Published in final edited form as:

Cancer J. 2015 ; 21(6): 486–491. doi:10.1097/PPO.0000000000000156.

Natural Killer Cell Adoptive Transfer Therapy: Exploiting the First Line of Defense Against Cancer

Zachary B. Davis, Ph.D¹, Martin Felices, Ph.D¹, Michael R. Verneris, MD², and Jeffrey S. Miller, MD¹

¹Blood and Marrow Transplant Program, Department of Pediatrics, University of Minnesota, Minneapolis, MN

²Blood and Marrow Transplant Program, Departments of Pediatric, University of Minnesota, Minneapolis, MN

Introduction

Natural killer (NK) cells are a critical component of the innate immune response against malignant cells. At their discovery in the 1970's, they were identified by their ability to kill tumor cells without prior sensitization to tumor antigens^{1–4}. This is distinct from the mechanism by which T-cells lyse tumor cells, which requires recognition of tumor antigens presented in the context of major histocompatibility class I or II by a specific T-cell receptor. Due to the delay in priming and expansion of T-cells bearing a particular tumor antigen specific receptor, NK cells act as a first line of defense against newly transformed cells.

Since NK cells do not require prior exposure to, or recognition of specific tumor antigens, their activity must be highly regulated to limit their responses against healthy tissue. Regulation of their activity occurs at two levels. The first level is through the expression of inhibitory receptors⁵. These germ-line encoded receptors come in three main varieties: 1) killer immunoglobulin-like receptors (KIRs), 2) the c-type lectin, NKG2A/CD94, and 3) leukocyte immunoglobulin-like receptors (LILRs)⁶. The ligands for these various inhibitory receptors are the ubiquitously expressed major histocompatibility class-I molecules (MHC-I). When MHC-I molecules are ligated by NK cell expressed inhibitory receptors, they provide an inhibitory signal to the NK cell that prevents it from becoming activated, blocking degranulation and cytokine production (Figure 1). This mechanism acts as a form of NK cell tolerance and protects healthy tissue from NK cell killing as healthy cells express normal levels of MHC-I molecules. Inhibitory receptor ligation with cognate MHC-I during NK cell development also serves as a mechanism by which NK cells are 'educated' to respond to MHC-I deficient cells⁷. However, this is not the only mechanism by which NK cell activity is regulated.

Natural killer cells express a complement of germ-line encoded activation receptors that include the natural cytotoxicity receptors (NCRs) (e.g., NKp30, NKp44, NKp46, and NKp80, etc.), the c-type lectins, NKG2D and NKG2C/CD94, the SLAM family receptors (e.g., 2B4, NTB-A) and the low affinity Fc receptor, CD16, among others⁸. While some of the ligands to these activation receptors are already present on healthy cells (e.g., CD48, NTB-A), the expression of many of them are induced upon cell stress. Under conditions where activation ligands are present on the cell surface, NK cell responses are dependent on the balance of inhibitory and activating signals^{9–11}. When activation signals outweigh inhibitory ones the NK cell can mediate a response against the target.

NK cells kill tumor targets through a variety of mechanisms. First, they destroy tumor cells through receptor-mediated cytotoxicity. Natural killer cells express a variety of germ-line encoded receptors such as the c-type lectin homodimer, NKG2D, which binds to stress induced ligands (e.g., ULBP's, MICA/MICB) typically expressed on tumor cells^{12, 13}. Upon ligation, NK cells degranulate, releasing perforin and granzymes to induce target cell apoptosis. NK cell degranulation can also be triggered through a process called antibody dependent cell-mediated cytotoxicity (ADCC)¹⁴. This process is dependent on the presence of tumor specific antibodies bound to tumor surface antigens. The Fc portion of these antibodies is bound by the low affinity Fc receptor, CD16, on NK cells and triggers degranulation. NK cells can also mediate target cell killing through death receptor-mediated apoptosis. NK cells can express the tumor-necrosis (TNF) family members FasL or TNF-related apoptosis inducing ligand (TRAIL), which can interact with their respective ligands Fas and TRAIL-receptor (TRAILR) expressed on tumor cells^{15–18}. While NK cells are capable of directly lysing tumor-transformed cells, they can also act as bridge between the innate and adaptive immune responses to enhance recognition and destruction of tumors by adaptive immune cells. This is achieved through the production and secretion of cytokines such as IFN- γ , which can restrict tumor angiogenesis as well as increase MHC-II expression on tumor cells and antigen-presenting cells thereby enhancing adaptive immune responses^{19, 20}.

