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NK Cells in Therapy of Cancer

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ABSTRACT: Natural killer (NK) cells recognize targets stressed by malignant transformation or infection and can be long-lived. They become educated by interacting with major histocompatibility antigen (MHC) class I molecules to gain function to kill targets and produce cytokines. In the clinic, haploidentical NK cells can be adoptively transferred to treat cancer. Persistence and *in vivo* expansion of NK cells depends on lymphodepleting chemotherapy to make space and induce release of endogenous IL-15. *In vivo* expansion is also enhanced by cytokine administration but IL-2 has the down side of stimulating $CD25^{hi}$ regulatory T cells (Tregs). Other limitations to NK-cell therapy include poor *in vivo* survival and lack of specificity. Bispecific or trispecific killer engagers that target CD16 on NK cells to enhance recognition of tumor antigens, and desintegrin and metalloproteinase 17 (ADAM17) inhibition that prevents CD16 shedding after NK-cell activation should promote enhanced killing of cancer with specificity. These are exciting times; more than 35 years after NK cells were initially described, we are exploiting their capacity for clinical therapy.

KEY WORDS: NK cells, adoptive transfer, allogeneic NK cells, cancer

ABBREVIATIONS: ADAM17: a desintegrin and metalloprotease-17; **AML:** acute myeloid leukemia; **BiKEs:** bispecific killer engagers; **CR:** complete remission; **IL:** interleukin; **KIR:** killer immunoglobulin receptors; **MHC:** major histocompatibility antigen; **NK:** natural killer; **Treg:** regulatory T cells.

I. INTRODUCTION

Both innate and adaptive immunity actively prevent neoplastic development in the process called "cancer immunosurveillance." In the early 1970s, Dr. Ronald Herberman and colleagues discovered a new class of lymphocytes that they called natural killer (NK) cells. Subsequently, these investigators were among the first to demonstrate that effector lymphocytes from athymic nude mice are highly reactive against several syngeneic and allogeneic tumors in a ^{51}Chromium-release cytotoxicity assay. They recognized that anti-tumor reactivity was not T-cell dependent.[1,2] Foreign, damaged, malignant and viral transformed cells may lose expression of major histocompatibility antigen (MHC) class I in a process of "loss of self" and, as a result, become "susceptible" to autologous NK-cell killing.[3–5] NK cells are educated by interaction with MHC class I molecules to gain potent function to kill malignantly transformed cells without prior sensitization by either direct cellular cytotoxicity, antibody-mediated cellular killing, or release of potent cytokines, such as interferon gamma (IFN-γ), that lyse susceptible targets.[6–8] We now better understand that NK cells can be long-lived and can remember past exposures.[9–12] Harnessing NK cells to treat cancer has evolved over the last 30 years to extend from strategies for unleashing autologous NK cells cytotoxicity by exogenous cytokines and activators to adoptive transfer of autologous or allogeneic NK cells.

The initial application of autologous NK-cell–enriched cellular products to treat cancer was pioneered at the National Cancer Institute in 1980.[13] Basic principles postulated in these trials laid the foundation for the adoptive cell therapy field. The potential role of allogeneic NK cells in cancer elimination has been more difficult to demonstrate. The most direct evidence comes from clinical observations following allogeneic donor stem cell transplantation. NK cells rapidly reconstitute after donor stem cell transplantation. In instances where haploidentical donor and recipients were mismatched in KIR–KIR ligands (class I HLA), donor NK cells mediated

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strong anti-leukemia cellular responses capable of protecting patients from leukemia relapse.[14,15] We demonstrated in proof-of-concept clinical studies that adoptively transferred HLA-haploidentical donor NK cells can expand following lympho-depleting chemotherapy and can eliminate chemotherapy-resistant acute myeloid leukemia that leads to clinical remissions.[16] Leukemia clearance correlated with the persistence and *in vivo* expansion of NK cells after adoptive transfer.

