Therapeutic applications: natural killer cells in the clinic

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Natural killer (NK) cells recognize targets stressed by malignant transformation or infection (particularly CMV). We now know that NK cells can be long-lived and remember past exposures. They become educated by interaction with MHC class I molecules to gain potent function to kill targets and produce cytokines. In the clinical setting, haploidentical NK cells can be transferred adoptively to treat cancer. Persistence and in vivo expansion of NK cells depends on lymphodepleting chemotherapy to make space for the release of endogenous IL-15. In vivo expansion is also enhanced by cytokine administration. IL-2 has been used at low doses to stimulate NK cells in vivo, but has the down side of stimulating CD25^{hi} regulatory T cells. IL-15 is now being tested and has the advantage of avoiding inhibitory regulatory T cell stimulation. In refractory acute myeloid leukemia, leukemia clearance is correlated with the persistence and in vivo expansion of NK cells after adoptive transfer. Limitations to NK cell therapy include poor in vivo survival and lack of specificity. Monoclonal antibodies and bispecific or trispecific killer engagers to target CD16 on NK cells to enhance recognition of various tumor antigens and ADAM17 inhibition to prevent CD16 shedding after NK cell activation should promote enhanced killing of cancer with specificity. Future strategies to exploit favorable donor immunogenetics or to expand NK cells ex vivo from blood, progenitors, or pluripotent progenitors may overcome immune barriers of adoptive transfer and comparative clinical trials will be needed to test these approaches.

Introduction

Natural killer (NK) cell activity was first described in mice in 1964 as activity in which lethally irradiated mice without prior sensitization could resist BM allografts.[1] More than 10 years later, NK cell activity was first detected in blood as non-MHC-restricted cytotoxicity toward transformed or virally infected target cells.[2] Karre et al later proposed the "missing self-hypothesis" to explain these findings such that NK cell cytotoxicity is triggered by the loss of MHC class I on the tumor cells.[3] After this observation, different families of receptors were identified on NK cells that recognize MHC class I to mediate tolerance in the host. Because of their ability to lyse tumors with aberrant MHC class I expression and to produce cytokines and chemokines upon activation, NK cells have great therapeutic potential to treat cancer and enhance the benefits of hematopoietic cell transplantation. Promising data suggest that NK cells are effective at preventing relapse or treating acute myeloid leukemia (AML) and ongoing trials are under way in many other disease settings.

In humans, NK cells express the adhesion marker CD56 and lack the TCR CD3. They are derived from CD34$^+$ progenitor cells in the BM and migrate upon differentiation to lymphoid tissue and peripheral blood. IL-15 is essential for NK cell development and homeostasis because IL-15–knockout mice lack NK cells. Furthermore, IL-15 activity is enhanced when trans-presented by IL-15 receptor alpha on cells such as dendritic cells.[4] Blood NK cells can be divided on the basis of surface density of CD56 into CD56^{bright} and CD56^{dim} NK cells. Resting CD56^{bright} regulatory NK cells are more proliferative, produce high levels of cytokines, and are poor mediators of NK cell natural cytotoxicity. In contrast, CD56^{dim} NK cells are potently cytotoxic and mediate antibody-dependent cellular cytotoxicity (ADCC) through CD16 (FcγRIII) without cytokine activation. NK cells produce a wide variety of cytokines and chemokines such as IFNγ, G-CSF, TNFα, TGF-β, macrophage inflammatory protein 1-beta (MIP-1β), and RANTES. It is still unclear whether the most important effect of NK cells is a result of direct cell killing or if it occurs indirectly through cytokine production to engage other arms of the immune system.

NK cell receptors

Under normal homeostatic conditions, a balance of activating and inhibitory signals tightly control NK cell function. These receptors can be divided into those that recognize class I MHC (classical or nonclassical) and those that are MHC independent. The most clinically relevant family of class I MHC-recognizing NK cell receptors in humans are the inhibitory killer-Ig–like receptors (KIRs) that interact with HLA-Bw4, HLA-C1 and HLA-C2 group ligands. Inhibitory KIRs are transmembrane molecules belonging to the Ig superfamily encoded for on chromosome 19. KIRs with long cytoplasmic tails contain immunoreceptor tyrosine-based inhibitory motifs. KIRs with short cytoplasmic tails result in activating function by association with adaptor molecules. *KIR* gene content can be divided into 2 broad haplotypes, *KIR-A* and *KIR-B*.[5] *KIR-A* haplotypes contain only one activating receptor, KIR2DS4, and are found in roughly one-third of whites in the United States, whereas *KIR-B* haplotypes possess 2 or more activating receptors and are found in two-thirds of whites. The frequency distribution between the *KIR-A* and *KIR-B* haplotypes varies between populations and geographic regions. In addition to content variability, *KIR* genes are highly polymorphic, which determines the affinity with which they bind polymorphic HLA alleles to determine function. Many activating KIR ligands have not been clearly identified, but some may bind their inhibitory counterparts; for example, KIR2DS1 can recognize HLA-C2 but with lower affinity.[6] Mice express analogous class

recognizing Ly49 receptors that also recognize class I MHC, but they do not express KIR.