Despite the mechanisms by which NK cells can recognize and destroy tumor-transformed cells, over the course of tumor progression the malignant cells develop opposing mechanisms through which they subvert or alter immune responses, including those from NK cells. Evasion of the NK cell response by tumor cells is achieved through the down-regulation of adhesion molecules, activation ligands or co-stimulatory molecules for activation receptors, up-regulation of MHC-I molecules or the shedding of soluble activation ligands^{21, 22}. Tumor cells can also effect NK cell function through the secretion of immunosuppressive factors such as IL-10, TGF- β or indoleamine 2,3-dioxygenase (IDO)²³. NK cell defects observed in cancer patients include decreased activation receptor expression, reduced expression of intracellular signaling molecules and over-expression of inhibitory receptors²⁴. Consequently these NK cells exhibit decreased cytotoxic and cytokine secreting functionality as well as decreased proliferation²². Considering NK cells play an important role in the first line of defense against malignantly transformed cells, the use of NK cells in human cancer immunotherapy has been tested in patients with high risk or chemotherapy refractory patients (Table 1) and will be described in this review.

Sources of natural killer cells for adoptive transfer

Autologous NK cells

Initial studies in NK adoptive transfer aimed to improve the autologous NK cell anti-tumor responses through the use of cytokines to enhance NK cell function and proliferation. Stimulation of NK cells with cytokines such as IL-2, IL-12, IL-15, IL-18 and type-I IFN's results in highly activated NK cells with increased expression of adhesion molecules, cytokine induced activating receptors (NKP44), perforin, granzymes, FasL, TRAIL as well as increased proliferation and cytokine production^{25–27}. While up-regulation of these effector molecules results in heightened activity against malignant cells, only limited clinical efficacy were observed in patients treated with the cytokines such as IL-2. Similarly inadequate clinical outcomes were observed with patients that received adoptive transfer of autologous lymphokine activated killer (LAK) cells or more enriched NK cells stimulated ex-vivo in combination IL-2 administration^{28, 29}. More recently we have learned that IL-2 therapy also results in activation and expansion of regulatory T-cells that compete for IL-2 as well as directly inhibits NK cell function and can induce NK activation induced cell death^{30–32}. In addition, cytokine enhancement of autologous NK cell function may still be impaired by inhibition though tumor expressed self-MHC or chronic immunosuppression by the host tumor. For these reasons, the use of haploidentical allogeneic NK cells from related donors were investigated for their efficacy in adoptive transfer.

Allogeneic NK cells

NK cells from healthy, related donors have the benefit of being educated in a non-immunosuppressive environment, and are therefore fully functional. Furthermore, the partial mismatch between the donor KIR repertoire and the recipient complement of MHC-I enable donor NK to respond to malignancies that retain normal MHC-I expression^{33, 34}. Our team at the University of Minnesota performed the first trial for this approach in 2005³⁵. This study included 43 patients with metastatic melanoma, renal cell carcinoma or poor prognosis AML patients. Apheresis was performed on haploidentical related donors to collect peripheral blood leukocytes, which were then depleted of CD3⁺ cells before an overnight stimulation with high-dose IL-2. Before NK cell infusion, patients were treated with a one of three preparatory chemotherapy regimens: high cyclophosphamide and fludarabine (Hi-Cy [60 mg/kg $\times$ 2 days]/Flu [25 mg/m² $\times$ 5 days]), low cyclophosphamide (750 mg/m²) and methylprednisone (1000 mg/m²) or fludarabine alone (25 mg/m² $\times$ 5 days). After infusion, patients received IL-2 infusions daily (1.75 $\times$ 10⁶ U/m²) for 14 days. This was later modified to 6 higher doses of 10 $\times$ 10⁶ U over two weeks. NK cell persistence and expansion was only observed in patients who received the Hi-Cy/Flu preparatory regimen, which is now defined by greater than 100 NK cells/ μ L of blood 12 to 16 days after infusion. There was a clear association between NK expansion and clinical responses and we continue to use this surrogate endpoint. This trial resulted in 30% of poor prognosis AML patients undergoing complete remission. However remission was not durable and the patients that were not well enough to undergo definitive treatment with allogeneic transplantation ultimately relapsed within a year. On a modified protocol, the addition of 400 cGy of total body irradiation was added to the Hi-Cy/Flu regimen in patients with refractory leukemia. The goal of this study was to further deplete host immune cells, thereby limiting the rejection of the transferred NK

cells and creating space for donor NK homeostatic proliferation (Figure 2). While this protocol required stem cell transplantation from the donor, it was done after the NK expansion endpoint. With this approach approximately 50% of patients showed NK cell expansion. Furthermore, 66% of patients with NK cell expansion cleared their leukemia suggesting that the adoptively transferred NK cells played a role in the anti-leukemia responses.

Our current approach at the University of Minnesota combines adoptively transferred NK cells, cytokines and lymphodepleting therapy (Hi-Cy/Flu) to achieve remission in refractory AML patients. As in prior studies, we have used NK cell persistence and *in vivo* expansion as a metric to gauge clinical efficacy as there is a strong correlation between leukemia clearance and the number of donor derived NK cells present 7 and 14 days after transfer. While effective in some cases, this approach could be more robust and several modifications to our current regimen are expected to improve clinical outcomes. Preclinical mouse models of T-reg depletion show enhanced responses to AML with antigen specific T-cell therapies. We piloted this approach by adding an IL-2 immunotoxin (Ontak) in the lymphodepleting chemotherapy preparative regimen. While T-reg depletion was incomplete, we found NK expansion in 30% of patients, which again correlated with higher leukemia clearance than previously observed³⁶. Since IL-2 induces expansion of T-regs, the use of IL-15 in place of IL-2 is an alternate approach to active NK and T cells, without T-reg stimulation and this is currently being tested. In addition, selection of donors with favorable KIR genes of the B haplotype may add additional benefit³⁷ but this requires formal clinical testing.