In this article, we review our collective experience at the University of Minnesota using NK cells in cancer therapy and present future directions using novel strategies such as the use of bispecific or trispecific killer engagers to simultaneously target CD16 on NK cells and various tumor antigens.[17,18] We also discuss recent strategies related to disintegrin and metaloprotease 17 (ADAM17) protease inhibition, which prevent CD16 shedding after NK cell activation and can promote killing of cancer with specificity.[17–19]

II. AUTOLOGOUS NK CELLS IN CANCER THERAPY

Human NK cell activity is under the control of signals from the killer immunoglobulin receptors (KIR) complex. KIRs are expressed on the NK cell surface and most commonly interact with the MHC class I molecule HLA-Bw4, HLA-C1, and HLA-C2 groups.[20,21] In most circumstances, autologous NK cells are under the dominance of inhibitory signals. NK cell cytotoxicity is triggered by the loss of MHC class I on tumor cells.[21] Under normal homeostatic conditions, a balance of activating and inhibitory signals tightly control NK cell function. Activating NK-cell receptors include natural cytotoxicity receptors NKp30, NKp44, and NK46 and, importantly, NKG2D and DNAM-1, which is constitutively expressed on all NK cells.[22,23] Activating receptors recognize stress-induced molecules, HLA class 1–related MICA and MICB, class I–like cytomegalovirus-homologous ULBP proteins, and ligands CD155 (Poliovirus receptor) and CD112 (Nectin -2), which are expressed on some tumors, making them sensitive to NK-cell–mediated killing.[24] *In vitro,* NK cells can mediate the direct killing of freshly isolated human tumor cells from acute myeloid leukemia, acute lymphoblastic leukemia, multiple myeloma, neuroblastoma, ovarian carcinoma, and colon, renal cell, and gastric carcinomas.[25,26] Many lymphoma tumors express high levels of HLA class I receptors and lack ligands that signal through activating NK-cell receptors. Such tumors may be resistant to the NK-cell–mediated lysis. After incubating NK cells with cytokines, particularly IL-2 or IL-15, NK cells acquire the capacity to lyse a broad array of fresh and cultured tumor targets not normally sensitive to NK lysis, including the Raji lymphoma cell lines.[27] Furthermore, IL-2–activated donor NK cells are synergistic with monoclonal antibodies against resistant cell lines *in vitro* and in mouse xenograft models.

The lymphokine-activated killer-cell infusions first tested were autologous peripheral blood mononuclear cells exogenously stimulated with IL-2 *in vitro*–induced killer cells. Subsequently, autologous tumor-infiltrating lymphocytes and tumor-specific cytotoxic T-cells were infused following lympho-depleting chemotherapy.[13] Meaningful responses were observed with tumor-infiltrating lymphocytes in few patients with melanoma. However, the importance of these early clinical observations lay in critical lessons learned: (1) High-dose IL-2 used *in vivo* with the aim of activating NK cells has unacceptable toxicity owing to severe capillary leak syndrome. (2) Low-dose subcutaneous IL-2 with and without autologous LAK cells is well tolerated. (3) Lympho-depleting chemotherapy combining high-dose cyclophosphamide and fludarabine leads to clearing of space and allows for *in vivo* expansion of autologous adoptively transferred cytotoxic T lymphocytes, leading to enhanced efficacy. Lymphopenia (or clearing space) changes the competitive balance between transferred lymphocytes and endogenous lymphocytes. Alternatively, lymphopenia induces survival factors or depletes inhibitory effects (cells or soluble factors).

In three clinical trials at the University of Minnesota, we tested use of *ex vivo* IL-2–activated autologous NK cells followed by daily subcutaneous IL-2 in patients with a variety of malignancies, including

non-Hodgkin's lymphoma and renal cell carcinoma.[28] Final analysis of the phase II studies using autologous NK cells failed to demonstrate efficacy. The results did, however, lead to the following important findings: (1) IL-2 can be administered safely. (2) IL-2 can induce an increase in circulating cytotoxic lymphocytes with a disproportionate increase in NK cells. (3) Recipients' lymphocytes can compete for cytokines and "space." (4) Autologous NK cells are inhibited by self-MHC. (5) Tumor-induced immunosupression of host immunity interferes with NK function. (6) Low-dose IL-2 stimulates host regulatory T cells (Tregs).