Both humans and mice encode lectin-like receptors composed of a common subunit, CD94, and a variable NKG2 subunit: NKG2A/B, NKG2C, NKG2E, or NKG2D/F (only in humans). CD94/NKG2A is a dominant inhibitory receptor in humans that binds to the nonclassical MHC HLA-E. As the expression of HLA-E is promoted by binding of peptides clipped from the leader sequence of classical HLA class I molecules, it is thought that HLA-E expression acts as a barometer of classical class I expression. CD94/NKG2C also recognizes HLA-E but is an activating receptor.[7] NKG2D is an activating homodimer that does not associate with CD94 stimulated by MICA and MICB and other non-MHC molecules such as ULBP1, ULBP2, and ULBP3 that are upregulated by during cell stress. The leukocyte Ig-like receptors are also expressed by NK cells and bind classical and nonclassical HLA class I molecules, including HLA-F and HLA-G. NK cell recognition is complex and is determined by a cadre of receptors that are class I independent, such as the natural cytotoxicity receptors (NCR) NKp30, NKp44 and NKp46, CD16, DNAM-1, CD160, NKp80, CD2, and CD244 (2B4). Excellent reviews have been written on these interactions and they will not be covered further here.[8,9]

Acquisition of NK cell function and memory

NK cells can express inhibitory receptors for both self- and non-self MHC class I molecules. To explain how NK cells acquire tolerance to self, several groups of investigators have proposed what is known as NK licensing,[10] NK arming,[11] or NK education.[12] Simply stated, this is the process by which NK cells acquire function through class I–recognizing inhibitory receptors during development. Mechanistically, this is somewhat paradoxical because it follows that inhibitory signals ultimately lead to a gain in function. The best proof of concept for NK cell education in humans is found in NK cells with receptors from an environment where cognate ligand is missing (eg, KIR3DL1$^+$ NK cells from a homozygous Bw6 individual because Bw4 is the ligand for KIR3DL1) or NK cells that lack inhibitory receptors (KIR$^-$NKG2A$^-$) for self-MHCs that are found to be functionally hyporesponsive.[12,13] It has been proposed that NK cells can be dynamically "tuned" by the strength of inhibitory signals[14] that could be determined by the affinity of their KIR alleles for specific class I alleles imparting a high degree of functional diversity by the KIR/MHC interaction.

In addition to education, emerging data have led to studies showing that NK cell function can be modulated by immune memory. Sun et al were the first to demonstrate NK cell–mediated viral immune memory.[15] During CMV infection in mice, NK cells expressing the activating receptor Ly49H preferentially expanded and persisted in high numbers after CMV infection and this response was driven through interaction of Ly49H with the CMV viral protein m157. These NK cells were able to mount a robust response to secondary CMV exposure and had higher constitutive IFNγ transcripts than naive NK cells. By analogy, we and others have shown that human NK cells expressing the activating receptor NKG2C expand after CMV reactivation in transplantation recipients.[16,17] These NKG2C$^+$ NK cells have been shown to express an inhibitory receptor for self-HLA; to progressively acquire CD57, a marker of NK cell maturity; and to have robust function against tumor targets. These memory NK cells are transplantable from CMV-seropositive adult donors, resulting in enhanced function when latent CMV is encountered in a CMV-seropositive recipient.[18]

