stimulated with CD137L (4-1BBL) and IL-12 switch to a $CD56^{bright}CD16^{-}$ phenotype [39]. Importantly, since mouse NK cells are not present in comparable large numbers in the LN, it has made definitive studies on the lineage of the two subsets difficult.

1.3. Differences between human and mouse NK cells

Mouse NK cells are normally defined by the expression of NK1.1 or DX5 which are not expressed on human NK cells; whereas human NK cells are characterized by CD56 expression. The lack of CD56 expression in mouse NK cells makes the comparison between human and mouse species difficult. There have been multiple attempts to link mouse NK cells with human NK cells through the use of markers that are shared between both species such as the TNF receptor family member CD27. Despite the higher cytotoxic capabilities shown by the mouse $CD27^{high}$ NK subset, cytokine production has also correlated $CD11b^{low}CD27^{high}$ and $CD11b^{high}$ $CD27^{low}$ with $CD56^{bright}$ and $CD56^{dim}$ respectively [27, 28, 40]. Furthermore in humans, a small population of $CD27^{high}$ belongs to $CD56^{bright}$ NK cells, and the contrary is true for peripheral blood $CD56^{dim}$ NK cells [41].

The presence of mouse NK cells in LN during steady-state conditions is low. However, upon stimulation there is a recruitment of NK cells to the LN. The production of high amounts of IFNγ by those NK cells has been involved in the induction of Th1 polarization at the LN suggesting that NK cells have an important role at early stages of an immune response [35]. Due to the higher cytokine production and location in secondary lymphoid organs, both mouse $CD11b^{low}CD27^{high}$ and human $CD56^{bright}$ NK cells similarly could be involved in the differentiation of naïve T cells toward a Th1 phenotype [35, 41].

As previously mentioned, another major difference between human and mouse NK cells is their ability to recognize MHC class I. NKG2/CD94 family members are a group of conserved inhibitory and activating receptors present on both species. However, KIRs are only found in humans, whereas Ly49 receptors are only observed in mice [10, 42]. Despite the structural differences between human KIRs and mouse Ly49s, both families perform similar functions: regulating NK activation. Human NK cells also express activating and inhibitory receptors that belong to the leukocyte immunoglobulin-like receptor (LILR) family. Two members of this family, LILRB1 and

LILRB2 are inhibitory receptors that also bind to MHC class I molecules. Nevertheless, CD94/ NKG2 and KIRs seems to play a more dominant role [10]. Some of the different activating and inhibitory NK receptors as well as the mechanism to control NK activation will be described in the next section.

Another difference between human and mouse NK cells is in regard to their cytolytic activity. Freshly isolated human NK cells from the peripheral blood exhibit higher cytolytic activity compared to resting splenic murine NK cells [43]. This necessitates the administration of exogenous activation signals such as IL-2 to allow murine NK cells to reach high cytotoxicity capabilities. Another difference between mouse and human NK cells resides in the ability to long-term *ex vivo* culture the cells. *In vitro* culture of mouse NK cells in the presence of IL-2 can only be maintained for approximately two weeks, while the culture of human NK cells can be sustained for longer periods of times with stable KIR expression [43]. The generation of human NK cell lines, such as NK-92, is another evidence of the capability of human NK cells to survive long periods of culture. Despite the differences between mouse and human NK cells, mouse models are still the predominant model used to study NK biology. Similarities between human and mouse NK cells regarding activating and inhibitory receptors mentioned earlier, mechanisms of action, signaling, and developmental pathways allow the use of mouse models as a bridge to study human NK cells. Furthermore, their small size, short lifespan, ease of accessibility to reagents, and multiple transgenic and gene-deficient models make the mouse a necessary platform to further advance the clinical application of NK-based immunotherapies.

2. NK activation

NK activation is achieved through a combination of the following: lack of recognition of self-MHC class I molecules displayed on the target cell surface, the presence of activating signals recognized by activating receptors, the cytokine environment, and finally the interaction with regulatory immune cells. All NK cells also have inhibitory receptors which can result in potent inhibitory signals that can over-ride activation.

2.1. Inhibitory receptors: NK licensing

The presence of inhibitory receptors on the NK cell surface that recognize MHC class I molecules on target cells play a very important role in NK tolerance. It has been shown that even in the presence of activating ligands, the inhibition of NK cells by inhibitory receptor engagement overrides the possible activating signals and a much higher activation is needed to overcome inhibition [44-46]. Initially it was suggested that NK function was MHC unrestricted due to the ability of NK cells to eliminate MHC class I deficient or allogeneic tumors [10]. The concept of “missing self ” also provided an explanation for the hybrid resistance exhibited by F1 hybrid NK cells after infusion of parental BM cells [47]. However Karre *et al*., demonstrated that NK cells recognized MHC class I and were indeed inhibited by the presence of self-MHC class I [48]. The inhibitory receptors contain immuno-receptor tyrosine-based inhibitory motifs (ITIMs) in their cytoplasmic domain, in contrast to the immunoreceptor tyrosine-based activating motifs (ITAMs) of the activating receptors [49, 50]. After MHC engagement, ITIMs recruit mainly SH2 domain-containing protein tyrosine phosphatase 1 (SHP-1) but also SHP-2 and SH2-domain-containing inositol-5-phosphatase (SHIP). The recruitment of SHP-1 has been associated with the dephosphorylation of the nucleotide exchange factor Vav1 [51] and/or the complex adaptor SLP-76 [52]; both are involved in NK activation. Other tyrosine kinase substrates have been found to be dephosphorylated after KIR engagement such as TCRζ, Syk, ZAP-70 or phospholipase Cγ [53]. Other studies suggest that the inhibitory capabilities are induced by the disruption of co-localization of different enzymes and substrates within the membrane lipid raft after MHC engagement thereby preventing further activation signaling [54]. Although not necessary for NK generation, the ability to lyse MHC class I deficient target cells is impaired in transgenic NK cells that express a dominant-negative form of SHP-1 suggesting that the acquisition of inhibitory receptors plays a role in gaining functional properties at the last stages of NK maturation [55, 56].

In mice, the inhibitory receptors belong to C-type lectin-like receptor family members, Ly49, and

the NKG2/CD94 family members, both of which have also activating receptor members that we will discuss later. Ly49s are type II membrane anchored glycoproteins that bind different MHC class I H-2 alleles. The structure of Ly49s is divided by a ligand-binding extracellular domain, a transmembrane domain and a cytoplasmic domain that contains the ITIM. Within the Ly49 family members, 13 inhibitory receptors (Ly49A, B, C, E, F, G, I, J, O, Q, S, T and V) have been described. In humans, KIRs are the best characterized family of NK receptors that belong to the immunoglobulin superfamily. KIRs were initially described as NK-associated transcripts (NKAT) [57] and also included both inhibitory and activating receptors. The inhibitory KIRs are defined by the presence of long cytoplasmic domains that also contain ITIMs and by the number of immunoglobulin-like domains; two or three (KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL4, KIR3DL1 and KIRD3DL2). Interestingly, KIR2DL4 contains both ITIM and ITAM, and therefore both activating and inhibitory properties have been attributed to this NK receptor [58]. CD94/NKG2 family is conserved in both species and recognize the non-classical MHC class 1b ligands; HLA-E in humans and $Qa1^b$ in mouse. The inhibitory receptors CD94/NKG2A and B contain ITIM motifs as well [10].

The acquisition of the different inhibitory receptors is not germ-line encoded. Therefore, within an organism there are NK cells with inhibitory receptors able to bind self-MHC as well as non-self-MHC. In order to explain how it was possible that NK cells with inhibitory receptors that do not recognize self-cells do not target these cells, several groups proposed what is known as NK “licensing”, NK “arming”, or NK “education” [59-61]. Initially licensing was demonstrated *in vitro* in mouse NK cells by the ability of NK cells that expressed inhibitory receptors for self-MHC to produce higher amounts of IFNγ [59]. Later NK education was observed in human NK cells where NK cells that expressed inhibitory receptors for non-self-MHC exhibited hyporesponsiveness after stimulation with MHC class I deficient targets or cytokines compared to “educated” NK cells [61]; illustrating another similarity between mouse and human NK cells. This hypothesis postulates that NK precursor cells are required to go through a maturation process to acquire self-tolerance where progenitor NK cells contact/interact with stroma cells that display self-MHC class I. During this process, an NK cell will become licensed when its inhibitory receptor recognizes self-MHC and unlicensed when it does not. Several *in vitro* studies have suggested that licensed NK cells are the main cytotoxic subset because of the production of higher amounts of IFNγ after NK1.1 stimulation compared with unlicensed NK cells [59]. *In vivo* evidence of the importance of licensed NK cells in mice can be found in studies where depletion of host licensed NK subsets resulted in improvement of allogeneic or MHC deficient BM cell engraftment after bone marrow transplantation (BMT) [62]. In humans, NK cells that display KIRs for non-self MHC class I are found to be less cytotoxic compared with NK cells expressing KIRs for self [61, 63]. The evidence of the existence of NK subsets with differential functions defined by the expression of inhibitory receptors represents a step forward in NK-based immunotherapy as now it is possible to preferentially select only those subsets that display the greatest activity and tumor protection.

The proposal of the rheostat model provided an additional explanation of how NK function is highly regulated by inhibitory receptors. This model suggests that as the amount of inhibitory receptors that bind self-MHC increases, the NK cells are more tightly regulated against self-attack and therefore are “armed” to become stronger killers [64, 65].

Despite advances in NK biology, the role of unlicensed NK cells is still unclear. Although they are known to be hyporesponsive in resting stage, during inflammatory conditions stimulatory signals are able to rescue unlicensed NK cells from their hyporesponsive state and activate them. Unlicensed NK cells have been shown to play a major role in the control of MCMV in self-MHC class I-expressed target cells [66]. Furthermore, a recent study has also suggested that unlicensed NK cells might have an important role against target tumor cells that express self-MHC molecules as well [67] and their presence seems to have a correlation with anti-tumor efficacy early after BMT [68]. Whether unlicensed NK

cells do or do not play a protective role during inflammatory conditions needs further investigation as we have recently shown that a particular subset of mouse NK cells, Ly49G2-positive NK cells, expands after cytokine stimulation, *Listeria monocytogenes* infection and early post-hematopoietic stem cell transplantation (HSCT) regardless of the H-2 haplotype [69]. Therefore, NK licensing may not play a regulatory role during NK activation in particular conditions.

2.2. Activating receptors

It is well-known that the signaling cascade resulting from engagement of activating receptors regulates NK activation, adhesion, and function. Some of these receptors are NKG2D, DNAX accessory molecule-1 (DNAM-1), CD16, 2B4, NKp80, the natural cytotoxicity receptors (NCR) NKp30, NKp44 and NKp46 and co-stimulatory receptors (NTB-A, CRACC, CD2, CD59) [8, 59]. The CD94/NKG2, KIR and Ly49 family members also contain activating receptors [10, 70]. In general, these activating receptors contain ITAM motifs or associate to ITAM-containing adaptor proteins such as DAP10 and DAP12. In mouse, activation of ITAMs results in tyrosine phosphorylation and subsequent recruitment of Syk and ZAP70 which results in degranulation and transcription of chemokines and cytokines required for NK effector functions [71].

The NKG2D activating receptor is involved in both CD8 T cell and NK-mediated tumor killing. Its role in tumor clearance was clearly demonstrated by the increased tumorigenesis observed in NKG2D deficient mice [72]. NKG2D is a type II transmembrane anchored C-type lectin-like glycoprotein that binds MHC class I related proteins [10]. NKG2D is not a specific NK activating receptor and can be found in NKT cells, CD8 T cells and some subsets of γδ T and CD4 T cells. NKG2D is expressed in all human CD8 T cells [73] but only found in activated mouse CD8 T cells [74]. It is not required to form dimers with CD94 as do other NKG2 family members, but associates with the adaptor proteins DAP10 (both human and mouse) or DAP12 (mouse only). NKG2D stimulation triggers the PI3K and AKT signaling pathways as well as phosphorylation of Janus kinase 2 (JAK2), STAT5, ERK1/2 and MEK1/2, resulting in NK activation and cytotoxicity due to cytokine secretion such as IFNγ and granzyme degranulation [10]. NKG2D ligands (NKG2DL) include the MHC class I related proteins A and B (MICA/B) and UL16-binding protein (ULBPs) in humans; retinoic acid early inducible-1 (Rae-1), minor histocompatibility antigen H60 and the murine UL16-binding protein like transcript-1 (MULT-1) in mice. Although NKG2D ligand expression has been observed in healthy tissues and normal mouse embryos, upregulation of these ligands is frequently correlated with stress and high proliferation rates [73, 75]. Gasser *et al*. suggested the activation of DNA damage response pathways as a mechanism for NKG2DL upregulation in tumor cells. In this study, the induction of DNA damage by genotoxic stress on non-tumor cell lines led to NKG2DL upregulation mediated by ATM (ataxia telangiectasia mutated) or ATR (ATM- and Rad3-rlated) protein kinases. Silencing of these proteins in tumor cell lines also abrogated NKG2DL expression suggesting a chronic alteration of the DNA damage pathway as a plausible internal mechanism to induce immune responses and protect from uncontrolled growth [76].

Natural cytotoxicity receptors (NCR) have also been shown to play an important role in NK function [77] as the use of blocking antibodies results in reduction of tumor clearance [78-80]. Furthermore, higher expression of NCRs is associated with enhanced NK-mediated cytolytic functions [78, 79]. NKp30 and NKp44 are only expressed in human cells whereas NKp46 can be found in both human and mouse [77]. NKp46 and NKp30 are constitutively expressed in NK cells whereas NKp44 expression is only observed after IL-2 stimulation [81]. The human leukocyte antigen-B associated transcript-3 (BAT-3) and B7-H6 have been identified as NKp30 ligands [82]. The cellular ligands for NKp46 and NKp44 remain unknown. Viral hemaglutinin has been suggested as a possible ligand for NKp46 confirming its implication in the elimination of viral infected cells [83]. In mouse models, the infusion of melanoma [84] or lymphoma [85] cells into NKp46 deficient mice resulted in impaired tumor growth control demonstrating the importance of NKp46.

DNAM-1 is a transmembrane glycoprotein constitutively expressed by NK cells, monocytes, T cells, a subset of B cells and platelets [77]. DNAM-1 interaction on NK cells results in activation, higher cytotoxicity, and cytokine production [10]. Poliovirus receptor (PVR or CD155) and nectin-2 (CD112), which in normal tissues is expressed by endothelial and epithelial cells, have been identified to bind DNAM-1. As adhesion molecules, they are involved in trans-epithelial migration, however, DNAM-1 is also found in tumor cells [10, 86-88]. DNAM-1 has been also involved in the regulation of NK migration [10].