Induced Pluripotent Stem Cell Derived NK cells (IPS)

While patient and related donors remain the primary sources of NK cells for adoptive transfer, stem cell derived NK cells are an active focus of investigation. Differentiation of mature, functional NK cells can be achieved through the culture of bone-marrow or umbilical cord blood derived CD34⁺ hematopoietic stem cells with IL-2/IL-15 along with allogeneic feeder cells and various growth factors such as stem cell factor and FLT-3L^{38, 39}. These NK cells exhibit an immature phenotype, similar to CD56^{bright} NK cells but show robust cytotoxicity and cytokine production⁴⁰. However, some cells show expression of KIRs and CD16. Additional studies have tested whether IPS cells can be differentiated into hematopoietic stem cells and then into NK cells⁴¹. This method is feasible and functionally, these NK cells seem to be more like the CD56^{dim} population as they mediate ADCC, as well as an effective anti-tumor response in *in vivo* xenogeneic mouse models⁴². Furthermore, IPS derived NK cells can serve as a renewable source of cells for therapy and can be genetically modified to express chimeric antigen receptors (CARs) against specific tumor antigens to enhance their efficacy⁴³.

NK cell Lines

Malignant NK cell lines are another area of investigation for clinical use. The potential benefit of these cell lines is their inability to be inhibited by recipient HLA due to low expression of KIR's or KIR-HLA mismatch in the recipient⁴⁴. The cell lines NK-92, NKL, KHYG-1 and NKG have well documented *in vitro* anti-tumor activity⁴⁴. Recently, the NK-92 cell line has been approved by the FDA for clinical testing in patients with renal cell

carcinoma and advanced malignant melanoma^{12, 45–47}. It is currently the only NK cell line to date that has entered clinical trials, however other established cell lines could be used in a similar manner. For instance, the KHYG-1 cell line exhibits greater cytotoxicity than the NK-92 cell line⁴⁸ and retains its enhanced cytolytic activity even when irradiated to inhibit proliferation⁴⁹. Whether inhibited proliferation is a desirable trait remains to be determined, but will certainly enhance safety. The NKL cell line exhibits functional and proliferative ability similar to the CD56^{dim}CD16⁺ NK cell subset⁵⁰. Their ability to mediate both ADCC and natural cytotoxicity makes them a prime candidate for use in adoptive immunotherapy. The main advantage to the use of NK cell lines is that they can be easily expanded and maintained in vitro under good manufacturing practice conditions for use in immunotherapy. Their function can also be enhanced through the use of cytokines⁴⁵. Thus, adoptive transfer of NK cell lines may prove a useful alternative to the isolation and transfer of autologous or allogeneic NK cells for tumor immunotherapy.

Memory-like NK cells

Recently, a population of NK cells has been identified that expands in response to Cytomegalovirus (CMV) infection⁵¹ and has anti-CMV activity as well as strong function against tumor targets⁵². Identified by their expression of the activation receptor NKG2C/CD94 and the maturation marker CD57, these NK cells have been shown to expand after CMV reactivation in transplant recipients^{53, 54}. We have also shown that these cells are transplantable, persist at least a year post transplant in CMV reactivated recipients and are potent producers of IFN- γ when exposed to K562 cells or through CD16 signaling^{55, 56}. The enhanced function of these cells makes them an area of interest for future use in tumor immunotherapy.

Enhancement of adoptive transfer therapies

Although NK cell adoptive transfers show a great deal of promise in the clinic, complimentary approaches or genetic manipulations geared at maximizing NK cell function, expansion, and persistence post transfer will be needed to move this form of immunotherapy forward. IL-15 alone or as a superagonist with IL-15R α ⁵⁷ to achieve trans-presentation holds great promise and may overcome the Treg stimulatory effects of IL-2. Other cytokines like IL-12 and IL-18 may induce priming of a memory-like NK cell with enhanced anti-tumor activity^{52, 58, 59}. In instances where NK cell adoptive transfers are utilized as a means for maximizing ADCC two strategies are being explored to improve this process, enhancing maintenance of the CD16 receptor and improving targeting through utilization of bi- and tri-specific molecules. NK cell activation, through CD16 or other means, leads to a disintegrin and metalloproteinase-17 (ADAM-17) mediated clipping of the CD16 receptor, which is critical for mediation of ADCC^{60–64}. To counter this matrix metalloproteinase (MMP) driven clipping, and thereby enhance CD16 maintenance and ADCC, ADAM-17 specific inhibitors, as well as broader MMP inhibitors, are being studied^{65, 66}. In vitro success with the ADAM-17 inhibitor has led to an ongoing clinical trial at the University of Minnesota examining the combinational use of the ADAM-17 inhibitor and rituximab in patients with refractory lymphoma. ADCC can be improved through utilization of bi- and tri-specific antibodies that bind directly to CD16, on the NK cell, and one or two tumor antigens, on the