NK cells in cancer therapeutics

The first trials in humans to harness the antitumor properties of NK cells focused on the use of IL-2 to activate autologous NK cells. Ex vivo IL-2–stimulated cell infusions enhanced recovery of NK cell cytotoxicity in vivo compared with IL-2 administration alone, but efficacy was probably limited by the following: (1) competition with the recipient's lymphocytes for cytokines and "space," (2) autologous NK cell inhibition by self-MHC, (3) chronic immunosuppression induced by the tumor on host immunity, and (4) the realization that low-dose IL-2 stimulated regulatory T cells (Tregs). As inhibitory KIR and their ligands were further characterized, the next approach to using NK cells as immunotherapy focused on allogeneic NK cells from healthy related donors. In this setting, allogeneic NK cells avoid tumor-induced suppression and have the advantage of being educated and fully functional. The first trial of this approach was published by a team from the University of Minnesota in 2005.[19] Forty-three patients with metastatic melanoma, metastatic renal cell carcinoma, or poor-prognosis AML were enrolled in the trial. Peripheral blood was collected by apheresis from haploidentical related donors and CD3 depleted before being incubated overnight in high-dose IL-2. Before NK cell infusion, patients underwent a regimen that involved 3 different chemotherapy preparative regimens: high cyclophosphamide and fludarabine (Hi-Cy/Flu), low cyclophosphamide and methylprednisone, or fludarabine alone. After infusion, patients received IL-2 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). NK cell expansion was only observed for patients receiving the preparatory regimen of Hi-Cy/Flu. Successful NK cell expansion in subsequent trials was then prospectively defined by us as having greater than 50 NK cells/μL of blood 12 to 16 days after infusion. On this initial protocol, 30% of poor-prognosis AML patients achieved a complete remission. However, this remission was not durable and patients ultimately relapsed. Because the lack of NK cell expansion may be the result of host mediated rejection of adoptively transferred cells, the addition of 400 cGy of total body irradiation was added to Hi-Cy/Flu to further deplete host immune cells and to create space for donor NK cells to expand. On this modified protocol that required stem cell transplantation, NK cell expansion was much more successful and 50% of patients achieved measurable expansion based on our current definition. Furthermore, leukemia clearance was observed in 66% of patients, which was higher than patients who did not expand NK cells in vivo, suggesting that the NK cells themselves played a role in the antileukemia response over and above the activity of the high-dose chemotherapy preparative regimen. It should be highlighted that the absolute level of in vivo NK cell expansion needed to induce a clinical response is unknown. It is possible that lower donor NK cell levels or donor chimerism for shorter time intervals (eg, day 7 but not day 14) may be sufficient for clinical efficacy. These parameters need to be measured and correlated with clinical response in all donor NK cell trials to address this question.

Our current strategy at the University of Minnesota is to use NK cells, cytokines, and lymphodepleting chemotherapy (Hi-Cy/Flu) as therapy to achieve remission in patients with refractory AML, a cohort that is generally not eligible for allogeneic transplantation. These trials use donor NK cell persistence and in vivo expansion as a surrogate to improve clinical efficacy given the correlation between leukemia clearance and donor-derived NK cells 7 and 14 days after adoptive transfer. Several modifications to our initial platform are expected to improve results. Building on preclinical data in the mouse, in which Treg depletion with IL-2 diphtheria

toxin (denileukin diftitox) induced enhanced responses to AML-specific T-cell therapy,[20] we piloted this approach as part of our NK cell adoptive transfer strategy. Although Treg precursor depletion was incomplete, NK cell expansion was found in 30% of patients and leukemia clearance was higher than we have seen previously, allowing 50% of patients to move on to best donor allogeneic transplantation.

The use of adoptive transfer of NK cells to treat various malignancies has resulted in mixed results. Shi et al infused haploidentical KIR-mismatched NK cells into 10 patients with relapsed multiple myeloma, which was followed 14 days later with an autologous stem cell graft.[21] Five patients achieved near complete remission. Bachanova et al treated 6 patients with non-Hodgkin lymphoma with infusion of haploidentical NK cells and found that NK cells poorly expanded in vivo and host Tregs were significantly increased after NK cell infusion and IL-2 administration.[22] Similarly, adoptively transferred NK cells failed to expand in patients with breast and ovarian cancers and a similar increase in host Tregs was also observed.[23]

Donor factors to consider that may enhance NK cell adoptive transfer

The importance of donor choice for NK cell–based therapies was first reported in the 2002 study from the Perugia group, who provided evidence that donor-alloreactive NK cells decreased graft rejection, enhanced engraftment, and mediated the GVL effect in the absence of GVHD after mismatched hematopoietic cell transplantation.[24] The potential for NK alloreactivity in the GVH direction was determined using what would become known as the KIR ligand incompatibility. The goal is to choose a donor with a KIR ligand that the recipient lacks so that alloreactive NK cells would not be inhibited in the recipient to mediate potent GVL. Multiple trials with differing results have highlighted the important of transplantation variables that serve as covariates for NK cell reconstitution, such as stem cell dose and source, T-cell depletion, preparative regimen, GVH prophylaxis, HLA match, and disease susceptibility.