CD94/NKG2F in humans and CD94/NKG2-C and -E are the activating receptors of the CD94/NKG2 family that associates with DAP12. Ly49H and Ly49D, the activating receptors of Ly49 family, also associate to DAP12 and less efficiently to DAP10 [71]. The activating receptors of KIRs contain a short cytoplasmic domain and associate with DAP12 (KIR2DS1-5 and KIR3DS1) [10].

2.3. NK activation regulated by cytokines

There are many cytokines that positively or negatively regulate NK activation. The production by DC, macrophages or T cells of type I interferon (IFN), IL-12, IL-18 and/or IL-15 results in NK activation [89]. IL-2 is a cytokine that induces the activation and proliferation of NK cells [7] and has been widely used to expand NK cells *in vivo* and *in vitro*. IL-15 has been proposed to be essential for NK maturation and survival as IL-15 deficient mice showed a defect in the NK cell population that was not observed in IL-2 knockout (KO) mice [22]. However, a robust $Ly49H^+$ NK proliferation was observed in IL-15 and IL-15Rα-deficient mice after MCMV infection suggesting that the need of IL-15 can be overridden following infection due to IL-12 and other signals [90].

As a negative regulator, transforming growth factor beta (TGF-β) has been shown to be involved in inhibition of NK cells [91]. TGF-β is secreted by most cells in the body but is utilized by regulatory T cells (Tregs) to control the immune system [91]. The negative impact of TGF-β in NK cells has been associated with decreased IFNγ production and diminished degranulation and cytotoxic functions [92-94]. Exogenous administration of TGF-β resulted in decreases of NKG2D and NKp30 expression in NK cells in addition to lower IFNγ production and tumor lysis [94]. Furthermore, the presence of TGF-β in the serum of cancer patients has been correlated with lower NK cytotoxic function further demonstrating the immunosuppressive role of this cytokine [95]. Blockade of TGF-β signaling on NK cells results in NK accumulation and restoration of NK functions such as IFNγ production [93].

IL-10, another cytokine that has been associated with immunosuppressive functions, is produced by many innate and adaptive immune cells [96]. IL-10 is known to play an important role in the induction of antiviral and antibacterial immunity during acute infection [97]. IL-10 can indirectly inhibit immune responses through downregulation of MHC class II and immunocostimulatory molecule (B7-1/B7-2) expression in monocytes and macrophages which reduces the production of proinflammatory cytokines important for NK and T cell activation such as IL-12, IL-18, IFNγ and TNFα [98-100]. Moreover, IL-10 inhibits IL-2 and IFNγ production on $CD4^+$ T cells [97], both of which are involved in NK activation.

However, despite this immunosuppressive role, IL-10 has been shown to be very important in NK survival during early infection of MCMV [101]. Blockade of IL-10R resulted in increased viral load and reduced NK cell numbers and cytotoxicity [101]. Similarly, *in vitro* studies have demonstrated that IL-10 improves NK proliferation, cytotoxicity and IFNγ production [102-105]. Biron *et al.* showed the importance of IL-10 production by NK cells to regulate CD8 T cell responses during MCMV infection [106]. Blockade of IL-10 in perforin deficient mice, although it did not suppress viral growth, resulted in a significant production of proinflammatory cytokines by CD8 T cells that resulted in the death of the mice. These studies highlight the significance of IL-10 in maintaining the NK cell population early during infection in order to regulate adaptive immune responses by suppressing overactivation of CD8 T cells. In addition to the molecules discussed, the tryptophan metabolizing

enzyme indoleamine 2,3-dioxygenase (IDO), prostaglandin E2 (PGE2), macrophage inhibitory factor (MIF) and reactive oxygen species (ROS) are also involved in NK suppression [94, 107-109].

3. Role of NK cells in cancer

There is a multitude of evidence implicating NK cells in metastatic and hematologic tumor clearance. *In vitro* studies demonstrated the ability of NK cells to recognize and eliminate tumor cell lines. The first *in vivo* correlation of number and function of NK cells with anti-tumor effects was published during the 1980's [110]. Since then, several groups have pointed out the relevance of NK cells in eliminating mouse and human tumors suggesting a role in cancer immunosurveillance [111-113]. An 11 year follow up study established an association of highly activated NK cells in peripheral blood with a decreased cancer risk [114]. The function and cytokine production capabilities of NK cells from patients with acute leukemia were also positively correlated with complete tumor regression [115-117]. Additionally, positive prognosis has been linked to the presence of NK infiltrating cells in several carcinomas [118] and $CD56^+$ cells were found in samples of metastatic melanoma [84]. There is strong evidence of the significant implication of NK cells in the control of hematological cancers such as leukemias and lymphomas as well as metastatic cancers such as breast or ovarian cancers [119, 120]. However, the low representation of NK infiltrating cells within the tumor site has suggested a low contribution of NK cells in solid tumors [121, 122].

An additional piece of evidence of the role of NK cells in tumor surveillance is the correlation that exists between tumor progression and defects in NK function [89]. The expression of activating receptors has been associated with improved cytotoxic functions [123] and there are multiple studies that have shown poor prognosis when NK cells have impaired activating receptor expression [123, 124]. For example, systemic NKG2D downregulation, observed in multiple tumors, has shown to negatively impact NK and CD8 responses [125]. In gastrointestinal sarcoma (GIST) and AML patients, the level of NKp30 and NKp46 expression has been negatively correlated with metastasis and cancer relapse, respectively [123, 124].

Recently, three different isoforms of NKp30 have been identified: NKp30a, NKp30b and NKp30c. NKp30c has been classified as an immunosuppressive isoform because of the production of IL-10 upon stimulation. In GIST patients, the overall expression of NKp30 is low, however within the small subset of NK cells that still express NKp30, there is a higher predominance of NKp30c [124]. Therefore, despite the general downregulation of NCRs observed in GIST patients, the preferential presence of NKp30c receptor results not only in lower NK-mediated cytolytic function but also in the immunosuppressor environment that favors tumor progression. These data indicate that NK cells can play a potentially significant role in human cancers but the various inhibitory and activating receptors seem to be critical in the responses to the particular cancers.

3.1. Tumor recognition and effector pathways

The identification of molecules expressed on tumor cells and their recognition by NK cells has allowed a better understanding of how NK cells and cancer cells interact [10, 126] as well as the application of this knowledge to improve NK-based immunotherapies. NK cells recognize tumor cells in a similar fashion to virally infected cells: through their modulation of MHC expression and expression of stress ligands. Downregulation of MHC class I has been observed in many tumors as a way to evade T cell-dependent immune recognition which further contributes to NK killing [127]. In those cases, the presence of NK activating ligands in conjunction with the lack of inhibitory receptor engagement led towards NK activation allowing for better recognition and attack of those tumors. Furthermore, it is likely that multiple activating receptors are responsible for the recognition of different ligands upregulated in tumor cells and coexpression of several activating receptors within a NK cell allows for a better cytotoxic function. This also limits the possibility of tumor evasion by downregulation of NK activating receptor ligands.

NKG2D ligands, such as MICA, MICB, ULBPs, have been found to be frequently upregulated in several mouse and human tumors [128-131]. Heinemann *et al.* recently demonstrated the role of microRNA in controlling NKG2DL expression by tumor cells. miR-34a and miR-34c were responsible for the degradation of UBLP2 on human melanoma cell lines, and therefore silencing this microRNA resulted in increased levels of ULBP2. It has been suggested that the loss of miR-34 expression, frequently observed in cancer cells, possibly regulates the levels of ULBPs on those cells allowing for tumor clearance [132].

An example of the consequences of NKG2DL expression in tumor cells arose from the NK resistance observed in RMA cells, which lack NKG2D ligands. The transfection of Rae-1 in those cells resulted in NK cell-mediated cytotoxicity [128, 133, 134]. The role of NKG2D in tumor immunosurveillance was confirmed by the development of NKG2D deficient mice. Lack of NKG2D allowed for the earlier and more aggressive appearance of spontaneous prostate tumors in the transgenic adenocarcinoma of mouse prostate (TRAMP) model of autochthonous prostate cancer development, but interestingly not greater numbers of metastasis [72]. This increased tumor growth was particularly correlated with NKG2D-dependent function, as other NK functions remained untouched in those mice. Moreover, Guerra *et al.* also suggested a possible selection of tumor cells with lower NKG2DL expression due to the selective pressure imparted by $NKG2D^+$ NK or T cells because the tumors arising from mice lacking NKG2D expressed significantly higher levels of NKG2DL [72].

Similar to NKG2D, DNAM-1 has also been involved in tumor surveillance. Iguchi-Manaka *et al.*, demonstrated that DNAM-1 defect results in accelerated tumor growth and those tumor cells displayed higher amounts of DNAM-1 ligands compared to wild type mice suggesting a selection of DNAM-1 ligand negative cells to override NK and T cell responses in a normal scenario and confirming the role of DNAM-1 in anti-tumor responses [123]. Although, Nectin-2 is ubiquitously expressed in many cells such as epithelial cells, neurons and fibroblasts, its expression has been linked to tumor metastasis. Myeloid leukemias and metastatic neuroblastomas are tumors where Nectin-2 is commonly found [86]. Additionally, PVR is expressed in ovarian carcinoma and has been shown to have an important role in the tumor recognition by DNAM-1/CD96 positive NK cells [135]. CD96, which also recognizes PVR and Nectin-2, has been show to regulate, along with DNAM-1, NK adhesion and activation [10]. However, it remains difficult to establish its implication in tumor recognition as blockade of CD96 did not abrogate NK-mediated killing of ovarian carcinoma cells through DNAM-1 inhibition [135].

The release of perforin and granzymes is the major cytotoxic pathway utilized by NK cells to eliminate target cells [136]. This pathway is predominant when NK killing capabilities are tested in short term assays [137]. However, in long term assays, NK cells have shown to also eliminate tumor cells through the expression of death receptors TRAIL and FasL that recognize DR5, DR4, and Fas on tumor cells respectively resulting in the induction of apoptosis [138].

3.2. Tumor evasion of NK cells

The multiple mechanisms that tumor cells have evolved to escape from NK recognition and killing serve as indirect evidence for the role of NK cells in tumor clearance. Abnormal NK cytotoxic function has been observed in multiple cancers [139]. This decrease of NK function may be due to several reasons. In some tumors, decreased NK killing has been linked to lower expression of NK activating receptors which also correlates with disease progression [123, 124, 138]. Overexpression of inhibitory receptors has been observed as well in cancer patients such as CD158a (KIR2DL1) in metastatic melanoma [140] or NKG2A in tongue cancer [141]. Combination of both downregulation of activating receptors and overexpression of inhibitory receptors has been observed in metastatic melanoma [140]. The release of immunosuppressive cytokines such as TGF-β or IL-10 by tumor cells and/or the recruitment of immunoregulatory cells such as Tregs, MDSC and M2 macrophages are further mechanisms that tumor cells have evolved to evade immune responses that directly impact NK

functions due to the creation of an immuno-suppressive environment. Tumor location can also limit NK accessibility as for example solid tumors have low number of infiltrated NK cells.

3.2.1. Decreased NK activating receptors

NKG2D, NKG2C, NKp30, NKp44 and NKp46 activating receptors have been found to be downregulated in NK cells from AML patients compared with healthy donors [118]. The loss of NKp30 has correlated with impaired NK function and shorter survival in AML patients [123]. NKG2D downregulation has also been observed in metastatic melanoma and hepatocellular carcinoma [142]. In GIST and melanoma patients, NKp46 and NKp30 are downregulated [124], whereas DNAM-1 expression is reduced in ovarian cancer patients [77]. Continuous stimulation with IL15/IL15Rα complex has shown to result in impaired NK function due to, in part, downregulation of NKG2D, 2B4, NKp46 and DNAM-1 [143]. Therefore, NK exhaustion due to sustained stimulation by tumor cells could also lead to the downregulation of activating receptors as well as lower functional capabilities.

Direct influence by cell-cell interactions with tumor cells has been suggested as the mechanism for the downregulation of NK activating receptors. For example, Fauriat *et al*. demonstrated the negative impact of leukemia cells in NK-mediated cytolytic functions. The co-culture of NK cells from healthy donors that expressed NKp30 and NKp46 with leukemia cells resulted in downregulation of these receptors. In this study, it was suggested that leukemia cells have a profound impact on NCR expression during NK differentiation but also in mature NK cells as co-culture of leukemia cells with NCR^{bright} NK cells downregulated NKp30, but not NKp46 whereas co-culture with CD34-derived NK cells resulted in defects of both NCRs [123]. Downregulation of NKG2D and NCRs impairs NK and CD8-mediated cytotoxic responses [125].

Similarly, melanoma cell lines were able to downregulate the expression of NKG2D, NKp30 and NKp44 on NK cells resulting in impaired NK-mediated cytolysis. In this study, the negative impact of melanoma cells on NK function was mediated by indoleamine 2, 3-dioxygenan (IDO) and prostaglandin E2 (PGE2) [82]. IDO is upregulated in mature DCs and is involved in the activation of Tregs having an important role during immune tolerance. Overexpression of IDO has been observed in tumor cells and antigen presenting cells (APC) located in the tumor draining LNs which acted as a tumor evasion mechanism. The production of the proinflammatory molecule PGE2 by cyclooxygenase 2 (COX-2) induces IDO. COX-2 is upregulated in activated macrophages and other cells at the site of inflammation. Elevated COX-2 has been associated with increased cancer progression [144]. It is of interest that IDO1 mRNA was only upregulated on melanoma cells after IFNγ treatment. One could speculate that IDO1 mRNA upregulation could be a response against immune activation and tumor cells could actively suppress in response to the cytokines produced by NK cells [82].

3.2.2. Alteration of activating receptor ligands

The liberation of soluble NKG2DL by many tumors has also been shown to have an important suppressive impact on NK cytotoxic function. *In vitro* studies that used soluble MICA (sMICA) demonstrated that the binding of sMICA led to endocytosis and degradation of NKG2D [145]. Moreover, CD8 T cells and NK cells isolated from PBMC from cancer patients with high levels of sMICA in the serum have NKG2D downregulation favoring immunoevasion. Sustained and localized stimulation of NKG2D by NKG2DL has also been shown to impair NK function [146]. In B-cell chronic lymphocytic leukemia and melanoma patients, the serum level of shed ULBP2 has also been associated with poor survival [147].