Following the discovery of inhibitory KIR and our evolving understanding of NK licensing and the role HLA class 1 plays in this process, we and others began to investigate the possibility of using allogeneic NK cells as opposed to autologous NK cells.

III. ALLOGENEIC NK CELLS IN ACUTE MYELOID LEUKEMIA THERAPY

Recent advances in the understanding of basic NK cell biology has shed light on the processes of NK cell education by which NK cells acquire self-tolerance and "alloreactivity." This developmental mechanism is an adaptive process that NK cells undergo in response to the HLA class 1 environment.[29,30] This "licensing" describes a terminal differentiation step by which NK cells become functionally competent only when they receive an appropriate signal via an inhibitory receptor ligating the cognate self-HLA. Several lines of evidence suggest that functional activity of mature NK cells can be reset when the cells are exposed to changed MHCs and that NK-cell education is a continuous process. These findings are important to NK-cell immunotherapy; they suggest that donor NK cells unlicensed by HLA alleles absent in the donor may become licensed by host HLA alleles, leading to activity of donor NK cells against host tumor cells lacking HLA expression.[31]

In clinical trials using allogeneic T-cell–depleted hematopoietic cell transplantation from haploidentical donors in patients with AML, Rugierri et al. showed that NK cell cytotoxicity is enhanced if KIR–HLA class I mismatch occurs. Remarkably, the potent anti-leukemia responses delivered by allogeneic donor-derived NK cells were not associated with graft-versus-host disease.[32,33] These observations led us to hypothesize that mature haploidentical NK cells alone without stem cell transplantation could represent a promising tool to achieve anti-tumor responses.

In a trial involving patients with refractory acute myelogenous leukemia, we used a lympho-depleting regimen with high-dose cyclophosphamide (60 mg/kg/day × 2) followed by IV fludarabine 25 mg/m^2/day × 5 days chemotherapy followed by infusion of adoptively transferred HLA-haploidentical NK cells.[16] IL-2 was administered daily (1.75 million units/m^2) for 14 days (subsequently modified to 6 higher doses [10 million units without m^2 correction] for 2 weeks). Administration of the regimen uniformly resulted in lymphopenia and marrow suppression. A marked increase in IL-15 concentration (up to 100 pg dl^{-1}) was detected in patients receiving high-dose cyclophosphamide and fludarabine. In addition, we reported the inverse correlation between absolute lymphocyte count and IL-15 concentration ($r=0.62$, p-value < 0.0001). Data suggest that decreasing numbers of mature lymphocytes, which utilize IL-15, result in elevated plasma IL-15 concentrations. We also found that 26% of poor-prognosis AML patients achieved complete hematologic remission (CR) after NK cell adoptive transfer. The apheresis product was CD3 depleted and activated with IL-2 *in vitro*. Cell processing resulted in a significant reduction of T cells in all products, decreasing from 60% in the apheresis product to 1% after CD3 depletion, yielding a final T-cell dose of $1.5\pm0.3 \times 10^5$ cells kg^{-1}, accompanied by an average of 40-fold less T cells than NK cells. Other components of the final product included monocytes (27%) and B lymphocytes (14%). All patients received subcutaneous IL-2 after infusions. *In vivo* NK-cell expansion was assessed using a PCR-based chimerism assay. Ten percent of the subjects in this trial met criteria for successful NK cell expansion defined as >100 NK cells microL^{-1} in peripheral blood at day 14 after NK-cell infusion.