tumor cell^{67, 68}. Direct binding to CD16, instead of CD16 binding to the Fc portion of an antibody bound to a tumor, provides stronger interactions and enhanced cytotoxicity⁶⁹. Our group has recently focused its efforts on smaller, more versatile, bi- and tri-specific molecules termed BiKEs and TriKEs^{70–73}. Incorporation of ADAM-17 inhibitor and/or Bi-specific engagers post adoptive transfer will likely substantially enhance ADCC in this system.

Another way to employ monoclonal antibodies (mAbs) in a post adoptive transfer setting is to use them to block signaling pathways that inhibit NK cell function. The inhibitory killer immunoglobulin-like receptors (KIR) are expressed in mature NK cells and block NK cell function upon encounter with cognate HLA class I ligands. Thus, efforts have been placed in the past to drive KIR-HLA mismatch in order to increase the graft vs. leukemia (GVL) effect in the allogeneic setting, and a blocking antibody is being tested in the clinic to block inhibitory KIR signaling in the autologous setting^{68, 74–77}. Another inhibitory receptor that should be targeted for blockade in the post transfer setting is NKG2A, since this receptor is upregulated post transplant and adoptive transfer of NK cells and is thought to exert a significant amount of inhibition in the graft versus tumor setting^{78, 79}. PD-1/PD-L1 axis blockade also warrants exploration in this setting since its expression is dynamically modulated post NK cell activation and signaling in this pathway has been shown to reduce NK cell anti-tumor effect⁸⁰.

Genetic manipulation of NK cells has great potential for improving NK cell adoptive transfer therapies. Utilizing this strategy, NK cells being transferred in could have enforced expression of genes coding for cytokines needed for expansion and survival (like IL-2, IL-15, and IL-21), chemokine receptors needed for homing of the NK cell to the tumor, co-stimulatory receptors needed for maximization of function, and siRNAs and miRNAs targeting inhibitory NK cell receptors⁸¹. Genetic manipulation of NK cells is also being explored for expression of chimeric antigen receptors (CARs) on the cells being transferred in, similar to approaches being taken with T cells, in order to maximize specific targeting of tumors and NK cell function post activation⁸². While the approach taken will likely depend on the type of cancer and stage at which the disease is detected, these exciting new methodologies are likely to revolutionize adoptive NK cells immunotherapies in the coming years.

Literature Cited

1. Herberman RB, Nunn ME, Holden HT, et al. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors. II. Characterization of effector cells. *International journal of cancer Journal international du cancer*. 1975; 16:230–239. [PubMed: 1080480]
2. Kiessling R, Klein E, Wigzell H. "Natural" killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Specificity and distribution according to genotype. *Eur J Immunol*. 1975; 5:112–117. [PubMed: 1234049]
3. Riccardi C, Santoni A, Barlozzari T, et al. In vivo natural reactivity of mice against tumor cells. *International journal of cancer Journal international du cancer*. 1980; 25:475–486. [PubMed: 6154658]
4. Barlozzari T, Reynolds CW, Herberman RB. In vivo role of natural killer cells: involvement of large granular lymphocytes in the clearance of tumor cells in anti-asialo GM1-treated rats. *J Immunol*. 1983; 131:1024–1027. [PubMed: 6863925]