It has been suggested that membrane and soluble carcinoembryonic antigen (CEA)-related cell adhesion molecule 1 (CEACAM1) can also negatively regulate NKG2DL expression by tumor cells. Initially, it was thought that CEACAM1 directly inhibited NK cell function in an MHC-independent manner [148]. However, using human and mouse tumor cell lines, Chen *et al*. demonstrated that levels of CEACAM1 were inversely correlated with the expression of MICA/B and RAE-1 respectively which resulted in poor NK and T cell cytotoxic function [149]. In this study, post-translational regulation of NKG2DL by CEACAM1 was demonstrated in the

murine colon carcinoma cell line MC38; CEACAM1 was responsible for retaining RAE1 intracellularly [149]. Although CEACAM1 was initially defined as a tumor suppressor because the loss of CEACAM1 expression in human colorectal and prostate cancers results in enhanced tumor growth [150], the expression of CEACAM1 by melanoma, lung, pancreas, colon, bladder and thyroid cancer cells has been correlated with poor prognosis and tumor metastasis as well [149]. A recent study showed high levels of CEACAM1 in the serum of malignant melanoma patients and reduced levels of serum CEACAM1 after treatments was correlated with improved survival [151].

NK cells promote tumor lysis predominantly through a perforin-dependent manner [136]. However, NK cells can mediate apoptosis of tumor cells by the recognition of DR5, DR4 or Fas on the surface of tumor cells that can be relevant in the case of impairment of perforin release [138]. Diminished expression of DR4 or DR5 has been observed in multiple malignancies such as ovarian cancer, colon cancer, squamous cell carcinoma, breast cancer and non-Hodgkin's lymphoma. These differences can be the result of reduced transportation of the ligands to the cell surface, intracellular retention, mutations that led to loss of function, or the release of decoy molecules that block DR4/DR5-TRAIL interaction among others and account for TRAIL-mediated killing resistance [152]. Some tumors have shown upregulation of anti-apoptotic proteins such as Bcl-2 in conjunction with downregulation of FasL resulting in an enhanced resistance to apoptosis [153]. The soluble forms of Fas (sFas) and FasL (sFasL) have been found at high levels in the serum of multiple cancers including hepatocellular, gastric, bladder and pancreatic carcinomas. These soluble forms promote tumor evasion two-fold: through the suppression of Fas-mediated killing; and the induction of CD8 or NK apoptosis. sFas levels has been correlated with tumor progression in breast cancer and other malignancies as well [153].

3.2.3. Promotion of tumor progression

NKG2DL expression by tumor cells and its role as a mechanism for immunosurveillance is well-known. The additional observations of higher levels of NKG2DL in NKG2D deficient mice also supported this concept suggesting a selection of NKG2DL negative tumors as an immune evasion tactic via immunoediting [72]. However, recent studies may have uncovered another function for NKG2DL to exist in the tumors: the induction of tumor proliferation and survival. It has been shown that the NKG2D-DAP10 receptor complex is expressed in breast, ovarian, cervical, prostate and colon cancer cells [154, 155] and when stimulated with NKG2DL, activation of MAP kinase cascade was produced, leading to increased cell proliferation within the tumor [154]. Therefore, sustained expression of NKG2DL can not only decrease NK and CD8 T cell cytotoxic function and mediate immunoevasion, but also provide survival signaling within the tumor cell facilitating tumor growth.

3.2.4. Alteration of NK population distribution

NK distribution is also altered in some cancer patients and might affect anti-tumor capabilities. Increased levels of $CD16^{dim}CD56^{bright}$ NK cells have been found in the serum of patients with metastatic melanoma [140] and GIST [124]. These NK cells have impaired ability to mediate antibody dependent cellular cytotoxicity (ADCC) and exhibit lower cytolytic ability. Downregulation of CD16 is also observed in NK cells from patients with ovarian cancer due to the expression of cell membrane bound mucin 16 (MUC16), a mucinous glycoprotein, or shed MUC16 resulting in poor ADCC NK function [156]. Moreover, MUC16 has shown to be a potent inhibitor of NK cells through suppression of immune synapse formation between NK and ovarian cancer cells [156].

The development of NK cells has also been shown to be affected by tumor cells. Comparison of the different stages of NK development in the BM between tumor-bearing mice and control mice revealed an impairment of mature NK cells that was correlated with defects in IL-15Rα expression [157]. Although no differences in NK cytotoxicity were found, those NK cells had defective IFNγ production [157]. The observation of impairment in NK maturation may provide an explanation for the presence of impaired NK cells in multiple cancers.

3.2.5. Induction of immunosuppression pathways that affect NK cells

The dual role of TGF-β in promotion or suppression of tumor growth has been the focus of many reviews [91, 158, 159]. It has been suggested that the secretion of TGF-β by tumor cells as a mechanism for immune evasion results in immune response inhibition by two means: direct effects of TGF-β in immune effector cells or indirectly through the recruitment of immunosuppressive cells. Overproduction of TGF-β is frequently associated with tumor metastasis because high levels of TGF-β enhance angiogenesis and vasculogenesis while preventing immunosurveillance by inhibiting activation of effector cells [91, 158]. In mouse models, overexpression of TGF-β-1 or its receptors in mammary epithelial cells increased lung metastasis confirming the role of TGF-β in tumor metastasis. High levels of TGF-β in the serum of cancer patients have been correlated with poor prognosis [91, 159]. The impact of TGF-β in the tumor microenvironment is due to direct and indirect inhibition of cytolytic functions of CD8 T cells and NK cells, suppression of IgA secretion by B cells, and promotion of the polarization towards tumor-associated type 2 macrophages (M2), type 2 neutrophils (N2), regulatory T cells and myeloid derived suppressor cells (MDSC). In NK cells, TGF-β has been shown to downregulate NKG2D and NKp30 expression correlating with reduced degranulation and IFNγ production and poor lytic activity [91]. In some cases of lung and colorectal cancer, the expression of NKG2D has been inversely correlated with the serum levels of TGF-β [95]. In glioma patients, NKG2D downregulation has been observed in NK and CD8 T cells [160] and resistance to tumor vaccines has been linked with TGF-β levels [161]. TGF-β can also affect NK activation by affecting the production of TNF, type I IFN, and IL-12 by DCs [91].

At the tumor site, there is an accumulation of multiple immune cells including tumor infiltrating lymphocytes (TILs), tumor-associated macrophages (TAMs), tumor-associated neutrophils (TANs) and myeloid derived suppressor cells (MDSC). Prognosis and survival of cancer patients has been associated with the presence of these immune cells at the tumor site. However, good or bad prognosis significantly depends on the type of TILs, TAMs and TANs localized within the tumor. The level of TGF-β within the tumor microenvironment has a profound impact on the composition of these cells. Besides the recruitment of natural Tregs, induced Tregs can be differentiated from naïve lymphocytes at the tumor site due to TGF-β. Increased numbers of Tregs at the tumor site, tumor draining LN and/or peripheral blood are found in breast, lung, ovary, pancreatic cancer [162] and hepatocellular carcinoma patients [163]. Increased numbers of Tregs have been correlated with poor survival in hepatocellular carcinoma patients due to impaired CD8 function [164]. An accumulation of Tregs has been observed in mouse cancer models as well [165]. We have previously shown that depletion of Tregs using a monoclonal antibody results in enhanced NK cell-mediated anti-tumor response when combined with cytokine stimulation [166]. NKG2D-mediated cytolysis is directly inhibited by Tregs in a TGF-β-dependent manner [27] and parental BM rejection mediated by F1 NK cells was also abrogated by the adoptive transfer of Tregs [167]. These and other studies confirmed the role of Tregs in inhibiting NK *in vitro* and *in vivo*.

In some cancers, there is a poor prognosis associated with the presence of TAMs and it has been shown that they can promote tumor progression and metastasis [158]. TGF-β can promote the polarization of TAMs towards type M2 while inhibiting type M1 differentiation which results in suppression rather than activation of immune responses [91]. In skin cancers, the recruitment of macrophages mediated by TGF-β has been shown to have an important role in tumor escape [91]. Similarly, the composition of TANs is also influenced by TGF-β as N2 polarization is favored by TGF-β resulting again in an immunosuppressive effect [158]. Angiogenesis and metastasis was also promoted by N2 [91]. Moreover, M2, N2, Tregs and MDSC collaborate with the tumor cells in the production of TGF-β among other immunosuppressive molecules such as IL-10, MMPs (matrix metalloproteinase), PGE2, IDO and ROS [82, 91] which further promote tumor escape and abrogate immune

activation. Additionally, the presence of inhibitory NK cells, for example NK cells expressing the inhibitory isoform NKp30c, could contribute to the creation of an immunosuppressive environment by the secretion of IL-10 [124].

It is also of interest to note the suppressive effect mediated by CD137L (4-1BBL)-CD137 (4-1BB) interactions. CD137L is expressed in carcinomas and lymphoma cells [168]. CD137 was found to be upregulated on human NK cells after IL-2 stimulation [168] and a genetically modified K562 cell line that expresses membrane bound IL-15 and CD137L was used to induce sustained and specific proliferation of human NK cells [169]. However, despite activation of NK cells, the bidirectional signaling of CD137L -CD137 caused the production of IL-10 and TNF by acute myeloid leukemia cells. These cytokines negatively regulate NK functions by reducing granule mobilization and IFNγ production [168].

4. NK-based immunotherapies

Throughout this review we have discussed the role of NK cells in tumor surveillance and, more importantly, their active role in tumor elimination. Multiple immunotherapies have been developed in order to exploit the anti-tumoral properties of NK cells. Moreover, the fact that NK cells can spontaneously induce cell death without prior immunization makes NK-based immunotherapies a very attractive and promising cancer therapy. We next will describe some of the strategies where NK cells are being utilized in cancer.

4.1. Modulation of NK function

As previously mentioned, NK activation can be modulated by multiple cytokines such as IL-2, IL-15, IL-12, and IL-21. Therefore, the use of these cytokines as a means to expand and improve cytotoxic functions of NK cells has been explored by multiple researchers.

Different mouse models have demonstrated the efficacy of IL-2 to expand and activate NK cells yielding NK-dependent anti-tumor responses [170, 171]. In advanced cancer patients, IL-2 treatment alone or in combination with lymphokine-activated killer cells (LAKs) resulted in anti-tumor responses [172, 173]. Because of the efficacy of IL-2 against tumors, it was approved by the FDA in 1992 for the treatment of renal cell carcinoma (RCC) [139] and in 1998 for metastatic melanoma [172, 174]. Increased numbers of circulating NK cells after *in vivo* treatment with IL-2 has been found in breast cancer, lymphoma, AIDS-associated lymphoma, metastatic melanoma, and metastatic RCC patients [139]. IL-2 was also able to improve NK cytotoxicity after autologous transplantation [175]. Unfortunately, although IL-2 was initially deemed as a promising cancer immunotherapy, the overall response in survival and cancer relapse has been rather limited [139, 176]. A meta-analysis of 14 clinical trials that used IL-2 as a remission maintenance therapy in patients with AML, found that ongoing complete remission did not occur. This was attributed to downregulation of NCR and NKG2D on NK cells mediated by phagocyte-derived ROS [176, 177].

Additionally, the high and frequent doses necessary to achieve positive results have limited the usage of this therapy due to toxicity, which is especially aggravated in aged patients. High doses of IL-2 are associated with vascular leak syndrome (VLS), myocardial infarction, and cardiac arrhythmia. VLS occurs when there is an abnormal increase in vascular permeability resulting in extravasation of fluids and proteins into tissues which in the most serious cases induces pulmonary edema and cardiovascular failure [178]. NK cells play an important role in the toxicity induced by high-doses of IL-2 as early studies demonstrated that depletion of NK cells in C57BL/6 using anti-NK1.1 attenuated the lethal toxicity associated to high-doses of IL-2 [179].

Another limitation of using IL-2 therapy is associated with its critical role in the expansion and maintenance of Tregs because of their constitutive expression of the high affinity IL-2 receptor (IL-2R) which is formed by the alpha (CD25), beta (CD122) and common gamma (CD132) chains in contrast to NK cells that express intermediate affinity IL-2R (CD122 and CD132). As previously mentioned, the release of TGF-β by Tregs has a profound impact on NK activation and function. The high affinity IL-2R is also found on $CD56^{bright}$ NK cells and activated conventional CD4 and CD8 T cells [139]. The use of low doses of IL-2 has been attempted to reduce IL-2-associated toxicity. However, after autologous

BMT, despite the increased number of total NK cells found in lymphoma and breast cancer patients after IL-2 treatment, the expansion was of the less cytotoxic $CD56^{bright}$ NK subset which may account for the lack of increased survival [180]. Current approaches involve using IL-2 in combinatorial therapies as a mean to improve other chemotherapeutic drugs or autologous and allogeneic BMT, which will be discussed in subsequent sections.

IL-15 has become a feasible alternative to IL-2 because of its involvement in the development, proliferation, survival and activation of NK cells and memory CD8 T cells. In order to function, IL-15, as opposed to IL-2, needs to be presented to NK cells and memory CD8 T cells by DCs. This trans-presentation is possible due to the expression of the high-affinity IL15Rα by DCs that will trans-present IL-15 to NK and memory CD8 T cells that express the intermediate-affinity IL2/15Rβγc [181]. IL-15 has also been shown to accelerate NK reconstitution after BMT [182, 183]. Moreover, IL-15 does not regulate Treg expansion and does not induce activation-induced cell death of effector T cells as IL-2 does [184]. Combining of IL-15 with IL-6 resulted in rescue of NK function after inhibition by TGF-β. Upregulation of NKG2D expression was also observed to be mediated by IL-15 resulting in improved NK cytotoxicity [184]. Currently, clinical trials are ongoing to demonstrate safety and efficiency of IL-15 as a cancer treatment for refractory metastatic melanoma and metastatic RCC. However, IL-15 has also been shown to upregulate inhibitory NK receptors, induce IL-10 secretion, and increase the expression of programmed cell death-1 (PD-1) receptor and PD-L1 on T cells [178, 184]. Additionally, a recent study has shown that sustained stimulation of NK cells with IL-15/IL-15Rα complexes results in accumulation of NK cells with impaired proliferation, activity and cytotoxic functions as well as alterations in the levels of activating and inhibitory receptors which has been correlated with induction of NK anergy [143]. These observations could explain the contradictory results obtained from different experimental tumor models. Similar to IL-2, it is believed that the best application for IL-15 will occur when it acts as an adjuvant with other cancer therapies [184]. The multiple approaches that are currently under investigation to improve IL-15-mediated anti-tumor responses have been reviewed elsewhere [178, 184]. IL-21 could potentially also be used to expand NK cells as it has been shown to improve NK cytotoxicity and stimulate $CD56^{dim}$ NK cells [81].