In subsequent applications of donor NK-cell infusions to treat non-Hodgkin's lymphoma, breast

cancer, and ovarian cancer, we and others have found that host regulatory T cells (Tregs) are resistant to cytotoxic therapy and expand rapidly when IL-2 is administered after NK-cell infusion.[34,35] Tregs are phenotypically distinct $CD4^+CD25^+Foxp3^+$ immunosuppressive lymphocytes residing in lymphoid organs and peripheral blood. In the setting of NK-cell adoptive transfer, however, we hypothesized that host Tregs interfere with NK-cell proliferation and expansion.[36] Because Tregs are uniquely dependent on the high-affinity IL-2 receptor alpha chain (CD25) for their function and survival, IL-2 mediates the strongest proliferative signal for Tregs.

In two subsequent clinical trials, we treated 23 additional AML patients with high dose cyclophosphamide/fludarabine lympho-depleting chemotherapy. Fifteen patients also received IL-2 diphtheria toxin, a recombinant cytotoxic fusion protein composed of the amino acid sequences for diphtheria toxin followed by truncated amino acid sequences for IL-2. We hypothesized that diphtheria toxin would selectively deplete IL-2 receptor (CD25+) expressing cells, including Tregs. Among the 15 patients treated with this regimen, 10 had detectable donor NK cells at day 7 (median 68% donor DNA). At day 14, 27% had successfully expanded NK cells *in vivo*, with median absolute donor-derived NK cell counts of 1000 cells μL^{-1} blood. These results improved our previous rate of 10% *in vivo* NK cell expansion observed with the same regimen but without Treg depletion. The absence of a bona fide Treg population at either day 7 or day 14 correlated with an *in vivo* NK cell expansion at day 14. Augmented lymphocyte and Treg depletion with diphtheria toxin resulted in 53% patients attaining CR, significantly better compared to strategies without diphtheria toxin (CR rate 26%; p=0.02). These outcomes suggest that the NK cells themselves played a role in the antileukemia response over and above the activity of the high-dose chemotherapy preparative regimen. Patients achieving remission also had a significantly higher proportion of circulating donor NK cells, further suggesting that persistence and expansion is required to observe clinical efficacy. However, it is important to recognize that the absolute level of *in vivo* NK cell expansion needed to induce a clinical response remains unknown. Our results suggest that lower donor NK cell levels or donor chimerism for shorter time intervals (e.g., day 7 but not day 14) may be sufficient for clinical efficacy. To address this question, these parameters need to be measured and correlated with clinical response in all donor NK cell trials.

IV. DONOR NK-CELL PRODUCTS

Three different processing methods were used to prepare NK cell products for infusion in AML trials. These included CD3 depletion alone (32 patients), CD3 depletion followed by CD56 selection (10 patients), and single-step CD3/CD19 depletion (15 patients). $CD19^+$ B-cell depletion was added after we observed an episode of severe hemolytic anemia mediated by NK-cell donor passenger B lymphocytes as well as three Epstein-Bar virus lymphoproliferative disease events. Importantly, we observed that the process of CD56 selection resulted in threefold fewer NK cells per product compared with CD3 depletion alone. All clinical products were highly cytotoxic against K562 targets. The highest NK cell doses (mean 26×10^6 NK cells kg^{-1}) were obtained with the CD3/CD19 depletion method, due to the reduced cell loss with the single Good Manufacturing Practice manipulation and extended 5-hour apheresis collection time (data not published). CD19 and CD3 depletion remains a standard in our NK-cell products.

Despite expectations, KIR–ligand mismatch status and KIR genotype did not correlate with clinical efficacy in these trials. This is not necessarily contradictory to other models because NK cells activated by endogenous IL-15 and IL-2 administration may act differently from NK cells after allogeneic transplantation. Moreover, higher serum IL-15 detected after denileukin-containing lympho-depleting chemotherapy correlated strongly with NK-cell expansion, confirming the role of IL-15 cytokine as a key cytokine maintaining NK-cell homeostasis. The most recent advance in allogeneic NK-cell therapy for AML includes an exogenous IL-15, which is currently being tested

in phase 1 dose-escalation trials at the University of Minnesota.