5. Lanier LL. Follow the leader: NK cell receptors for classical and nonclassical MHC class I. *Cell*. 1998; 92:705–707. [PubMed: 9529246]
6. Lanier LL. Up on the tightrope: natural killer cell activation and inhibition. *Nat Immunol*. 2008; 9:495–502. [PubMed: 18425106]
7. Anfossi N, Andre P, Guia S, et al. Human NK cell education by inhibitory receptors for MHC class I. *Immunity*. 2006; 25:331–342. [PubMed: 16901727]
8. Lanier LL. Activating and inhibitory NK cell receptors. *Adv Exp Med Biol*. 1998; 452:13–18. [PubMed: 9889954]
9. Roder JC, Pross HF. The biology of the human natural killer cell. *J Clin Immunol*. 1982; 2:249–263. [PubMed: 6292259]
10. Cerwenka A, Lanier LL. Natural killer cells, viruses and cancer. *Nat Rev Immunol*. 2001; 1:41–49. [PubMed: 11905813]
11. Miller JS. The biology of natural killer cells in cancer, infection, and pregnancy. *Exp Hematol*. 2001; 29:1157–1168. [PubMed: 11602317]
12. Ljunggren HG, Malmberg KJ. Prospects for the use of NK cells in immunotherapy of human cancer. *Nat Rev Immunol*. 2007; 7:329–339. [PubMed: 17438573]
13. Waldhauer I, Steinle A. NK cells and cancer immunosurveillance. *Oncogene*. 2008; 27:5932–5943. [PubMed: 18836474]
14. Smyth MJ, Hayakawa Y, Takeda K, et al. New aspects of natural-killer-cell surveillance and therapy of cancer. *Nat Rev Cancer*. 2002; 2:850–861. [PubMed: 12415255]
15. Bradley M, Zeytun A, Rafi-Janajreh A, et al. Role of spontaneous and interleukin-2-induced natural killer cell activity in the cytotoxicity and rejection of Fas+ and Fas- tumor cells. *Blood*. 1998; 92:4248–4255. [PubMed: 9834230]
16. Screpanti V, Wallin RP, Ljunggren HG, et al. A central role for death receptor-mediated apoptosis in the rejection of tumors by NK cells. *J Immunol*. 2001; 167:2068–2073. [PubMed: 11489989]
17. Takeda K, Hayakawa Y, Smyth MJ, et al. Involvement of tumor necrosis factor-related apoptosis-inducing ligand in surveillance of tumor metastasis by liver natural killer cells. *Nat Med*. 2001; 7:94–100. [PubMed: 11135622]
18. Cretney E, Takeda K, Yagita H, et al. Increased susceptibility to tumor initiation and metastasis in TNF-related apoptosis-inducing ligand-deficient mice. *J Immunol*. 2002; 168:1356–1361. [PubMed: 11801676]
19. Street SE, Cretney E, Smyth MJ. Perforin and interferon-gamma activities independently control tumor initiation, growth, and metastasis. *Blood*. 2001; 97:192–197. [PubMed: 11133760]
20. Smyth MJ, Crowe NY, Pellicci DG, et al. Sequential production of interferon-gamma by NK1.1(+) T cells and natural killer cells is essential for the antimetastatic effect of alpha-galactosylceramide. *Blood*. 2002; 99:1259–1266. [PubMed: 11830474]
21. Pietra G, Manzini C, Rivara S, et al. Melanoma cells inhibit natural killer cell function by modulating the expression of activating receptors and cytolytic activity. *Cancer Res*. 2012; 72:1407–1415. [PubMed: 22258454]
22. Vitale M, Cantoni C, Pietra G, et al. Effect of tumor cells and tumor microenvironment on NK-cell function. *Eur J Immunol*. 2014; 44:1582–1592. [PubMed: 24777896]
23. Della Chiesa M, Carlomagno S, Frumento G, et al. The tryptophan catabolite L-kynurenine inhibits the surface expression of NKp46- and NKG2D-activating receptors and regulates NK-cell function. *Blood*. 2006; 108:4118–4125. [PubMed: 16902152]
24. Sutlu T, Alici E. Natural killer cell-based immunotherapy in cancer: current insights and future prospects. *J Intern Med*. 2009; 266:154–181. [PubMed: 19614820]
25. Farag SS, Caligiuri MA. Cytokine modulation of the innate immune system in the treatment of leukemia and lymphoma. *Adv Pharmacol*. 2004; 51:295–318. [PubMed: 15464915]
26. Smyth MJ, Cretney E, Kershaw MH, et al. Cytokines in cancer immunity and immunotherapy. *Immunol Rev*. 2004; 202:275–293. [PubMed: 15546400]
27. Becknell B, Caligiuri MA. Interleukin-2, interleukin-15, and their roles in human natural killer cells. *Adv Immunol*. 2005; 86:209–239. [PubMed: 15705423]