Another strategy to improve NK function is to modulate tumor cells to make them more susceptible to NK-mediated cytolysis. Molecular targeting agents typically used to eliminate highly proliferating tumor cells have been shown to upregulate death receptors (Fas, TNFR1, DR4, DR5, DR6 and DR3) [152] and/or NK activating receptor ligands. The proteasome inhibitor bortezomib has been shown to increase FasL-mediated NK cytolysis by increasing the expression of DR5 [45, 138, 185] or prevention of caspase 8 degradation in tumor cells [186]. HSP90 inhibitors (Celastrol) have been shown to increase TRAIL-induced apoptosis by downregulating c-FLIP or anti-apoptotic proteins such as Bcl-2 and upregulate DR5 expression [152]. Thalidomide and histone deacetylase inhibitors (HDAC) enhance the levels of death receptors on tumor cells facilitating tumor clearance as well [187].

The induction of DNA damage has also proven to be a mechanism for the increase of stress ligands that are recognized by activating receptors on NK cells [76, 188]. Doxorubicin, melphalan, bortezomib, and HDACs are some of the drugs that have shown to increase the levels of DNAM-1L and/or NKG2DL in multiple cancers improving NK-mediated killing [188-190]. Blockade of shedded NKG2DL with metalloproteinase inhibitors may also be used as a therapy to improve NK function by restoring NKG2D-dependent cytotoxicity [190]. Combinations of myeloid growth factors and IFNγ with 5-aza-2'-deoxycytidine, trichostanti A, all-trans retinoic acid (ATRA), or vitamin D3 have been shown to increase the levels of UBLPs on AML cell lines and ALL blasts as well. [104, 139].

Frequently, improving NK numbers, activation, and function is not enough to observe clinical benefits. An explanation for the limited results of

NK-based immunotherapies may be found in the presence of an immunosuppressive environment that overrides NK activation. The use of therapies to enhance NK function in combination with strategies to inhibit immunosuppression might be more efficient in achieving anti-tumor responses. As previously explained, TGF-β is involved in immune evasion in multiple cancers and TGF-β blockade has already been demonstrated to be effective as a cancer therapy for glioblastoma patients [191]. TGF-β blockade could be also used to modulate NK function by restoring NKG2DL expression in tumor cells and enhancing NKG2D-mediated NK killing [160]. Amplification of NKG2D-mediated killing is also observed after IFNα, IL-2, and IL-12 treatment [104]. Elimination of Tregs can improve the NK population by limiting immunosuppression. However, this approach should be carefully analyzed as autoimmune disorders and/or toxicity could emerge because of exacerbated immune activation.

4.2. Hematopoietic stem cell transplantation

Hematopoietic stem cell transplantation (HSCT) is a therapy frequently used to treat blood born cancers, such as leukemia or lymphoma, as well as other hematological diseases. HSCT can be myeloid ablative or non-myeloid ablative. During myeloid ablative HSCT, patients receive a conditioning regimen of irradiation and/or chemotherapy. Following this conditioning regimen, hematopoietic stem cells (HSCs) are then administered to repopulate the patient's immune system. HSCs can either be isolated from the recipient prior to conditioning (autologous) or from related or unrelated HLA-matched or mismatched donors (allogeneic). Despite the benefits that this therapy has shown in the battle against cancer, there are several shortcomings that need to be improved. Following HSCT the patient is immunocompromised while the immune system recovers making the patient susceptible to opportunistic infections such as CMV which can account for a significant portion of mortality and morbidity of patients receiving this treatment [191]. Given the lack of immune defense, in addition to opportunistic infections, tumor cells resistant to chemotherapy or radiotherapy conditioning frequently lead to cancer relapse. Lastly, another major problem associated with HSCT, especially allogeneic HSCT, is graft versus host disease (GvHD). In order to minimize GvHD, T cell depleted grafts are frequently used in HSCT which, unfortunately, are often accompanied by reduced graft versus tumor (GvT) effects. NK cells have shown to be the first lymphoid population to recover after HSCT [192, 193]. Importantly, donor NK cells have been demonstrated to suppress GvHD while maintain GvT following allogeneic HSCT [194]. Therefore strategies that accelerate NK reconstitution could result in improved survival and reduced cancer relapse by increasing the protection against opportunistic infections, enhancing GvT, and reducing GvHD.

Enhanced survival was correlated with rapid and early recovery of NK cells after autologous HSCT for patients with non-Hodgkin's lymphoma, Hodgkin's disease, ALM, MM and metastatic breast cancer [195-197]. $CD56^{bright}CD16^{low}$ NK subset has shown to predominate early after HLA-matched HSCT. This subset, a difference from donor $CD56^{brigh}CD16^{low}$ NK cells shows an intermediate mature phenotype with higher IFNγ production and degranulation properties and upregulation of CD94/NKG2A, NKG2D and NKp46. Despite the early reconstitution of this immature NK subset, no defects were found in their cytotoxic functions [198]. However, defects on ADCC would be expected due to the lower expression of CD16.

Studies carried out by Ruggeri *et al.*, revealed an association of improved disease-free survival and reduced relapse rate in AML patients, but not adult ALL, that underwent haploidentical HSCT with donor NK cells that displayed KIRs for MHC class I ligands not expressed by the hosts [120, 199, 200]. The presence of alloreactive NK cells showed decreased relapse rates in pediatric ALL after haploidentical HSCT [201]. Alloreactive NK cells have also been correlated with the suppression of GvHD by the elimination of not only host T cells and granulocytes, but also hosts DCs which are responsible for the activation of donor T cells involved in GvHD [199]. Furthermore, *in vitro* studies suggest that the production of TGF-β by NK cells ameliorate GvHD by inhibiting T cell responses [194]. These studies introduced the possible use of alloreactive

NK cells in less myeloid ablation condition regimens and without the need of donor T cell depletion which should result in a stronger GvT effect.

However, KIR-mismatch has not always correlated with better outcomes [202-204]. This difference can be due to different transplantation protocols: including conditioning regimens, doses and source of HSC, presence of T cells in the graft, and post-transplantation immunosuppression protocols as cyclosporine A has been demonstrated to suppress NK cell development [81]. It has been suggested that the efficiency of KIR-mismatched NK cells could better be observed in T cell depleted grafts [127] as it has been shown that T cells affect KIR expression during NK reconstitution after unrelated donor transplantation [204]. When un-manipulated hematopoietic stem cell transplants were used, recovered NK cells showed reduced KIR expression compared with their donors whereas T cell depleted grafts were less affected. Additionally, these NK cells also demonstrated higher cytotoxic functions by increased IFNγ production. In this study, increased IFNγ production and reduced KIR expression were correlated with more acute GvHD and inferior survival respectively [204]. Thus, although the presence of T cells during immune reconstitution after HSCT seems to significantly improve NK effector functions by reduced KIR expression and increased IFNγ production, the appearance of acute GvHD negates the possible enhanced anti-tumor responses resulting in an overall reduced survival.

Alloreactive NK cells can also be found in HLA-matched HSCT or autologous HSCT as by definition, alloreactivity of NK cells could be achieved when NK cells are lacking KIRs for host HLA ligands independent of the HLA type of the donor. This concept leads to the proposal of the missing ligand model [202, 205, 206]. Several studies have demonstrated better outcomes in patients lacking the class I ligand for donor alloreactive NK cells [63, 200, 201, 205, 207]. Survival rates have been strongly associated with missing ligand in patients with stage IV neuroblastoma that went through autologous HSCT. In this study, the presence of NK cells that lack one or more KIRs for self-HLA resulted in a 46% lower risk of death and 34% lower risk of cancer progression [67].

NK education or licensing can also play a role in outcomes after HSCT. Mouse HSCT studies showed that host licensed NK cells play a predominant role in the rejection of allogeneic or MHC class I deficient allografts [62]. In humans and mice it has been demonstrated that unlicensed NK cells, which by definition can potentially be alloreactive NK cells, are normally hyporesponsive [59, 61]. However, upon activation, unlicensed host NK cells can efficiently eliminate MHC class I deficient BM cells [62]. Moreover, a mouse model showed that unlicensed NK cells played a predominant role in the elimination of MCMV-infected cells [66].

In unrelated HSCT it has been suggested that NK education is driven by donor ligands and therefore donor alloreactive NK cells can become licensed which promotes sustained GvT after HLA-mismatched HSCT [208]. Furthermore, unlicensed NK cells with KIRs for other ligands than host HLA could potentially have an important anti-tumor role as well. Hsu *et al*. demonstrated that in HLA-matched HSCT, NK cells devoid of KIRs for host and donor HLA (unlicensed), exhibited effector functions early post-HSCT and only later (+200 post-HSCT) tolerance to self was achieved [68]. According to this study, in HLA-mismatched HSCT, alloreactive unlicensed NK cells could cooperate with alloreactive licensed NK cells in eliminating tumors early post-HSCT. In autologous HSCT settings, unlicensed NK cells could potentially promote anti-tumor responses early post-HSCT as well.

4.2.1. NK adoptive transfer

Cancer relapse is a major problem for patients that have undergone HSCT. NK cells have an important role in HSCT outcomes, therefore NK adoptive transfer may potentiate the benefits of HSCT [209]. NK adoptive transfer therapy has been used alone, as an adjuvant for allogeneic or autologous HSCT to prevent cancer relapse, or when cancer relapse has occurred after HSCT.

The adoptive transfer of autologous NK cells after *ex vivo* activation has proven to be safe and well tolerated in lymphomas, breast and lung cancer,

CRC, and metastatic RCC patients resulting in *in vivo* expansion of NK cells. Unfortunately, clinical benefits from the adoptive transfer of activated NK cells were not observed in all cases [139, 180]. The requirements of additional stimulation by cytokines for the survival and function of *ex vivo* activated NK cells can account for the unsuccessful effect of NK adoptive transfer therapy. For example, the use of IL-2 in some cases to expand and maintain the NK repertoire post-infusion may have also negatively affected the anti-tumor response by increasing the Treg population or by preferentially expanding the less cytotoxic $CD56^{bright}CD16^{low}$ NK population due to its expression of the high affinity IL-2R [180]. Moreover, sustained cytokine stimulation of adoptive transfer NK cells could result in NK anergy or NK exhaustion similar to *in vivo* administration of IL-15/IL-15Rα [143]. NK exhaustion has also been recently observed during homeostatic proliferation and tumor exposure of adoptively transferred NK cells [210]. Additionally, it has been postulated that the failure of autologous NK adoptive transfer lies in the expression of inhibitory receptors that recognize self-HLA displayed on tumor cells overriding NK activation [139].

Because of the improved results obtained after allogeneic HSCT [199], allogeneic NK adoptive transfer may also become a promising alternative to autologous NK adoptive transfer or donor lymphocyte infusion because of the reduced GvHD risk. Miller *et al*. were able to successfully infuse haploidentical NK cells in advanced cancer patients. In general, an expansion of NK cells was observed after IL-2 administration due to increased levels of endogenous IL-15 which resulted in improved survival rates without GvHD. More importantly, complete remission was achieved in 5 of 19 AML patients [211]. In a recent phase I pilot study, the repetitive infusion of allogeneic IL-15 activated NK cells in combination with chemotherapy was safe and clinically effective against non-small cell lung cancer [212]. In another study where haploidentical NK cells were transferred in AML patients after relapse from haploidentical HSCT, a persistent and massive expansion of allogeneic NK cells was observed for weeks after infusion. However, improved persistence of activated NK cells did not translate into prolonged survival as patients relapsed approximately 80 days post NK transfer [213]. IL-2 (10^7 IU/week) was given in this study to maintain the NK population. Although a $CD56^{bright}$ expansion was not observed in the treated patients, IL-2 administration could have led to Treg expansion thereby inhibiting NK function. In another phase II clinical trial where allogeneic NK therapy was used in patients with recurrent ovarian and breast cancer, an expansion of Tregs was also detected and correlated with the limited benefit of the therapy despite the transitory expansion of haploidentical NK cells and increased IL-15 serum levels [214]. This study illustrates the relevance of immunosuppression in controlling immune activation.

To further improve allogeneic and autologous NK alloreactivity, antibodies that block inhibitory KIR have been developed with the goal of enhancing cytotoxic functions. A novel human anti-KIR, 1-7F9, has been shown to increase NK-mediated cytotoxicity of HLA-matched AML blasts *in vitro* and *in vivo* [145]. The combination of this antibody with lenalidomide, which augments NK function by increasing both activating NK receptor expression on NK cells and activating ligands on MM target cells, further improved NK function of patient-derived NK cells against autologous MM target cells [215]. In a mouse HSCT model, the blockade of Ly49 inhibitory receptors that recognized self-MHC displayed by tumor cells improved anti-tumor responses [216]. An alternative to the use of blockade antibodies against inhibitory receptors could rest on the preferential expansion, activation and adoptive transfer of those NK cells that do not express inhibitory receptors for self-MHC.

The infusion of NK-92, an NK cell line, is also a possible alternative to allogeneic or autologous NK cells. NK-92, which lacks KIR expression, can be grown in good manufacturing practice (GMP) conditions and has been shown to efficiently eliminate leukemia, lymphoma and CML *in vitro* [139]. Clinical studies have demonstrated the safety and possible clinical benefit of using NK-92 infusions in advanced RCC and melanoma [217].

One of the major limitations for NK adoptive transfer lies in the generation of large numbers of

NK cells to be used therapeutically. Short-term activated NK cells have been obtained after *in vitro* culture with IL-2 or IL-15. However, the number reached after short-term activation is limited. Therefore there have been multiple attempts to generate a large-scale expansion of highly purified, GMP grade NK cells by long term *in vitro* expansion. Additionally, it is been suggested that long term activated NK cells express higher levels of IL-2Rα [79] making these NK cells a better target for IL-2 *in vivo* expansion. IL-2 and IL-15 have been used to expand NK cells for long periods of time [218] as well. However, the addition of feeder cells can further augment NK expansion. The use of a genetically modified K562 cell line that expresses a membrane-bound form of IL-15 and 41BB ligand (CD137L) for *in vitro* culture of NK cells resulted in a major expansion compared with NK cells stimulated with IL-2, IL-12, IL-15, or IL-21. These NK cells were also more cytotoxic and detectable for up to a month when injected into immunodeficient mice [169]. Epstein-Barr virus-transformed lymphoblastoid cells (EBV-LCL) have also been used to expand NK cells. Co-culture of isolated NK cells with EBV-LCL and IL-2 for 21 days upregulated the expression of TRAIL, FasL and NKG2D on NK cells [189]. Membrane-bound (mb) IL-21 has also demonstrated to enhance *ex vivo* human NK expansion resulting in NK cells with higher proliferative capabilities and cytotoxic functions [219].