V. ALLOGENEIC NK CELL IN OTHER CANCER THERAPIES

Based on success observed in AML, we are currently testing the adoptively transferred allogeneic NK cells in other malignancies. Ovarian and breast cancer have been shown to be highly sensitive to NK-cell killing *in vitro*.[26] Geller at al. reported 13 patients with advanced chemotherapy-refractory disease.[35] Nine patients had detectable donor NK cells at day 7, but none met the definition of NK cell expansion at day 14. In a subsequent attempt to promote NK-cell expansion and eliminate Treg, we intensified the preparative regimen by adding 200 cGy of total body irradiation to our regimen. Unfortunately, the myeloid suppression and toxicity of this therapy proved to be excessive. Nevertheless, one patient experienced prolonged NK-cell persistence despite interruption of IL-2 therapy and corticosteroid use, suggesting that the expansion of allogeneic NK cells in patients with solid tumor is possible.

In a pilot study, Bachanova et al. evaluated infusion of haploidentical donor NK cells with rituximab and IL-2 for antitumor efficacy in patients with advanced chemotherapy-refractory lymphoma.[34] At 2 months, four patients showed a demonstrable clinical response. We observed evidence of donor DNA in nodal sites, suggesting a tissue-homing capacity of infused NK cells. All patients showed substantial increases in host-regulatory T cells (Treg) after NK-cell and IL-2 therapy (180–80 cells microL^{-1} at day 14 vs. baseline: 58–24 cells microL^{-1}, $p = 0.04$). These data suggest that inadequate immunodepletion and Treg persistence may contribute to a hostile milieu for NK-cell survival and expansion. Our recent collective experience using donor-derived NK cells with IL-2 in solid tumor patients suggests that future cell-therapy trials should incorporate effective strategies to interfere with suppressive elements such as Treg and myeloid suppressor cells.

VI. FUTURE PERSPECTIVES AND PRE-CLINICAL STRATEGIES

Several tumor-targeted antibody strategies have been proposed to enhance NK-cell activity or targeting. These approaches are intended to interrupt NK-cell inhibition, provide co-stimulation, or to enhance targeting through CD16. Each of these strategies has the potential to augment the therapeutic benefit of NK cells and to broaden the impact of their use beyond hematologic malignancies.

The Fc receptor CD16 is present on most peripheral blood NK cells. Upon recognition of antibody-coated tumor cells, CD16 delivers potent activating signals to NK cells, leading to target elimination through direct killing and cytokine production. Our group recently demonstrated that activated NK cells lose Fc receptor gamma (CD16) and homing receptor CD62L that is clipped by disintegrin and metalloprotease-17 (ADAM17).[19] Inhibition of ADAM17 enhanced CD16-mediated NK-cell function by preserving CD16 on the NK-cell surface increased killing of rituximab-coated lymphoma cells. Notably, ADAM family enzymes, including ADAM10 and ADAM17, are highly expressed in lymphoma tumor stroma. Lymphoma-associated stress ligands (e.g., ULB, MICA, MICB, and B7-H6) are also ADAM17 protease targets.[37] These findings demonstrate that novel therapeutic targets, such as ADAM17, can be explored clinically to augment the efficacy of monoclonal antibody-dependent NK-cell tumor-cell killing.

In addition to monoclonal antibodies, we at the University of Minnesota have focused on a platform using bispecific killer engagers (BiKEs) constructed with a single-chain Fv against CD16 and a single-chain Fv against a tumor-associated antigen.[17,18] Using CD16x19 BiKEs and a trispecific CD16x19x22 (TriKE), we have shown that CD16 signaling is potent and delivers a different signal compared with natural recognition of rituximab, especially in regard to cytokine production. One advantage to the BiKE and TriKE platform is its flexibility and ease of production. We have recently developed a CD16x33 BiKE to target myeloid malignancies (AML and myelodysplastic syndrome). One of the

most remarkable properties of this drug is its potent signaling. In refractory acute leukemia, we found that CD16x33 BiKE overcomes inhibitory KIR signaling, leading to potent killing and production of cytokines by NK cells.[17] Interestingly, ADAM17 inhibition enhances CD16x33 BiKE responses against primary AML targets. These immunotherapeutics will be developed for clinical testing for hematologic malignancies and will allow for NK-cell activation via CD16 while approximating NK cells in direct contact with targeted tumor cells.