28. Rosenberg SA. Interleukin-2 and the development of immunotherapy for the treatment of patients with cancer. *Cancer J Sci Am*. 2000; 6(Suppl 1):S2–S7. [PubMed: 10685650]
29. Burns LJ, Weisdorf DJ, DeFor TE, et al. IL-2-based immunotherapy after autologous transplantation for lymphoma and breast cancer induces immune activation and cytokine release: a phase I/II trial. *Bone Marrow Transplant*. 2003; 32:177–186. [PubMed: 12838283]
30. Ghiringhelli F, Menard C, Terme M, et al. CD4+CD25+ regulatory T cells inhibit natural killer cell functions in a transforming growth factor-beta-dependent manner. *The Journal of experimental medicine*. 2005; 202:1075–1085. [PubMed: 16230475]
31. Rodella L, Zamaï L, Rezzani R, et al. Interleukin 2 and interleukin 15 differentially predispose natural killer cells to apoptosis mediated by endothelial and tumour cells. *Br J Haematol*. 2001; 115:442–450. [PubMed: 11703348]
32. Verneris MR. Natural killer cells and regulatory T cells: how to manipulate a graft for optimal GVL. *Hematology Am Soc Hematol Educ Program*. 2013; 2013:335–341. [PubMed: 24319201]
33. Ruggeri L, Capanni M, Urbani E, et al. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. *Science*. 2002; 295:2097–2100. [PubMed: 11896281]
34. Ruggeri L, Mancusi A, Perruccio K, et al. Natural killer cell alloreactivity for leukemia therapy. *J Immunother*. 2005; 28:175–182. [PubMed: 15838373]
35. Miller JS, Soignier Y, Panoskaltsis-Mortari A, et al. Successful adoptive transfer and in vivo expansion of human haploidentical NK cells in patients with cancer. *Blood*. 2005; 105:3051–3057. [PubMed: 15632206]
36. Bachanova V, Cooley S, DeFor TE, et al. Clearance of acute myeloid leukemia by haploidentical natural killer cells is improved using IL-2 diphtheria toxin fusion protein. *Blood*. 2014; 123:3855–3863. [PubMed: 24719405]
37. Cooley S, Trachtenberg E, Bergemann TL, et al. Donors with group B KIR haplotypes improve relapse-free survival after unrelated hematopoietic cell transplantation for acute myelogenous leukemia. *Blood*. 2009; 113:726–732. [PubMed: 18945962]
38. Miller JS, Alley KA, McGlave P. Differentiation of natural killer (NK) cells from human primitive marrow progenitors in a stroma-based long-term culture system: identification of a CD34+7+ NK progenitor. *Blood*. 1994; 83:2594–2601. [PubMed: 7513206]
39. Mrozek E, Anderson P, Caligiuri MA. Role of interleukin-15 in the development of human CD56+ natural killer cells from CD34+ hematopoietic progenitor cells. *Blood*. 1996; 87:2632–2640. [PubMed: 8639878]
40. Grzywacz B, Kataria N, Kataria N, et al. Natural killer-cell differentiation by myeloid progenitors. *Blood*. 2011; 117:3548–3558. [PubMed: 21173117]
41. Woll PS, Martin CH, Miller JS, et al. Human embryonic stem cell-derived NK cells acquire functional receptors and cytolytic activity. *J Immunol*. 2005; 175:5095–5103. [PubMed: 16210613]
42. Woll PS, Grzywacz B, Tian X, et al. Human embryonic stem cells differentiate into a homogeneous population of natural killer cells with potent in vivo antitumor activity. *Blood*. 2009; 113:6094–6101. [PubMed: 19365083]
43. Knorr DA, Kaufman DS. Pluripotent stem cell-derived natural killer cells for cancer therapy. *Transl Res*. 2010; 156:147–154. [PubMed: 20801411]
44. Cheng M, Zhang J, Jiang W, et al. Natural killer cell lines in tumor immunotherapy. *Front Med*. 2012; 6:56–66. [PubMed: 22460449]
45. Tonn T, Becker S, Esser R, et al. Cellular immunotherapy of malignancies using the clonal natural killer cell line NK-92. *J Hematother Stem Cell Res*. 2001; 10:535–544. [PubMed: 11522236]
46. Klingemann HG. Natural killer cell-based immunotherapeutic strategies. *Cytotherapy*. 2005; 7:16–22. [PubMed: 16040380]
47. Malmberg KJ, Bryceson YT, Carlsten M, et al. NK cell-mediated targeting of human cancer and possibilities for new means of immunotherapy. *Cancer Immunol Immunother*. 2008; 57:1541–1552. [PubMed: 18317755]
48. Suck G, Branch DR, Smyth MJ, et al. KHYG-1, a model for the study of enhanced natural killer cell cytotoxicity. *Exp Hematol*. 2005; 33:1160–1171. [PubMed: 16219538]