Other characteristics to take under consideration when *ex vivo* expanded NK cells are used are the phenotype and the activation status of these NK cells. $CD56^{bright}$ NK cells express the high affinity IL-2Rαβγ making them more susceptible to expansion by IL-2 administration [220]. Additionally, longer telomeres have been observed in the $CD56^{bright}$ NK subset [38] which can be correlated with their higher proliferative capabilities. In contrast, $CD56^{dim}$ NK cells show low proliferative capabilities when stimulated with IL-2 *in vitro* [221, 222]. CD62L expression, however, has been recently identified to further differentiate a $CD56^{dim}$ subpopulation that displays higher proliferation, cytokine production and cytotoxicity [223]. A preferential expansion of $CD56^{bright}$ NK cells after *in vivo* or *in vitro* stimulation with IL-2 has been observed. Stimulation with mbIL-15, mbIL-21 or IL-12 and CD137L has also shown to preferentially expand $CD56^{bright}$ [39, 219]. The effect of activation in $CD56^{bright}$ NK cells and their functional capabilities is still unclear as there is no consensus regarding the expression of KIRs, activating receptors and CD16. A predominance of the $CD56^{bright}KIR^{-}CD16^{-/low}$ NK subset with poor cytolytic activity was observed after *in vivo* and *in vitro* NK stimulation [224]. However, acquisition of KIR and CD16 expression has also been associated to cytokine stimulated $CD56^{bright}$ NK cells [38, 225]. NCR, CD16 and NKG2D upregulation on $CD56^{bright}$ NK cells was detected after stimulation with mbIL-21 [219]. These NK cells also displayed superior cytokine production and ADCC-dependent cytotoxicity [219]. Similarly, NK stimulation with IL-12 and CD137L resulted in a significant expansion of $CD56^{bright}$ NK cells that display higher cytotoxic function and IFNγ production against K562 target cells [39]. Interestingly, Dowell *et al*. were able to promote differentiation towards $CD56^{bright}$ $CD16^{-}$ phenotype after IL-12 and CD137L stimulation from $CD56^{dim}CD16^{+}$ peripheral blood sorted NK cells, challenging the unidirectional differentiation of $CD56^{dim}$ NK cells from $CD56^{bright}$ NK cells [39].

In summary, there seem to be major differences regarding the NK phenotype and function of the NK cells obtained from *in vivo* and *ex vivo* expansion. These discrepancies can be the result of using different stimulation strategies. However, as Denman *et al*. have suggested, the NK phenotype after activation may not correlate with NK function in the same way that it does in resting NK cells [219]. Nevertheless, characterization of NK phenotype and especially NK function prior to infusion of activated NK cells is necessary to determine the potential NK anti-tumor responses. Typically, to determine NK activation prior to adoptive transfer a short-term lytic assay against K562 targets is performed. This assay, although possibly indicating NK function, might not adequately represent the potential NK activity post-transfer as NK cells can employ multiple anti-tumor mediators (ie perforin, TRAIL, fas L, interferon, TNF, etc). Additionally, multiple parameters can suppress NK activity, such as

MHC expression or immunosuppression by tumor cells. Finally, toxicities that might arise from adoptive NK transfer therapy on normal hematopoietic cells or normal tissues need to be carefully evaluated.

4.3. ADCC- combination of NK cells with antibodies

NK-mediated ADCC can also be exploited to improve antibody-dependent immunotherapies. For example, ADCC is one of the most important mechanisms of action for rituximab, a chimeric mouse/human antibody that recognizes CD20 antigen expressed on mature B lymphocytes [226]. NK-dependent ADCC is also part of the effector mechanisms used by Herceptin, which potentially could also benefit from therapies that target NK activation. Therefore, improvement of NK-dependent ADCC may further enhance anti-tumor responses with these drugs. Combination regimens such as rituximab with IL-2 with and without short term activated NK cells resulted in NK expansion and higher ADCC function [227]. The administration of IL-2 with rituximab allowed for the rejection of rituximab-resistant tumors in an ADCC-dependent manner demonstrating the synergistic effect of using this combinatorial therapy [213]. NK activation with IL-15 was also shown to increase rituximab-mediated ADCC against CLL *in vitro* [226]. Furthermore, this combination could override NK inhibition mediated by TGF-β. Despite these observations, however, no clinical benefits have been reported [228].

Currently, bispecific antibodies are under development to promote NK cell targeting of tumor cells. Antibodies specific for CD16 to induce NK activation in combination with CD19 for B-cell lymphoma, ERBB2 for breast cancer, or CD30 for Hodgkin's lymphoma have shown promising results [81]. Recently, a bispecific NK receptor fusion protein that targets both T cells and tumor cells was shown to increase IFNγ production and cytotoxicity against NKG2DL-positive tumor cells and increase tumor survival in mouse models [229]. A single chain variable fragment (scFv) of anti-CD3ε was fused to the extracellular domain of NKG2D receptor (scFv-NKG2D antibody) creating a receptor able to bind to NKG2DL-positive tumor cells and activate T cells via CD3ε cross-linking. A similar approach could be employed to improve NK-mediated cytotoxicity using anti-CD16 to activate NK cells. The use of antibodies fused with NKG2D, NKp46, NKp30 or NKp44 could be of particular benefit in promotion of NK activation and NK-mediated tumor recognition for cancer patients whose NK cells have shown impaired function due to downregulation of NKG2D or other NCRs.

CONCLUSIONS

While NK cell based immunotherapeutic approaches may be of potential benefit in cancer, it is clear that it is likely contingent on the type of cancer being targeted with hematologic malignancies being most promising. In addition, there is still much to learn regarding the biology of NK cells, their subsets as well as regulation and subsequently how to exploit them in cancer. Tumor evasion remains a significant hurdle that must be overcome (Figure 1). Potential toxicities that can arise by using cytokines that activate NK cells are also an issue. Nevertheless the most promising results have been obtained from the combination of NK-based immunotherapies with other cancer therapies and therefore the current tendency is the use of combinatorial therapies that attack cancer cells from multiple angles [139, 189, 230]. However, such an activating environment could potentially result in high levels of inflammatory cytokines resulting in autoimmunity and/or toxicities, and therefore these types of approaches should proceed cautiously.

Important questions remain regarding the use of NK cells in cancer: What cancers should be targeted? What NK cell subsets should be employed? What is the optimal means of activation that allows for sustained effects? How can we get the NK cells to traffic where the tumor is? How does the tumor evade NK cell attack? All of these are important to address. In addition, there is more and more evidence that NK cells themselves can be immunosuppressive, in particular to T cells and DCs and this may result in mixed responses in cancer.

Strategies to further improve NK function can also have an important impact on NK-based immunotherapy outcomes. A clear example is IL-2, which not only promotes Tregs resulting in

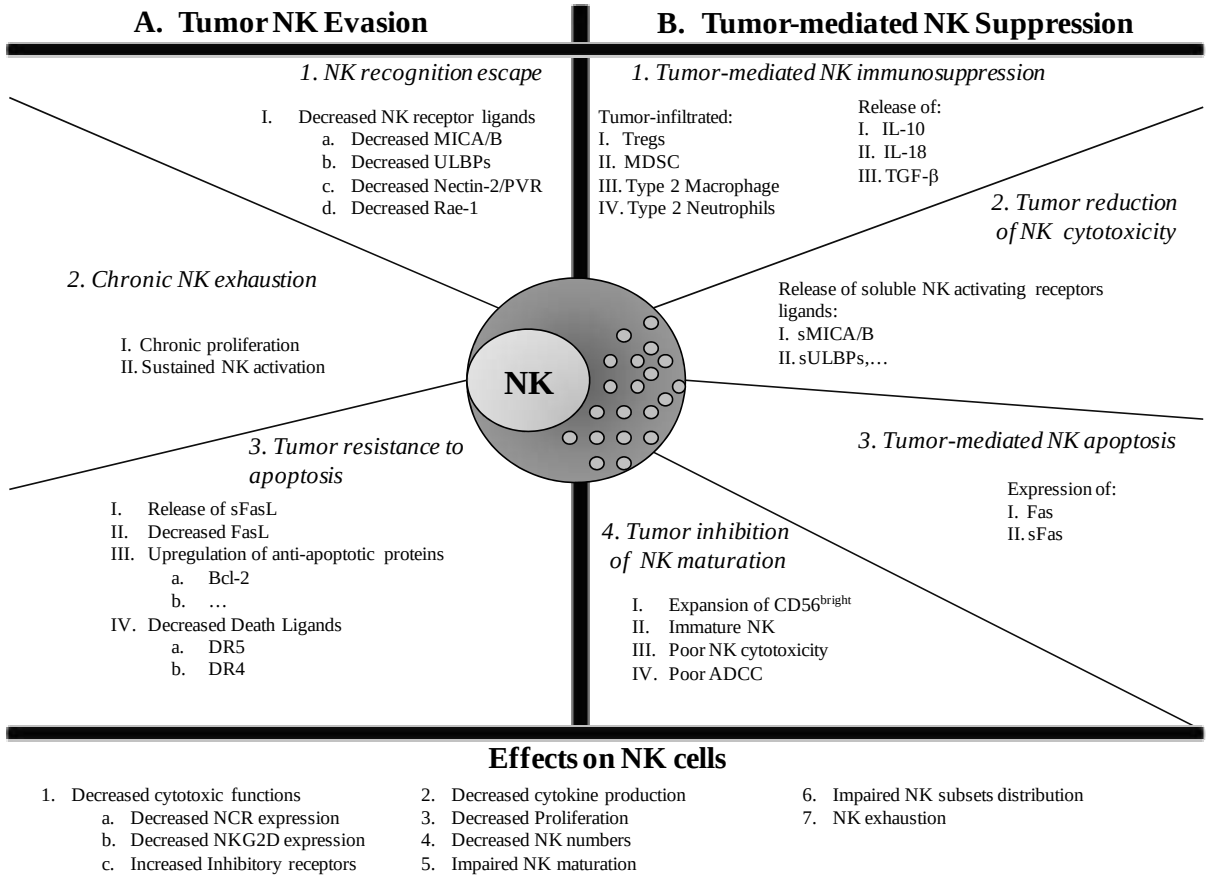

Figure 1. Mechanisms evolved by tumor cells to evade NK cells. **A.** Tumor cells can evade NK-mediated killing by: 1. Abrogation of NK recognition through downregulation of NK receptor ligands such as MICA/B or ULBPs, ligands for NKG2D, or Nectin-2 and PVR (ligands for DNAM-1). 2. Induction of chronic NK exhaustion with low NK cytotoxic functions due to sustained contact with tumor cells. 3. Resistance to NK-mediated apoptosis by the release of soluble (s)FasL or reduction of surface expression of FasL or death ligands (DR5/DR4) to block Fas-mediated or TRAIL-mediated NK killing. Resistance to apoptosis can occur by upregulating anti-apoptotic proteins such as Bcl-2. **B.** Tumor cells can mediate direct NK suppression by: 1. Promotion of NK immunosuppresion due to recruitment of tumor-associated regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSC), type 2 macrophages and type 2 neutrophils at the tumor site; and enhancement of IL-10, IL-18 and TGFβ produced by tumor cells or tumor-associated immunosuppressor cells. 2. Reduction of NK cytotoxicity by the tumor release of soluble NK activating receptor ligands such as sMICA/B which mediates NKG2D downregulation on NK cells. 3. Induction of NK apoptosis by the expression of Fas or sFas. 4. Inhibition of NK maturation which alters NK subsets and favors a more immature and less cytotoxic NK population. All together these mechanisms can lead to poor NK cytotoxic functions due to downregulation of NCR and NKG2D activating receptors and upregulation of inhibitory receptors, poor cytokine production, low proliferation and expansion, impaired NK maturation, and altered subset distribution and/or NK exhaustion.

NK suppression, but also might promote a selective expansion of the less cytotoxic $CD56^{bright}$ population which might account for reduced tumor eradication. Therefore the introduction of other cytokines such as IL-15, IL-12, or IL-21, which do not promote Tregs, could show benefits where IL-2 administration has failed. Another factor that needs to be taken under consideration is the impact of these cytokines in long term NK activation because sustained NK activation by IL-15 was demonstrated to induce NK exhaustion in a similar way that chronic virus infections promote immune exhaustion [143] thereby limiting NK-dependent anti-tumor responses.

NK adoptive transfer has demonstrated potential promising benefits alone or in combination with other cancer therapies such as chemotherapeutic drugs or HSCT. A limitation for autologous NK adoptive transfer could depend on the presence of MHC class I on tumor cells that inhibit NK function, but the development of strategies to block inhibitory receptors could potentially override this inhibition and allow autologous NK cells to eliminate tumor cells that display self-MHC and activating receptor ligands. Additionally, the combination of autologous HSCT with adoptive transfer of autologous unlicensed NK cells could also be used to improve responses as unlicensed NK cells have been shown to be responsive early post-HSCT and play an important role in the clearance of self-HLA expressing tumor cells [68]. Regarding allogeneic NK adoptive transfer, it would be important to develop strategies to properly select, activate and expand those donor NK cells that have shown the highest alloreactivity against the patient's tumor yet not contribute to GvHD when used in allogeneic HSCT.

Recently, several studies have suggested the existence of NK cells displaying memory-like properties. These NK cells are characterized by longer survival and stronger secondary responses upon rechallenge. Thus far, memory-like NK cells have been identified after induction of hapten-specific contact hypersensitivity [231, 232], virus infection [14, 232] or cytokine stimulation [233]. The induction of memory NK cells in other conditions, such as tumor elimination, is still unknown. However, if an NK subset with memory properties prevails after a first tumor encounter, identifying those therapies that target and expand this subset may aid in attacking the remaining tumor cells and reduce tumor relapse. Clearly, NK cells have come a long way from initially being considered a rather simplistic cell of the innate immune system.

ACKNOWLEGEMENTS

We thank Monja Metcalf and Angela Baptiste for assisting in the preparation of this manuscript. This publication has been funded in whole or in part by National Institute of Health (NIH) Grant R01-HL089905.