VII. CONCLUSIONS

The studies discussed here provide clinical evidence of a solid basis for development of future strategies to manipulate NK-cell product, host, and target. The ultimate goal is to enhance the therapeutic benefit of NK-cell–based cancer therapy while minimizing risks and toxicities. Important questions remain to be answered, including determination of minimum NK expansion needed for clinical anti-tumor activity. At present, the success of NK-cell expansion interventions remain unpredictable, particularly for solid tumors in which an immunosuppressive tumor-induced microenvironment dominates and interferes with immune responses. The product characteristics and effective cytokine cocktail proportions vary for different tumor types and patient populations. Future clinical trials will be designed to exploit strategies to overcome the host immune barriers of NK anti-tumor reactivity. Likewise, strategies to exploit favorable donor immunogenetics and NK-cell expansion *ex vivo* from blood, lymphoid progenitors, or pluripotent progenitors will be explored. Limitations to implementation of complex NK-cell therapies include requirements for Good Manufacturing Practice facilities and expertise as well as significant financial resources. Enormous progress has been made in the nearly four decades since Dr. Herberman and his colleagues first identified and characterized the NK cell. Ongoing basic and clinical research is progressively translating into promising approaches for more successful outcomes in the treatment of malignant disease.[38–40]

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REFERENCES

1. Herberman RB, Nunn ME, Holden HT, Lavrin DH. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors. II. characterization of effector cells. Int J Cancer. 1975;16(2):230–9.
2. Herberman RB, Nunn ME, Lavrin DH. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic acid allogeneic tumors. I. distribution of reactivity and specificity. Int J Cancer. 1975;16(2):216–29.
3. Lanier LL. Up on the tightrope: Natural killer cell activation and inhibition. Nat Immunol. 2008;9(5):495–502.
4. Roder JC, Karre K, Kiessling R. Natural killer cells. Prog Allergy. 1981;28:66–159.
5. Ljunggren HG, Karre K. Host resistance directed selectively against H-2-deficient lymphoma variants. analysis of the mechanism. J Exp Med. 1985;162(6):1745–59.
6. Lanier LL, Ruitenberg JJ, Phillips JH. Functional and biochemical analysis of CD16 antigen on natural killer cells and granulocytes. J Immunol. 1988;141(10):3478–85.
7. Pan G, O'Rourke K, Chinnaiyan AM, Gentz R, Ebner R, Ni J, Dixit VM. The receptor for the cytotoxic ligand TRAIL. Science. 1997;276(5309):111–3.
8. Raulet DH, Vance RE. Self-tolerance of natural

killer cells. Nat Rev Immunol. 2006;6(7): 520–31.