49. Suck G, Branch DR, Keating A. Irradiated KHYG-1 retains cytotoxicity: potential for adoptive immunotherapy with a natural killer cell line. *Int J Radiat Biol.* 2006; 82:355–361. [PubMed: 16782653]
50. Robertson MJ, Cochran KJ, Cameron C, et al. Characterization of a cell line, NKL, derived from an aggressive human natural killer cell leukemia. *Exp Hematol.* 1996; 24:406–415. [PubMed: 8599969]
51. Sun JC, Beilke JN, Lanier LL. Adaptive immune features of natural killer cells. *Nature.* 2009; 457:557–561. [PubMed: 19136945]
52. Romee R, Schneider SE, Leong JW, et al. Cytokine activation induces human memory-like NK cells. *Blood.* 2012; 120:4751–4760. [PubMed: 22983442]
53. Lopez-Verges S, Milush JM, Schwartz BS, et al. Expansion of a unique CD57(+)NKG2Chi natural killer cell subset during acute human cytomegalovirus infection. *Proc Natl Acad Sci U S A.* 2011; 108:14725–14732. [PubMed: 21825173]
54. Foley B, Cooley S, Verneris MR, et al. Human cytomegalovirus (CMV)-induced memory-like NKG2C(+) NK cells are transplantable and expand in vivo in response to recipient CMV antigen. *J Immunol.* 2012; 189:5082–5088. [PubMed: 23077239]
55. Foley B, Cooley S, Verneris MR, et al. Cytomegalovirus reactivation after allogeneic transplantation promotes a lasting increase in educated NKG2C+ natural killer cells with potent function. *Blood.* 2012; 119:2665–2674. [PubMed: 22180440]
56. Davis ZB, Cooley SA, Cichocki F, et al. Adaptive Natural Killer Cell and Killer Cell Immunoglobulin-Like Receptor-Expressing T Cell Responses are Induced by Cytomegalovirus and Are Associated with Protection against Cytomegalovirus Reactivation after Allogeneic Donor Hematopoietic Cell Transplantation. *Biol Blood Marrow Transplant.* 2015
57. Wong HC, Jeng EK, Rhode PR. The IL-15-based superagonist ALT-803 promotes the antigen-independent conversion of memory CD8 T cells into innate-like effector cells with antitumor activity. *Oncoimmunology.* 2013; 2:e26442. [PubMed: 24404427]
58. Ni J, Miller M, Stojanovic A, et al. Sustained effector function of IL-12/15/18-preactivated NK cells against established tumors. *J Exp Med.* 2012; 209:2351–2365. [PubMed: 23209317]
59. Cooper MA, Elliott JM, Keyel PA, et al. Cytokine-induced memory-like natural killer cells. *Proc Natl Acad Sci U S A.* 2009; 106:1915–1919. [PubMed: 19181844]
60. Borrego F, Lopez-Beltran A, Pena J, et al. Downregulation of Fc gamma receptor IIIA alpha (CD16-II) on natural killer cells induced by anti-CD16 mAb is independent of protein tyrosine kinases and protein kinase C. *Cell Immunol.* 1994; 158:208–217. [PubMed: 8087866]
61. Edsparr K, Speetjens FM, Mulder-Stapel A, et al. Effects of IL-2 on MMP expression in freshly isolated human NK cells and the IL-2-independent NK cell line YT. *J Immunother.* 2010; 33:475–481. [PubMed: 20463600]
62. Grzywacz B, Kataria N, Verneris MR. CD56(dim)CD16(+) NK cells downregulate CD16 following target cell induced activation of matrix metalloproteinases. *Leukemia.* 2007; 21:356–359. author reply 359. [PubMed: 17251901]
63. Harrison D, Phillips JH, Lanier LL. Involvement of a metalloprotease in spontaneous and phorbol ester-induced release of natural killer cell-associated Fc gamma RIII (CD16-II). *J Immunol.* 1991; 147:3459–3465. [PubMed: 1834741]
64. Liu Q, Sun Y, Rihn S, et al. Matrix metalloprotease inhibitors restore impaired NK cell-mediated antibody-dependent cellular cytotoxicity in human immunodeficiency virus type 1 infection. *J Virol.* 2009; 83:8705–8712. [PubMed: 19553339]
65. Romee R, Foley B, Lenvik T, et al. NK cell CD16 surface expression and function is regulated by a disintegrin and metalloprotease-17 (ADAM17). *Blood.* 2013; 121:3599–3608. [PubMed: 23487023]
66. Zhou Q, Gil-Krzewska A, Peruzzi G, et al. Matrix metalloproteinases inhibition promotes the polyfunctionality of human natural killer cells in therapeutic antibody-based anti-tumour immunotherapy. *Clin Exp Immunol.* 2013; 173:131–139. [PubMed: 23607800]
67. Ferrini S, Cambiaggi A, Cantoni C, et al. Targeting of T or NK lymphocytes against tumor cells by bispecific monoclonal antibodies: role of different triggering molecules. *Int J Cancer Suppl.* 1992; 7:15–18. [PubMed: 1428398]