REFERENCES

1. Kiessling, R., Klein, E., Pross, H., and Wigzell, H. 1975, Eur. J. Immunol., 5, 117.
2. Kiessling, R., Klein, E., and Wigzell, H. 1975, Eur. J. Immunol., 5, 112.
3. Herberman, R. B., Nunn, M. E., Holden, H. T., and Lavrin, D. H. 1975, Int. J. Cancer, 16, 230.
4. Herberman, R. B., Nunn, M. E. and Lavrin, D. H. 1975, Int. J. Cancer, 16, 216.
5. Karre, K., Ljunggren, H. G., Piontek, G., and Kiessling, R. 1986, Nature, 319, 675.
6. Cudkowicz, G. and Bennett, M. 1971, J. Exp. Med., 134, 1513.
7. Trinchieri, G. 1989, Adv. Immunol., 47, 187.
8. DiSanto, J. P., Muller, W., Guy-Grand, D., Fischer, A., and Rajewsky, K. 1995, Proc. Natl. Acad. Sci. USA, 92, 377.
9. Ljunggren, H. G. and Malmberg, K. J. 2007, Nat. Rev. Immunol., 7, 329.
10. Lanier, L. L. 2005, Annu. Rev. Immunol., 23, 225.
11. Shinkai, Y., Rathbun, G., Lam, K. P., Oltz, E. M., Stewart, V., Mendelsohn, M., Charron, J., Datta, M., Young, F., Stall, A. M., and Alt, F. W. 1992, Cell, 68, 855.
12. de Villartay, J. P., Fischer, A., and Durandy, A. 2003, Nat. Rev. Immunol., 3, 962.
13. Paust, S., Senman, B., and von Andrian, U. H. 2010, Immunol. Rev., 235, 286.
14. Sun, J. C., Beilke, J. N., and Lanier, L. L. 2009, Nature, 457, 557.
15. Di Santo, J. P. 2006, Annu. Rev. Immunol., 24, 257.
16. Lanier, L. L. 2008, Nat. Rev. Immunol., 8, 259.
17. Revilleza, M. J., Wang, R., Mans, J., Hong, M., Natarajan, K., and Margulies, D. H. 2011, J. Biomed. Biotechnol., 2011, 724607.
18. Kim, Y., Park, B., Cho, S., Shin, J., Cho, K., Jun, Y., and Ahn, K. 2008, PLoS Pathog., 4, e1000123.
19. Colucci, F., Caligiuri, M. A., and Di Santo, J. P. 2003, Nat. Rev. Immunol., 3, 413.
20. Prlic, M., Blazar, B. R., Farrar, M. A., and Jameson, S. C. 2003, J. Exp. Med., 197, 967.

21. Kennedy, M. K., Glaccum, M., Brown, S. N., Butz, E. A., Viney, J. L., Embers, M., Matsuki, N., Charrier, K., Sedger, L., Willis, C. R., Brasel, K., Morrissey, P. J., Stocking, K., Schuh, J. C., Joyce, S., and Peschon, J. J. 2000, J. Exp. Med., 191, 771.
22. Cooper, M. A., Bush, J. E., Fehniger, T. A., VanDeusen, J. B., Waite, R. E., Liu, Y., Aguila, H. L., and Caligiuri, M. A. 2002, Blood, 100, 3633.
23. Koka, R., Burkett, P. R., Chien, M., Chai, S., Chan, F., Lodolce, J. P., Boone, D. L., and Ma, A. 2003, J. Exp. Med., 197, 977.
24. Castillo, E. F., Stonier, S. W., Frasca, L., and Schluns, K. S. 2009, J. Immunol., 183, 4948.
25. McCullar, V., Oostendorp, R., Panoskaltsis-Mortari, A., Yun, G., Lutz, C. T., Wagner, J. E., and Miller, J. S. 2008, Exp. Hematol., 36, 598.
26. Vosshenrich, C. A., Ranson, T., Samson, S. I., Corcuff, E., Colucci, F., Rosmaraki, E. E., and Di Santo, J. P. 2005, J. Immunol., 174, 1213.
27. Hayakawa, Y. and Smyth, M. J. 2006, J. Immunol., 176, 1517.
28. Chiossone, L., Chaix, J., Fuseri, N., Roth, C., Vivier, E., and Walzer, T. 2009, Blood, 113, 5488.
29. Caligiuri, M. A. 2008, Blood, 112, 461.
30. Dussault, I. and Miller, S. C. 1993, Natural immunity, 12, 66.
31. Ellis, T. M. and Fisher, R. I. 1989, J. Immunol., 142, 2949.
32. Frey, M., Packianathan, N. B., Fehniger, T. A., Ross, M. E., Wang, W. C., Stewart, C. C., Caligiuri, M. A., and Evans, S. S. 1998, J. Immunol., 161, 400.
33. Hazenbos, W. L., Gessner, J. E., Hofhuis, F. M., Kuipers, H., Meyer, D., Heijnen, I. A., Schmidt, R. E., Sandor, M., Capel, P. J., Daeron, M., van de Winkel, J. G., and Verbeek, J. S. 1996, Immunity, 5, 181.
34. Fehniger, T. A., Cooper, M. A., Nuovo, G. J., Cella, M., Facchetti, F., Colonna, M., and Caligiuri, M. A. 2003, Blood, 101, 3052.
35. Martin-Fontecha, A., Thomsen, L. L., Brett, S., Gerard, C., Lipp, M., Lanzavecchia, A., and Sallusto, F. 2004, Nat. Immunol., 5, 1260.
36. De Maria, A., Bozzano, F., Cantoni, C., and Moretta, L. 2011, Proc. Natl. Acad. Sci. USA, 108, 728.
37. Chan, A., Hong, D. L., Atzberger, A., Kollnberger, S., Filer, A. D., Buckley, C. D., McMichael, A., Enver, T., and Bowness, P. 2007, J. Immunol., 179, 89.
38. Romagnani, C., Juelke, K., Falco, M., Morandi, B., D'Agostino, A., Costa, R., Ratto, G., Forte, G., Carrega, P., Lui, G., Conte, R., Strowig, T., Moretta, A., Munz, C., Thiel, A., Moretta, L., and Ferlazzo, G. 2007, J. Immunol., 178, 4947.
39. Dowell, A. C., Oldham, K. A., Bhatt, R. I., Lee, S. P., and Searle, P. F. 2012, Cancer Immunol. Immunother., 61, 615.
40. Hayakawa, Y., Huntington, N. D., Nutt, S. L., and Smyth, M. J. 2006, Immunol. Rev., 214, 47.
41. Silva, A., Andrews, D. M., Brooks, A. G., Smyth, M. J., and Hayakawa, Y. 2008, Int. Immunol., 20, 625.
42. Trowsdale, J. 2001, Immunity, 15, 363.
43. Hallett, W. H. and Murphy, W. J. 2006, Semin. Cancer Biol., 16, 367.
44. Long, E. O. 2008, Immunol. Rev., 224, 70.
45. Lundqvist, A., Abrams, S. I., Schrump, D. S., Alvarez, G., Suffredini, D., Berg, M., and Childs, R. 2006, Cancer Res., 66, 7317.
46. Veillette, A., Latour, S., and Davidson, D. 2002, Annu. Rev. Immunol., 20, 669.
47. Murphy, W. J., Kumar, V., and Bennett, M. 1987, J. Exp. Med., 166, 1499.
48. Ljunggren, H. G. and Karre, K. 1985, J. Exp. Med., 162, 1745.
49. Long, E. O. 1999, Annu. Rev. Immunol., 17, 875.
50. Lanier, L. L. 1998, Annu. Rev. Immunol., 16, 359.
51. Hoglund, P. and Brodin, P. 2010, Nat. Rev. Immunol., 10, 724.
52. Binstadt, B. A., Billadeau, D. D., Jevremovic, D., Williams, B. L., Fang, N., Yi, T., Koretzky, G. A., Abraham, R. T., and Leibson, P. J. 1998, J. Biol. Chem., 273, 27518.
53. Binstadt, B. A., Brumbaugh, K. M., Dick, C. J., Scharenberg, A. M., Williams, B. L., Colonna, M., Lanier, L. L., Kinet, J. P., Abraham, R. T., and Leibson, P. J. 1996, Immunity, 5, 629.

54. Lou, Z., Jevremovic, D., Billadeau, D. D., and Leibson, P. J. 2000, J. Exp. Med., 191, 347.
55. Lowin-Kropf, B., Kunz, B., Beermann, F., and Held, W. 2000, J. Immunol., 165, 1314.
56. Liao, N. S., Bix, M., Zijlstra, M., Jaenisch, R., and Raulet, D. 1991, Science, 253, 199.
57. Colonna, M. and Samaridis, J. 1995, Science, 268, 405.
58. Apps, R., Gardner, L., and Moffett, A. 2008, Trends Immunol., 29, 313.
59. Kim, S., Poursine-Laurent, J., Truscott, S. M., Lybarger, L., Song, Y. J., Yang, L., French, A. R., Sunwoo, J. B., Lemieux, S., Hansen, T. H., and Yokoyama, W. M. 2005, Nature, 436, 709.
60. Fernandez, N. C., Treiner, E., Vance, R. E., Jamieson, A. M., Lemieux, S., and Raulet, D. H. 2005, Blood, 105, 4416.
61. Anfossi, N., Andre, P., Guia, S., Falk, C. S., Roetynck, S., Stewart, C. A., Breso, V., Frassati, C., Reviron, D., Middleton, D., Romagne, F., Ugolini, S., and Vivier, E. 2006, Immunity, 25, 331.
62. Sun, K., Alvarez, M., Ames, E., Barao, I., Chen, M., Longo, D. L., Redelman, D., and Murphy, W. J. 2012, Blood, 119, 1590.
63. Cooley, S., Xiao, F., Pitt, M., Gleason, M., McCullar, V., Bergemann, T. L., McQueen, K. L., Guethlein, L. A., Parham, P., and Miller, J. S. 2007, Blood, 110, 578.
64. Joncker, N. T., Fernandez, N. C., Treiner, E., Vivier, E., and Raulet, D. H. 2009, J. Immunol., 182, 4572.
65. Brodin, P., Lakshmikanth, T., Johansson, S., Karre, K., and Hoglund, P. 2009, Blood, 113, 2434.
66. Orr, M. T., Murphy, W. J., and Lanier, L. L. 2010, Nat. Immunol., 11, 321.
67. Venstrom, J. M., Zheng, J., Noor, N., Danis, K. E., Yeh, A. W., Cheung, I. Y., Dupont, B., O'Reilly, R. J., Cheung, N. K., and Hsu, K. C. 2009, Clin. Cancer Res., 15, 7330.
68. Yu, J., Venstrom, J. M., Liu, X. R., Pring, J., Hasan, R. S., O'Reilly, R. J., and Hsu, K. C. 2009, Blood, 113, 3875.
69. Barao, I., Alvarez, M., Ames, E., Orr, M. T., Stefanski, H. E., Blazar, B. R., Lanier, L. L., Anderson, S. K., Redelman, D., and Murphy, W. J. 2011, Blood, 117, 7032.
70. Bryceson, Y. T., Chiang, S. C., Darmanin, S., Fauriat, C., Schlums, H., Theorell, J., and Wood, S. M. 2011, J. Innate Immun., 3, 216.
71. Lanier, L. L. 2009, Immunol. Rev., 227, 150.
72. Guerra, N., Tan, Y. X., Joncker, N. T., Choy, A., Gallardo, F., Xiong, N., Knoblaugh, S., Cado, D., Greenberg, N. M., and Raulet, D. H. 2008, Immunity, 28, 571.
73. George, T. C., Mason, L. H., Ortaldo, J. R., Kumar, V., and Bennett, M. 1999, J. Immunol., 162, 2035.
74. Nakamura, M. C., Linnemeyer, P. A., Niemi, E. C., Mason, L. H., Ortaldo, J. R., Ryan, J. C., and Seaman, W. E. 1999, J. Exp. Med., 189, 493.
75. Dimasi, N. and Biassoni, R. 2005, Immunol. Cell Biol., 83, 1.
76. Gasser, S., Orsulic, S., Brown, E. J., and Raulet, D. H. 2005, Nature, 436, 1186.
77. Burke, S., Lakshmikanth, T., Colucci, F., and Carbone, E. 2010, Trends Immunol., 31, 339.
78. Pende, D., Parolini, S., Pessino, A., Sivori, S., Augugliaro, R., Morelli, L., Marcenaro, E., Accame, L., Malaspina, A., Biassoni, R., Bottino, C., Moretta, L., and Moretta, A. 1999, J. Exp. Med., 190, 1505.
79. Sivori, S., Pende, D., Bottino, C., Marcenaro, E., Pessino, A., Biassoni, R., Moretta, L., and Moretta, A. 1999, Eur. J. Immunol., 29, 1656.
80. Mason, L. H., Willette-Brown, J., Mason, A. T., McVicar, D., and Ortaldo, J. R. 2000, J. Immunol., 164, 603.
81. Malmberg, K. J., Bryceson, Y. T., Carlsten, M., Andersson, S., Bjorklund, A., Bjorkstrom, N. K., Baumann, B. C., Fauriat, C., Alici, E., Dilber, M. S., and Ljunggren, H. G. 2008, Cancer Immunol. Immunother., 57, 1541.
82. Pietra, G., Manzini, C., Rivara, S., Vitale, M., Cantoni, C., Petretto, A., Balsamo, M., Conte, R., Benelli, R., Minghelli, S., Solari, N., Gualco, M., Queirolo, P., Moretta, L., and Mingari, M. C. 2012, Cancer Res., 72, 5430.
83. Hanke, T. and Raulet, D. H. 2001, J. Immunol., 166, 3002.