9. Lopez-Verges S, Milush JM, Pandey S, York VA, Arakawa-Hoyt J, Pircher H, Norris PJ, Nixon DF, Lanier LL. CD57 defines a functionally distinct population of mature NK cells in the human CD56dimCD16+ NK-cell subset. Blood. 2010;116(19):3865–74.
10. Foley B, Cooley S, Verneris MR, Curtsinger J, Luo X, Waller EK, anasetti C, Weisdorf D, Miller JS. 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(10):5082–8.
11. Sun JC, Lopez-Verges S, Kim CC, DeRisi JL, Lanier LL. NK cells and immune "memory". J Immunol. 2011;186(4):1891–7.
12. Sun JC, Beilke JN, Bezman NA, Lanier LL. Homeostatic proliferation generates long-lived natural killer cells that respond against viral infection. J Exp Med. 2011;208(2):357–68.
13. Rosenberg SA, Lotze MT, Muul LM, Chang AE, Avis FP, Leitman S, Linehan WM, Robertson CN, Lee RE, Rubin JT, Seipp CA, Simpson CG, White DE. A progress report on the treatment of 157 patients with advanced cancer using lymphokine-activated killer cells and interleukin-2 or high-dose interleukin-2 alone. N Engl J Med. 1987;316(15):889–97.
14. Ruggeri L, Capanni M, Urbani E, Perruccio K, Shlomchik WD, Tosti A, Posati S, Rogaia D, Frassoni F, Aversa F, Mertelli MF, Velardi A. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science. 2002;295(5562):2097–100.
15. Ruggeri L, Capanni M, Casucci M, Volpi I, Tosti A, Perruccio K, Urbani E, Negrin RS, Martelli MF, Velardi A. Role of natural killer cell alloreactivity in HLA-mismatched hematopoietic stem cell transplantation. Blood. 1999;94(1):333–9.
16. Miller JS, Soignier Y, Panoskaltsis-Mortari A, McNearney SA, Yun GH, Fautsch SK, McKenna D, Le C, Defor TE, Burns LJ, Orchard PJ, Blazar BR, Wagner JE, Slungaard A, Weisdorf DJ, Okazaki IJ, McGlave PB. Successful adoptive transfer and in vivo expansion of human haploidentical NK cells in patients with cancer. Blood. 2005;105(8): 3051–7.
17. Wiernik A, Foley B, Zhang B, Verneris MR, Warlick E, Gleason MK Ross JA, Luo X, Weisdorf DJ, Walcheck B, Vallera DA, Miller JS. Targeting natural killer cells to acute myeloid leukemia in vitro with a CD16 x 33 bispecific killer cell engager and ADAM17 inhibition. Clin Cancer Res. 2013;19(14):3844–55.
18. Gleason MK, Verneris MR, Todhunter DA, Zhang B, McCullar V, Zhou SX, Panoskaltsis-Mortari A, Weiner LM, Vallera DA, Miller JS. 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(12):2674–84.
19. Romee R, Foley B, Lenvik T, Wang Y, Zhang B, Ankarlo D, Luo X, Cooley S, Verneris M, Walcheck B, Miller J. NK cell CD16 surface expression and function is regulated by a disintegrin and metalloprotease-17 (ADAM17). Blood. 2013;121(18):3599–608.
20. Lanier LL. NK cell recognition. Annu Rev Immunol. 2005;23:225–74.
21. Moretta L, Moretta A. Killer immunoglobulin-like receptors. Curr Opin Immunol. 2004;16(5):626–33.
22. Grzywacz B, Kataria N, Sikora M, Oostendorp RA, Dzierzak EA, Blazar BR, Miller JS, Verneris MR. Coordinated acquisition of inhibitory and activating receptors and functional properties by developing human natural killer cells. Blood. 2006;108(12):3824–33.
23. Rohner A, Langenkamp U, Siegler U, Kalberer CP, Wodnar-Filipowicz A. Differentiation-promoting drugs up-regulate NKG2D ligand expression and enhance the susceptibility of acute myeloid leukemia cells to

natural killer cell-mediated lysis. Leuk Res. 2007;31(10):1393–402.