68. Foley B, Felices M, Cichocki F, et al. The biology of NK cells and their receptors affects clinical outcomes after hematopoietic cell transplantation (HCT). *Immunol Rev.* 2014; 258:45–63. [PubMed: 24517425]
69. Moore GL, Bautista C, Pong E, et al. A novel bispecific antibody format enables simultaneous bivalent and monovalent co-engagement of distinct target antigens. *MAbs.* 2011; 3:546–557. [PubMed: 22123055]
70. Gleason MK, Ross JA, Warlick ED, et al. CD16×CD33 bispecific killer cell engager (BiKE) activates NK cells against primary MDS and MDSC CD33+ targets. *Blood.* 2014; 123:3016–3026. [PubMed: 24652987]
71. Gleason MK, Verneris MR, Todhunter DA, et al. Bispecific and trispecific killer cell engagers directly activate human NK cells through CD16 signaling and induce cytotoxicity and cytokine production. *Mol Cancer Ther.* 2012; 11:2674–2684. [PubMed: 23075808]
72. Vallera DA, Zhang B, Gleason MK, et al. Heterodimeric bispecific single-chain variable-fragment antibodies against EpCAM and CD16 induce effective antibody-dependent cellular cytotoxicity against human carcinoma cells. *Cancer Biother Radiopharm.* 2013; 28:274–282. [PubMed: 23611188]
73. Wiernik A, Foley B, Zhang B, et al. Targeting natural killer cells to acute myeloid leukemia in vitro with a CD16 × 33 bispecific killer cell engager and ADAM17 inhibition. *Clin Cancer Res.* 2013; 19:3844–3855. [PubMed: 23690482]
74. Benson DM Jr, Cohen AD, Jagannath S, et al. A Phase I Trial of the Anti-KIR Antibody IPH2101 and Lenalidomide in Patients with Relapsed/Refractory Multiple Myeloma. *Clin Cancer Res.* 2015
75. Korde N, Carlsten M, Lee MJ, et al. A phase II trial of pan-KIR2D blockade with IPH2101 in smoldering multiple myeloma. *Haematologica.* 2014; 99:e81–e83. [PubMed: 24658821]
76. Benson DM Jr, Hofmeister CC, Padmanabhan S, et al. A phase 1 trial of the anti-KIR antibody IPH2101 in patients with relapsed/refractory multiple myeloma. *Blood.* 2012; 120:4324–4333. [PubMed: 23033266]
77. Benson DM Jr, Bakan CE, Zhang S, et al. IPH2101, a novel anti-inhibitory KIR antibody, and lenalidomide combine to enhance the natural killer cell versus multiple myeloma effect. *Blood.* 2011; 118:6387–6391. [PubMed: 22031859]
78. Nguyen S, Dhedin N, Vernant JP, et al. NK-cell reconstitution after haploidentical hematopoietic stem-cell transplantations: immaturity of NK cells and inhibitory effect of NKG2A override GvL effect. *Blood.* 2005; 105:4135–4142. [PubMed: 15687235]
79. Godal R, Bachanova V, Gleason M, et al. Natural killer cell killing of acute myelogenous leukemia and acute lymphoblastic leukemia blasts by killer cell immunoglobulin-like receptor-negative natural killer cells after NKG2A and LIR-1 blockade. *Biol Blood Marrow Transplant.* 2010; 16:612–621. [PubMed: 20139023]
80. Benson DM Jr, Bakan CE, Mishra A, et al. The PD-1/PD-L1 axis modulates the natural killer cell versus multiple myeloma effect: a therapeutic target for CT-011, a novel monoclonal anti-PD-1 antibody. *Blood.* 2010; 116:2286–2294. [PubMed: 20460501]
81. Carlsten M, Childs RW. Genetic Manipulation of NK Cells for Cancer Immunotherapy: Techniques and Clinical Implications. *Front Immunol.* 2015; 6:266. [PubMed: 26113846]
82. Hermanson DL, Kaufman DS. Utilizing chimeric antigen receptors to direct natural killer cell activity. *Front Immunol.* 2015; 6:195. [PubMed: 25972867]

Figure 1. Regulation of NK cell responses

A) Expression of self-MHC and lack of activation ligands (stress induced) by healthy cells results in a dominant inhibitory signal preventing NK killing of normal cells. **B)** Tumor transformation may result in decreased MHC-I expression and increased expression of cell stress induced activation ligands. The expression of activation ligands and down-modulation of MHC-I results in a dominant activation signal resulting in NK killing of the tumor target.

Figure 2. Schema for adoptive transfer of haploidentical NK cells

Lymphocytes are apheresed from a healthy donor with a haploidentical HLA type to the recipient and depleted of B- and T-cells to minimize a graft vs. host disease. NK cells are then activated *ex vivo* with cytokines to enhance function prior to adoptive transfer. Before transplantation, the recipient undergoes a course of lymphodepleting chemotherapy to make space for the adoptively transferred cells. Activated donor NK cells are then infused and the recipient is treated with IL-2 or IL-15 to encourage donor cell expansion.

Table 1

Selected NK adoptive transfer clinical trials

Disease	Status	Trial Phase	Cell Product	Combined Therapy	ClinicalTrials.gov Identifier
Advanced melanoma and renal cell carcinoma	Completed	Phase 2	Autologous NK Cells	IL-2 + Lymphodepleting Chemotherapy	NCT00328861
Acute myeloid leukemia	Ongoing	Phase 1	NK-92 (Neukoplast™)	None	NCT00900809
Acute myeloid leukemia	Ongoing	Phase 1/2	<i>Ex vivo</i> stimulated mIL-21 allogeneic NK	G-CSF or Lymphodepleting Chemotherapy	NCT01787474
Lymphoma and solid tumors	Completed	Phase 1	<i>Ex vivo</i> expanded allogeneic NK cells	None	NCT01212341
Non-Hodgkins lymphoma and chronic lymphocytic leukemia	Ongoing	Phase 2	Allogeneic NK cells	Rituximab + IL-2 + Lymphodepleting Chemotherapy	NCT01181258
Non-B lineage hematologic malignancies	Completed	Phase 1	Allogeneic NK cells	Chemotherapy + IL-2	NTC00640796
Her2+ breast and gastric cancer	Ongoing	Phase 1/2	Autologous NK cells	Trastuzumab	NCT02030561
Acute myeloid leukemia	Completed	Phase 2	Allogeneic NK cells	IL-2 + Lymphodepleting Chemotherapy	NCT00274846