84. Lakshmikanth, T., Burke, S., Ali, T. H., Kimpfler, S., Ursini, F., Ruggeri, L., Capanni, M., Umansky, V., Paschen, A., Sucker, A., Pende, D., Groh, V., Biassoni, R., Hoglund, P., Kato, M., Shibuya, K., Schadendorf, D., Anichini, A., Ferrone, S., Velardi, A., Karre, K., Shibuya, A., Carbone, E., and Colucci, F. 2009, J. Clin. Invest., 119, 1251.
85. Halfteck, G. G., Elboim, M., Gur, C., Achdout, H., Ghadially, H., and Mandelboim, O. 2009, J. Immunol., 182, 2221.
86. Fuchs, A. and Colonna, M. 2006, Semin. Cancer Biol., 16, 359.
87. Reymond, N., Imbert, A. M., Devilard, E., Fabre, S., Chabannon, C., Xerri, L., Farnarier, C., Cantoni, C., Bottino, C., Moretta, A., Dubreuil, P., and Lopez, M. 2004, J. Exp. Med., 199, 1331.
88. Pende, D., Bottino, C., Castriconi, R., Cantoni, C., Marcenaro, S., Rivera, P., Spaggiari, G. M., Dondero, A., Carnemolla, B., Reymond, N., Mingari, M. C., Lopez, M., Moretta, L., and Moretta, A. 2005, Mol. Immunol., 42, 463.
89. Vivier, E., Tomasello, E., Baratin, M., Walzer, T., and Ugolini, S. 2008, Nat. Immunol., 9, 503.
90. Sun, J. C., Ma, A., and Lanier, L. L. 2009, J. Immunol., 183, 2911.
91. Flavell, R. A., Sanjabi, S., Wrzesinski, S. H., and Licona-Limon, P. 2010, Nat. Rev. Immunol., 10, 554.
92. Kopp, H. G., Placke, T., and Salih, H. R. 2009, Cancer Res., 69, 7775.
93. Laouar, Y., Sutterwala, F. S., Gorelik, L., and Flavell, R. A. 2005, Nat. Immunol., 6, 600.
94. Castriconi, R., Cantoni, C., Della Chiesa, M., Vitale, M., Marcenaro, E., Conte, R., Biassoni, R., Bottino, C., Moretta, L., and Moretta, A. 2003, Proc. Natl. Acad. Sci. USA, 100, 4120.
95. Lee, J. C., Lee, K. M., Kim, D. W., and Heo, D. S. 2004, J. Immunol., 172, 7335.
96. O'Garra, A., Barrat, F. J., Castro, A. G., Vicari, A., and Hawrylowicz, C. 2008, Immunol. Rev., 223, 114.
97. Couper, K. N., Blount, D. G., and Riley, E. M. 2008, J. Immunol., 180, 5771.
98. Moore, K. W., de Waal Malefyt, R., Coffman, R. L., and O'Garra, A. 2001, Annu. Rev. Immunol., 19, 683.
99. Chiu, B. C., Stolberg, V. R., and Chensue, S. W. 2008, J. Immunol., 181, 3156.
100. Scott, M. J., Hoth, J. J., Turina, M., Woods, D. R., and Cheadle, W. G. 2006, Cytokine, 33, 79.
101. Stacey, M. A., Marsden, M., Wang, E. C., Wilkinson, G. W., and Humphreys, I. R. 2011, J. Immunol., 187, 2944.
102. Carson, W. E., Lindemann, M. J., Baiocchi, R., Linett, M., Tan, J. C., Chou, C. C., Narula, S., and Caligiuri, M. A. 1995, Blood, 85, 3577.
103. Cai, G., Kastelein, R. A., and Hunter, C. A. 1999, Eur. J. Immunol., 29, 2658.
104. Mocellin, S., Panelli, M., Wang, E., Rossi, C. R., Pilati, P., Nitti, D., Lise, M., and Marincola, F. M. 2004, Genes Immun., 5, 621.
105. Qian, C., Jiang, X., An, H., Yu, Y., Guo, Z., Liu, S., Xu, H., and Cao, X. 2006, Blood, 108, 2307.
106. Lee, S. H., Kim, K. S., Fodil-Cornu, N., Vidal, S. M., and Biron, C. A. 2009, J. Exp. Med., 206, 2235.
107. Dokun, A. O., Kim, S., Smith, H. R., Kang, H. S., Chu, D. T., and Yokoyama, W. M. 2001, Nat. Immunol., 2, 951.
108. Della Chiesa, M., Carlomagno, S., Frumento, G., Balsamo, M., Cantoni, C., Conte, R., Moretta, L., Moretta, A., and Vitale, M. 2006, Blood, 108, 4118.
109. Spaggiari, G. M., Capobianco, A., Abdelrazik, H., Becchetti, F., Mingari, M. C., and Moretta, L. 2008, Blood, 111, 1327.
110. Riccardi, C., Santoni, A., Barlozzari, T., Puccetti, P., and Herberman, R. B. 1980, Int. J. Cancer, 25, 475.
111. Smyth, M. J., Hayakawa, Y., Takeda, K., and Yagita, H. 2002, Nature reviews Cancer, 2, 850.
112. Wu, J. and Lanier, L. L. 2003, Advances in cancer research, 90, 127.
113. Guimaraes, F., Guven, H., Donati, D., Christensson, B., Ljunggren, H. G., Bejarano, M. T., and Dilber, M. S. 2006, Leukemia: Official journal of the Leukemia Society of America, Leukemia Research Fund, UK, 20, 833.

114. Imai, K., Matsuyama, S., Miyake, S., Suga, K., and Nakachi, K. 2000, Lancet., 356, 1795.
115. Pizzolo, G., Trentin, L., Vinante, F., Agostini, C., Zambello, R., Masciarelli, M., Feruglio, C., Dazzi, F., Todeschini, G., Chilosi, M., Veneri, D., Zanotti, R., Bemedetti, F., Perona, G., and Semenzato, G. 1988, British journal of cancer, 58, 368.
116. Archimbaud, E., Thomas, X., Campos, L., Magaud, J. P., Fiere, D., and Dore, J. F. 1991, Leukemia: Official journal of the Leukemia Society of America, Leukemia Research Fund, UK, 5, 967.
117. Archimbaud, E., Bailly, M., and Dore, J. F. 1991, British journal of haematology, 77, 328.
118. Levy, E. M., Roberti, M. P., and Mordoh, J. 2011, J. Biomed. Biotechnol., 2011, 676198.
119. Hsu, K. C., Keever-Taylor, C. A., Wilton, A., Pinto, C., Heller, G., Arkun, K., O'Reilly, R. J., Horowitz, M. M., and Dupont, B. 2005, Blood, 105, 4878.
120. Ruggeri, L., Mancusi, A., Burchielli, E., Aversa, F., Martelli, M. F., and Velardi, A. 2007, Current opinion in oncology, 19, 142.
121. Albertsson, P. A., Basse, P. H., Hokland, M., Goldfarb, R. H., Nagelkerke, J. F., Nannmark, U., and Kuppen, P. J. 2003, Trends Immunol., 24, 603.
122. Esendagli, G., Bruderek, K., Goldmann, T., Busche, A., Branscheid, D., Vollmer, E., and Brandau, S. 2008, Lung Cancer, 59, 32.
123. Iguchi-Manaka, A., Kai, H., Yamashita, Y., Shibata, K., Tahara-Hanaoka, S., Honda, S., Yasui, T., Kikutani, H., Shibuya, K., and Shibuya, A. 2008, J. Exp. Med., 205, 2959.
124. Delahaye, N. F., Rusakiewicz, S., Martins, I., Menard, C., Roux, S., Lyonnet, L., Paul, P., Sarabi, M., Chaput, N., Semeraro, M., Minard-Colin, V., Poirier-Colame, V., Chaba, K., Flament, C., Baud, V., Authier, H., Kerdine-Romer, S., Pallardy, M., Cremer, I., Peaudecerf, L., Rocha, B., Valteau-Couanet, D., Gutierrez, J. C., Nunes, J. A., Commo, F., Bonvalot, S., Ibrahim, N., Terrier, P., Opolon, P., Bottino, C., Moretta, A., Tavernier, J., Rihet, P., Coindre, J. M., Blay, J. Y., Isambert, N., Emile, J. F., Vivier, E., Lecesne, A., Kroemer, G., and Zitvogel, L. 2011, Nat. Med., 17, 700.
125. Wiemann, K., Mittrucker, H. W., Feger, U., Welte, S. A., Yokoyama, W. M., Spies, T., Rammensee, H. G., and Steinle, A. 2005, J. Immunol., 175, 720.
126. El-Sherbiny, Y. M., Meade, J. L., Holmes, T. D., McGonagle, D., Mackie, S. L., Morgan, A. W., Cook, G., Feyler, S., Richards, S. J., Davies, F. E., Morgan, G. J., and Cook, G. P. 2007, Cancer Res., 67, 8444.
127. Romagne, F. and Vivier, E. 2011, F1000 Med. Rep., 3, 9.
128. Cerwenka, A., Bakker, A. B., McClanahan, T., Wagner, J., Wu, J., Phillips, J. H., and Lanier, L. L. 2000, Immunity, 12, 721.
129. Groh, V., Rhinehart, R., Secrist, H., Bauer, S., Grabstein, K. H., and Spies, T. 1999, Proc. Natl. Acad. Sci. USA, 96, 6879.
130. Dunn, C., Chalupny, N. J., Sutherland, C. L., Dosch, S., Sivakumar, P. V., Johnson, D. C., and Cosman, D. 2003, J. Exp. Med., 197, 1427.
131. Schreiber, R. D., Old, L. J., and Smyth, M. J. 2011, Science, 331, 1565.
132. Heinemann, A., Zhao, F., Pechlivanis, S., Eberle, J., Steinle, A., Diederichs, S., Schadendorf, D., and Paschen, A. 2012, Cancer Res., 72, 460.
133. Diefenbach, A., Jensen, E. R., Jamieson, A. M., and Raulet, D. H. 2001, Nature, 413, 165.
134. Cerwenka, A., Baron, J. L., and Lanier, L. L. 2001, Proc. Natl. Acad. Sci. USA, 98, 11521.
135. Carlsten, M., Bjorkstrom, N. K., Norell, H., Bryceson, Y., van Hall, T., Baumann, B. C., Hanson, M., Schedvins, K., Kiessling, R., Ljunggren, H. G., and Malmberg, K. J. 2007, Cancer Res., 67, 1317.
136. Kagi, D., Ledermann, B., Burki, K., Seiler, P., Odermatt, B., Olsen, K. J., Podack, E. R., Zinkernagel, R. M., and Hengartner, H. 1994, Nature, 369, 31.

137. Ortaldo, J. R., Winkler-Pickett, R. T., Nagashima, K., Yagita, H., and Okumura, K. 1992, J. Leukoc Biol., 52, 483.
138. Hallett, W. H., Ames, E., Motarjemi, M., Barao, I., Shanker, A., Tamang, D. L., Sayers, T. J., Hudig, D., and Murphy, W. J. 2008, J. Immunol., 180, 163.
139. Sutlu, T. and Alici, E. 2009, J. Intern. Med., 266, 154.
140. Konjevic, G., Mirjacic Martinovic, K., Jurisic, V., Babovic, N., and Spuzic, I. 2009, Biomarkers: Biochemical indicators of exposure, response, and susceptibility to chemicals, 14, 258.
141. Katou, F., Ohtani, H., Watanabe, Y., Nakayama, T., Yoshie, O., and Hashimoto, K. 2007, Cancer Res., 67, 11195.
142. Kamimura, H., Yamagiwa, S., Tsuchiya, A., Takamura, M., Matsuda, Y., Ohkoshi, S., Inoue, M., Wakai, T., Shirai, Y., Nomoto, M., and Aoyagi, Y. 2012, Journal of hepatology, 56, 381.
143. Elpek, K. G., Rubinstein, M. P., Bellemare-Pelletier, A., Goldrath, A. W., and Turley, S. J. 2010, Proc. Natl. Acad. Sci. USA, 107, 21647.
144. Katz, J. B., Muller, A. J., and Prendergast, G. C. 2008, Immunol. Rev., 222, 206.
145. Romagne, F., Andre, P., Spee, P., Zahn, S., Anfossi, N., Gauthier, L., Capanni, M., Ruggeri, L., Benson, D. M. Jr., Blaser, B. W., Della Chiesa, M., Moretta, A., Vivier, E., Caligiuri, M. A., Velardi, A., and Wagtmann, N. 2009, Blood, 114, 2667.
146. Oppenheim, D. E., Roberts, S. J., Clarke, S. L., Filler, R., Lewis, J. M., Tigelaar, R. E., Girardi, M., and Hayday, A. C. 2005, Nat. Immunol., 6, 928.
147. Arnon, T. I., Markel, G., and Mandelboim, O. 2006, Semin. Cancer Biol., 16, 348.
148. Champsaur, M. and Lanier, L. L. 2010, Immunol. Rev., 235, 267.
149. Chen, Z., Chen, L., Baker, K., Olszak, T., Zeissig, S., Huang, Y. H., Kuo, T. T., Mandelboim, O., Beauchemin, N., Lanier, L. L., and Blumberg, R. S. 2011, J. Exp. Med., 208, 2633.
150. Leung, N., Turbide, C., Olson, M., Marcus, V., Jothy, S., and Beauchemin, N. 2006, Oncogene, 25, 5527.
151. Sivan, S., Suzan, F., Rona, O., Tamar, H., Vivian, B., Tamar, P., Jacob, S., Gal, M., and Michal, L. 2012, Clinical & developmental immunology, 2012, 290536.
152. Mellier, G., Huang, S., Shenoy, K., and Pervaiz, S. 2010, Mol. Aspects Med., 31, 93.
153. Kim, R., Emi, M., Tanabe, K., and Arihiro, K. 2006, Cancer Res., 66, 5527.
154. Benitez, A. C., Dai, Z., Mann, H. H., Reeves, R. S., Margineantu, D. H., Gooley, T. A., Groh, V., and Spies, T. 2011, Proc. Natl. Acad. Sci. USA, 108, 4081.
155. Weiss-Steider, B., Soto-Cruz, I., Martinez-Campos, C. A., and Mendoza-Rincon, J. F. 2011, Journal of experimental & clinical cancer research: CR, 30, 37.
156. Gubbels, J. A., Felder, M., Horibata, S., Belisle, J. A., Kapur, A., Holden, H., Petrie, S., Migneault, M., Rancourt, C., Connor, J. P., and Patankar, M. S. 2010, Molecular cancer, 9, 11.
157. Richards, J. O., Chang, X., Blaser, B. W., Caligiuri, M. A., Zheng, P., and Liu, Y. 2006, Blood, 108, 246.
158. Smith, H. R., Heusel, J. W., Mehta, I. K., Kim, S., Dorner, B. G., Naidenko, O. V., Iizuka, K., Furukawa, H., Beckman, D. L., Pingel, J. T., Scalzo, A. A., Fremont, D. H., and Yokoyama, W. M. 2002, Proc. Natl. Acad. Sci. USA, 99, 8826.
159. Nam, J. S., Terabe, M., Mamura, M., Kang, M. J., Chae, H., Stuelten, C., Kohn, E., Tang, B., Sabzevari, H., Anver, M. R., Lawrence, S., Danielpour, D., Lonning, S., Berzofsky, J. A., and Wakefield, L. M. 2008, Cancer Res., 68, 3835.
160. Crane, C. A., Han, S. J., Barry, J. J., Ahn, B. J., Lanier, L. L., and Parsa, A. T. 2010, Neuro Oncol., 12, 7.
161. Liau, L. M., Prins, R. M., Kiertscher, S. M., Odesa, S. K., Kremen, T. J., Giovannone, A. J., Lin, J. W., Chute, D. J., Mischel, P. S., Cloughesy, T. F., and Roth, M. D. 2005, Clin. Cancer Res., 11, 5515.
162. Lee, S. K., Kim, J. Y., Jang, B. W., Hur, S. E., Na, B. J., Lee, M., Fukui, A., Gilman-Sachs, A., and Kwak-Kim, J. 2011, Human immunology, 72, 621.