24. Pende D, Spaggiari GM, Marcenaro S, Martini S, Rivera P, Capobianco A, Flaco M, Lanino E, Pierri I, Zambello R, Bacigalupo A, Mingari MC, Moretta A, Moretta L. Analysis of the receptor-ligand interactions in the natural killer-mediated lysis of freshly isolated myeloid or lymphoblastic leukemias: Evidence for the involvement of the poliovirus receptor (CD155) and nectin-2 (CD112). Blood. 2005;105(5):2066–73.
25. Godal R, Bachanova V, Gleason M, McCullar V, Yun GH, Cooley S, Verneris MR, McGlave PB, Miller JS. 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(5):612–21.
26. Cooley S, Burns LJ, Repka T, Miller JS. Natural killer cell cytotoxicity of breast cancer targets is enhanced by two distinct mechanisms of antibody-dependent cellular cytotoxicity against LFA-3 and HER2/neu. Exp Hematol. 1999;27(10):1533–41.
27. Lopes de Menezes DE, Denis-Mize K, Tang Y, Ye H, Kunich JC, Garrett EN, Peng J,Cousens LS, Gelb AB, Heise C, Wilson SE, Jallal B, Aukerman SL. Recombinant interleukin-2 significantly augments activity of rituximab in human tumor xenograft models of B-cell non-hodgkin lymphoma. J Immunother. 2007;30(1):64–74.
28. Burns LJ, Weisdorf DJ, DeFor TE, Vesole DH, Repka TL, Blazar BR, Burger SR, Panoskaltsis-Mortari A, Keever-Taylor CA, Zhang MJ, Miller JS. 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(2):177–86.
29. Raulet DH. Missing self recognition and self tolerance of natural killer (NK) cells. Semin Immunol. 2006;18(3):145–50.
30. Parham P. Taking license with natural killer cell maturation and repertoire development. Immunol Rev. 2006;214:155–60.
31. Joncker NT, Shifrin N, Delebecque F, Raulet DH. Mature natural killer cells reset their responsiveness when exposed to an altered MHC environment. J Exp Med. 2010;207(10):2065–72.
32. Ruggeri L, Capanni M, Urbani E, Perruccio K, Shlomchik WD, Tosti A, Posati S, Rogaia D, Frassoni F, Aversa F, Martelli MF, Velardi A. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science. 2002;295(5562):2097–100.
33. Velardi A, Ruggeri L, Mancusi A. Killer-cell immunoglobulin-like receptors reactivity and outcome of stem cell transplant. Curr Opin Hematol. 2012;19(4):319–23.
34. Bachanova V, Burns LJ, McKenna DH, Curtsinger J, Panoskaltsis-Mortari A, Lindgren BR, Cooley S, Weisdorf D, Miller JS. Allogeneic natural killer cells for refractory lymphoma. Cancer Immunol Immunother. 2010;59(11):1739–44.
35. Geller MA, Cooley S, Judson PL, Ghebre R, Carson LF, Argenta PA, Jonson AL, Panoskaltsis-Mortari A, Curtsinger J, McKenna D, Dusenbery K, Bliss R, Downs LS, Miller JS. A phase II study of allogeneic natural killer cell therapy to treat patients with recurrent ovarian and breast cancer. Cytotherapy. 2011;13(1):98–107.
36. Simon AK, Jones E, Richards H, Wright K, Betts G, Godkin A, Screaton G, Gallimore A. Regulatory T cells inhibit fas ligand-induced innate and adaptive tumour immunity. Eur J Immunol. 2007;37(3):758–67.
37. Chitadze G, Lettau M, Bhat J, Wesch D, Steinle A, Fürst D, Mytilineos J, Kalthoff H, Janssen O, Oberg HH, Kabelitz D. Shedding of endogenous MHC class I-related chain molecules A and B from different human tumor entities: Heterogeneous involvement of the “a

disintegrin and metalloproteases" 10 and 17. Int J Cancer. 2013;133(7):1557–66.

38. Tolar J, Curtsinger J, McElmurry R, McCullar V, Verneris MR, van Rhee F, Lapteva N, Rooney CM, Wagner JE, Blazar BR, Miller JS., Optimal xenogeneic adoptive transfer of human NK cells: Fresh NK cells and IL-15 administration are superior to frozen NK cells and IL-2. ASH Annual Meeting Abstracts. 2012;120(21):346.
39. Kaufman DS. Toward clinical therapies using hematopoietic cells derived from human pluripotent stem cells. Blood. 2009;114(17):3513–23.
40. Cooley S, Weisdorf DJ, Guethlein LA, Klein JP, Wang T, Le CT, Marsh SG, Geraghty D, Spellman S, Haagenson MD, Ladner M, Trachtenberg E, Parham P, Miller JS. Donor selection for natural killer cell receptor genes leads to superior survival after unrelated transplantation for acute myelogenous leukemia. Blood. 2010;116(14):2411–9.