163. Zhou, J., Ding, T., Pan, W., Zhu, L. Y., Li, L., and Zheng, L. 2009, Int. J. Cancer, 125, 1640.
164. Gao, Q., Qiu, S.J., Fan, J., Zhou, J., Wang, X. Y., Xiao, Y. S., Xu, Y., Li, Y. W., and Tang, Z. Y. 2007, J. Clin. Oncol., 25, 2586.
165. Li, M. O., Wan, Y. Y., Sanjabi, S., Robertson, A. K., and Flavell, R. A. 2006, Annu. Rev. Immunol., 24, 99.
166. Hallett, W. H., Ames, E., Alvarez, M., Barao, I., Taylor, P. A., Blazar, B. R., and Murphy, W. J. 2008, Biol. Blood Marrow Transplant, 14, 1088.
167. Barao, I., Hanash, A. M., Hallett, W., Welniak, L. A., Sun, K., Redelman, D., Blazar, B. R., Levy, R. B., and Murphy, W. J. 2006, Proc. Natl. Acad. Sci. USA, 103, 5460.
168. Baessler, T., Charton, J. E., Schmiedel, B. J., Grunebach, F., Krusch, M., Wacker, A., Rammensee, H. G., and Salih, H. R. 2010, Blood, 115, 3058.
169. Fujisaki, H., Kakuda, H., Shimasaki, N., Imai, C., Ma, J., Lockey, T., Eldridge, P., Leung, W. H., and Campana, D. 2009, Cancer Res., 69, 4010.
170. Whiteside, T. L., Chikamatsu, K., Nagashima, S., and Okada, K. 1996, Anticancer research, 16, 2357.
171. Whiteside, T. L., Sung, M. W., Nagashima, S., Chikamatsu, K., Okada, K., and Vujanovic, N. L. 1998, Clin. Cancer Res., 4, 1135.
172. Rosenberg, S. A., Yang, J. C., Topalian, S. L., Schwartzentruber, D. J., Weber, J. S., Parkinson, D. R., Seipp, C. A., Einhorn, J. H., and White, D. E. 1994, JAMA: The journal of the American Medical Association, 271, 907.
173. Kammula, U. S., White, D. E., and Rosenberg, S. A. 1998, Cancer, 83, 797.
174. Kirkwood, J. M., Tarhini, A. A., Panelli, M. C., Moschos, S. J., Zarour, H. M., Butterfield, L. H., and Gogas, H. J. 2008, J. Clin. Oncol., 26, 3445.
175. Miller, J. S., Tessmer-Tuck, J., Pierson, B. A., Weisdorf, D., McGlave, P., Blazar, B. R., Katsanis, E., Verfaillie, C., Lebkowski, J., Radford, J. Jr., and Burns, L. J. 1997, Biol. Blood Marrow Transplant, 3, 34.
176. Buyse, M., Squifflet, P., Lange, B. J., Alonzo, T. A., Larson, R. A., Kolitz, J. E., George, S. L., Bloomfield, C. D., Castaigne, S., Chevret, S., Blaise, D., Maraninchi, D., Lucchesi, K. J., and Burzykowski, T. 2011, Blood, 117, 7007.
177. Romero, A. I., Thoren, F. B., Brune, M., and Hellstrand, K. 2006, British journal of haematology, 132, 91.
178. Steel, J. C., Waldmann, T. A., and Morris, J. C. 2012, Trends in pharmacological sciences, 33, 35.
179. Peace, D. J. and Cheever, M. A. 1989, J. Exp. Med., 169, 161.
180. Burns, L. J., Weisdorf, D. J., DeFor, T. E., Vesole, D. H., Repka, T. L., Blazar, B. R., Burger, S. R., Panoskaltsis-Mortari, A., Keever-Taylor, C. A., Zhang, M. J., and Miller, J. S. 2003, Bone Marrow Transplant, 32, 177.
181. Waldmann, T. A. 2006, Nat. Rev. Immunol., 6, 595.
182. Barao, I., Alvarez, M., Redelman, D., Weiss, J. M., Ortaldo, J. R., Wiltrout, R. H., and Murphy, W. J. 2011, Biol. Blood Marrow Transplant, 17, 1754.
183. Huntington, N. D., Legrand, N., Alves, N. L., Jaron, B., Weijer, K., Plet, A., Corcuff, E., Mortier, E., Jacques, Y., Spits, H., and Di Santo, J. P. 2009, J. Exp. Med., 206, 25.
184. Jakobisiak, M., Golab, J., and Lasek, W. 2011, Cytokine Growth Factor Rev., 22, 99.
185. Ames, E., Hallett, W. H., and Murphy, W. J. 2009, Clinical and experimental immunology, 155, 504.
186. Kandasamy, K. and Kraft, A. S. 2008, Mol. Cancer Ther., 7, 1091.
187. Falschlehner, C., Schaefer, U., and Walczak, H. 2009, Immunology, 127, 145.
188. Soriani, A., Zingoni, A., Cerboni, C., Iannitto, M. L., Ricciardi, M. R., Di Gialleonardo, V., Cippitelli, M., Fionda, C., Petrucci, M. T., Guarini, A., Foa, R., and Santoni, A. 2009, Blood, 113, 3503.
189. Srivastava, S., Lundqvist, A., and Childs, R. W. 2008, Cytotherapy, 10, 775.
190. Huang, B., Sikorski, R., Sampath, P., and Thorne, S. H. 2011, J. Immunother., 34, 289.

191. Coomes, S. M. and Moore, B. B. 2010, Transplantation, 90, 1139.
192. Symann, M., Bosly, A., Gisselbrecht, C., Brice, P., and Franks, C. 1989, Cancer treatment reviews, 16(Suppl A), 15.
193. Vitale, C., Chiossone, L., Morreale, G., Lanino, E., Cottalasso, F., Moretti, S., Dini, G., Moretta, L., and Mingari, M. C. 2004, Eur. J. Immunol., 34, 455.
194. Asai, O., Longo, D. L., Tian, Z. G., Hornung, R. L., Taub, D. D., Ruscetti, F. W., and Murphy, W. J. 1998, J. Clin. Invest., 101, 1835.
195. Porrata, L. F., Ingle, J. N., Litzow, M. R., Geyer, S., and Markovic, S. N. 2001, Bone Marrow Transplant, 28, 865.
196. Porrata, L. F., Inwards, D. J., Lacy, M. Q., and Markovic, S. N. 2001, Bone Marrow Transplant, 28, 673.
197. Olsen, G. A., Gockerman, J. P., Bast, R. C. Jr., Borowitz, M., and Peters, W. P. 1988, Transplantation, 46, 57.
198. Dulphy, N., Haas, P., Busson, M., Belhadj, S., Peffault de Latour, R., Robin, M., Carmagnat, M., Loiseau, P., Tamouza, R., Scieux, C., Rabian, C., Di Santo, J. P., Charron, D., Janin, A., Socie, G., and Toubert, A. 2008, J. Immunol., 181, 2227.
199. Ruggeri, L., Capanni, M., Urbani, E., Perruccio, K., Shlomchik, W. D., Tosti, A., Posati, S., Rogaia, D., Frassoni, F., Aversa, F., Martelli, M. F., and Velardi, A. 2002, Science, 295, 2097.
200. Ruggeri, L., Mancusi, A., Capanni, M., Urbani, E., Carotti, A., Aloisi, T., Stern, M., Pende, D., Perruccio, K., Burchielli, E., Topini, F., Bianchi, E., Aversa, F., Martelli, M. F., and Velardi, A. 2007, Blood, 110, 433.
201. Stern, M., Ruggeri, L., Mancusi, A., Bernardo, M.E., de Angelis, C., Bucher, C., Locatelli, F., Aversa, F., and Velardi, A. 2008, Blood, 112, 2990.
202. Velardi, A. 2008, Curr. Opin. Immunol., 20, 581.
203. Sun, J. Y., Gaidulis, L., Dagis, A., Palmer, J., Rodriguez, R., Miller, M. M., Forman, S. J., and Senitzer, D. 2005, Bone Marrow Transplant, 36, 525.
204. Cooley, S., McCullar, V., Wangen, R., Bergemann, T. L., Spellman, S., Weisdorf, D. J., and Miller, J. S. 2005, Blood, 106, 4370.
205. Hsu, K. C., Gooley, T., Malkki, M., Pinto-Agnello, C., Dupont, B., Bignon, J. D., Bornhauser, M., Christiansen, F., Gratwohl, A., Morishima, Y., Oudshoorn, M., Ringden, O., van Rood, J. J., and Petersdorf, E. 2006, Biol. Blood Marrow Transplant, 12, 828.
206. Sobecks, R. M., Ball, E. J., Maciejewski, J. P., Rybicki, L. A., Brown, S., Kalaycio, M., Pohlman, B., Andresen, S., Theil, K. S., Dean, R., and Bolwell, B. J. 2007, Bone Marrow Transplant, 39, 417.
207. Clausen, J., Wolf, D., Petzer, A. L., Gunsilius, E., Schumacher, P., Kircher, B., Gastl, G., and Nachbaur, D. 2007, Clinical and experimental immunology, 148, 520.
208. Haas, P., Loiseau, P., Tamouza, R., Cayuela, J.M., Moins-Teisserenc, H., Busson, M., Henry, G., Falk, C. S., Charron, D., Socie, G., Toubert, A., and Dulphy, N. 2011, Blood, 117, 1021.
209. Parham, P. and McQueen, K. L. 2003, Nat. Rev. Immunol., 3, 108.
210. Gill, S., Vasey, A. E., De Souza, A., Baker, J., Smith, A. T., Kohrt, H. E., Florek, M., Gibbs, K. D. Jr., Tate, K., Ritchie, D. S., and Negrin, R. S. 2012, Blood, 119, 5758.
211. Miller, J. S., Soignier, Y., Panoskaltsis-Mortari, A., McNearney, S. A., Yun, G. H., Fautsch, S. K., McKenna, D., Le, C., Defor, T. E., Burns, L. J., Orchard, P. J., Blazar, B. R., Wagner, J. E., Slungaard, A., Weisdorf, D. J., Okazaki, I. J., and McGlave, P. B. 2005, Blood, 105, 3051.
212. Iliopoulou, E. G., Kountourakis, P., Karamouzis, M. V., Doufexis, D., Ardavanis, A., Baxevanis, C. N., Rigatos, G., Papamichail, M., and Perez, S. A. 2010, Cancer Immunol. Immunother., 59, 1781.
213. Nguyen, S., Beziat, V., Norol, F., Uzunov, M., Trebeden-Negre, H., Azar, N., Boudifa, A., Bories, D., Debre, P., Vernant, J. P., Vieillard, V., and Dhedin, N. 2011, Transfusion, 51, 1769.

214. Geller, M. A., Cooley, S., Judson, P. L., Ghebre, R., Carson, L. F., Argenta, P. A., Jonson, A. L., Panoskaltsis-Mortari, A., Curtsinger, J., McKenna, D., Dusenbery, K., Bliss, R., Downs, L. S., and Miller, J. S. 2011, Cytotherapy, 13, 98.
215. Benson, D. M. Jr., Bakan, C. E., Zhang, S., Collins, S. M., Liang, J., Srivastava, S., Hofmeister, C. C., Efebera, Y., Andre, P., Romagne, F., Blery, M., Bonnafous, C., Zhang, J., Clever, D., Caligiuri, M. A., and Farag, S. S. 2011, Blood, 118, 6387.
216. Koh, C. Y., Blazar, B. R., George, T., Welniak, L. A., Capitini, C. M., Raziuddin, A., Murphy, W. J., and Bennett, M. 2001, Blood, 97, 3132.
217. Klingemann, H. 2008, Cytotherapy, 10, 225.
218. Suck, G., Oei, V. Y., Linn, Y. C., Ho, S. H., Chu, S., Choong, A., Niam, M., and Koh, M. B. 2011, Exp. Hematol., 39, 904.
219. Denman, C. J., Senyukov, V. V., Somanchi, S. S., Phatarpekar, P. V., Kopp, L. M., Johnson, J. L., Singh, H., Hurton, L., Maiti, S. N., Huls, M. H., Champlin, R. E., Cooper, L. J., and Lee, D. A. 2012, PLoS One, 7, e30264.
220. Caligiuri, M. A., Zmuidzinas, A., Manley, T. J., Levine, H., Smith, K. A., and Ritz, J. 1990, J. Exp. Med., 171, 1509.
221. Cooper, M. A., Fehniger, T. A., and Caligiuri, M. A. 2001, Trends Immunol., 22, 633.
222. Wendt, K., Wilk, E., Buyny, S., Buer, J., Schmidt, R. E., and Jacobs, R. 2006, J. Leukoc Biol., 80, 1529.
223. Juelke, K., Killig, M., Luetke-Eversloh, M., Parente, E., Gruen, J., Morandi, B., Ferlazzo, G., Thiel, A., Schmitt-Knosalla, I., and Romagnani, C. 2010, Blood, 116, 1299.
224. Jacobs, R., Hintzen, G., Kemper, A., Beul, K., Kempf, S., Behrens, G., Sykora, K. W., and Schmidt, R. E. 2001, Eur. J. Immunol., 31, 3121.
225. Caligiuri, M. A., Murray, C., Robertson, M. J., Wang, E., Cochran, K., Cameron, C., Schow, P., Ross, M. E., Klumpp, T. R., Soiffer, R. J., Kendall, A. S., and Ritz, J. 1993, J. Clin. Invest., 91, 123.
226. Moga, E., Canto, E., Vidal, S., Juarez, C., Sierra, J., and Briones, J. 2011, Exp. Hematol., 39, 1064.
227. Terme, M., Ullrich, E., Delahaye, N. F., Chaput, N., and Zitvogel, L. 2008, Nat. Immunol., 9, 486.
228. Khan, K. D., Emmanouilides, C., Benson, D. M. Jr., Hurst, D., Garcia, P., Michelson, G., Milan, S., Ferketich, A. K., Piro, L., Leonard, J. P., Porcu, P., Eisenbeis, C. F., Banks, A. L., Chen, L., Byrd, J. C., and Caligiuri, M. A. 2006, Clin. Cancer Res., 12, 7046.
229. Zhang, T. and Sentman, C. L. 2011, Cancer Res., 71, 2066.
230. Zitvogel, L., Apetoh, L., Ghiringhelli, F., and Kroemer, G. 2008, Nat. Rev. Immunol., 8, 59.
231. O'Leary, J. G., Goodarzi, M., Drayton, D. L., and von Andrian, U. H. 2006, Nat. Immunol., 7, 507.
232. Paust, S., Gill, H. S., Wang, B. Z., Flynn, M. P., Moseman, E. A., Senman, B., Szczepanik, M., Telenti, A., Askenase, P. W., Compans, R. W., and von Andrian, U. H. 2010, Nat. Immunol., 11, 1127.
233. Cooper, M. A., Elliott, J. M., Keyel, P. A., Yang, L., Carrero, J. A., and Yokoyama, W. M. 2009, Proc. Natl. Acad. Sci. USA, 106, 1915.