Activating KIRs may be involved in the GVL effect as KIR2DS1 expressing alloreactive NK clones have been shown to lyse targets expressing C2 and can override NKG2A-mediated inhibition.[25] In the transplantation setting, Pende et al demonstrated KIR2DS1-mediated lysis of C2 homozygous leukemic blasts and confirmed that NKG2A-inhibitory signaling could be overcome.[26] We reported on a large study of 1409 unrelated AML transplantations evaluating the role of donor and recipient *KIR* genes and found that transplantations from a *KIR-B* haplotype donor (containing more activating KIR) resulted in lower relapse and improved survival.[27] The effect was not seen for patients with acute lymphoblastic leukemia. As described earlier, *KIR-A* haplotypes consist predominately of inhibitory KIRs and only one activating KIR, KIR2DS4. *KIR-B* haplotypes, however, have various activating *KIR* gene content. To isolate which segment of the haplotype may be involved in the protection effects observed, genes with *KIR-B* haplotypes were divided into either centromeric or telomeric regions. Donors homozygous for centromeric *KIR-B* haplotype (*Cen-B*) genes were associated with the lowest level of relapse and highest overall survival. A prospective clinical trial is ongoing in which donors are being selected for *KIR-B* haplotype genes designated into 3 donor groups predicting favorable clinical outcome: best (*Cen-B* homozygous), better (greater than 2 *B*-motifs without *Cen B/B*), and neutral donors (no or 1 *B*-motif). It remains unclear why recipients who receive a graft from a Cen-B homozygous donor have preferable outcomes. Our own preliminary data suggest that donor KIRs interact with HLA in the donor and recipient to modify function so that recipient HLA-C1 content further adds to the benefit of donor *KIR-B* haplotypes compared with recipients who are HLA-C2 homozygous. In addition, Ventrom et al found that the presence of donor KIR2DS1 (a *Tel-B* gene) interacts with HLA-C2 to diminish NK cell alloreactivity, leading to greater relapse.[28]

CMV may also be an important donor factor to potentiate donor NK cell function to eliminate malignant cells. Although CMV causes asymptomatic or mild illness in healthy individuals, for patients who are immunosuppressed due to HIV infection or solid organ/hematopoietic cell transplantation, CMV is a potentially life-threatening complication. CMV remains latent in the host and latent CMV reservoirs have been found in cells of the myeloid lineage and endothelial cells. Recently, CMV reactivation after allogeneic transplantation has been shown to be beneficial. Elmaagacli et al reported that early CMV reactivation is associated with a reduced risk of relapse in AML patients undergoing allogeneic transplantation from HLA-matched sibling or unrelated donors.[29] The risk of leukemic relapse was 9% at 10 years after allogeneic transplantation compared with 42% in patients who did not reactivate CMV; CMV reactivation was not detrimental to overall survival. It is also possible that the lower risk of relapse reported in umbilical cord blood transplantation[30] are associated with CMV reactivation, which is common. In recipients of umbilical cord blood transplantation, CMV reactivation induces a rapid reconstitution of fully functional, educated NK cells with increased survival capacity and the ability to respond rapidly with cytokines.[17] In the absence of CMV reactivation, NK cells remain hyporesponsive and recovery of NK cell effector function does not occur fully for 6 to 12 months.[31]

Much of our knowledge of donor factors comes from studies of allogeneic transplantation. In aggregate, these studies suggest that immunogenetics and KIRs or KIR-ligand differences between the donor and recipient might play a role in adoptive transfer of NK cells. It is also increasingly clear that even distant CMV reactivation leads to long-lived changes in the NK cell repertoire, even in healthy subjects. Although clinical trial data are lacking to support extrapolation from transplantation data definitively, perhaps selection of donors who are CMV positive with *KIR-B* genes into recipients with HLA-C1 or who are KIR ligand (MHC) incompatible will be proven to induce a functionally superior NK cell with clinical advantages for adoptive transfer. It is also possible that NK cell activation after adoptive transfer is functionally different from NK cells reconstituting from stem cell transplantation. These ideas will require clinical testing to understand the role of NK cell donor choice.

Additional donor factors worth highlighting are those related to the NK cell product collection and processing. We have gone through several sequential stages in our fresh overnight IL-2–activated NK cell product. Initially, we started off with a 3-hour apheresis collection and $CD3^+$ T-cell depletion alone. Due to one severe episode of severe hemolytic anemia mediated by NK cell donor passenger B lymphocytes[32] and a few EBV lymphoproliferative disease events (J.S.M., D.H. McKenna, S. Cooley, unpublished data, 2010), $CD19^+$ B-cell depletion is now standard in our NK cell products. Finally, because infusion reactions are transient and easily manageable, we have extended our apheresis to a 5-hour collection to increase donor NK cell doses to 20×10^6/kg, which may in part explain the improved clinical responses seen in AML over time. However, definitive dose correlations with clinical response have not been established. Because our current strategy is to achieve

in vivo NK cell expansion, starting cell doses may be less important as in vivo expansion methods improve. Improved in vivo expansion may allow the possibility of multiple small cell infusions from one apheresis product as prolonged maintenance therapy. In addition, the possibility of third-party "off the shelf" products is being explored with ex vivo–expanded blood NK cells, umbilical cord blood progenitors,[33] and even embryonic stem cells or induced pluripotent stem cells[34] that allow the advantage of unlimited sources of cells to improve the "druggability" of cell therapy.

One strategy to overcome barriers of NK cell adoptive transfer is to simply give more cells obtained by ex vivo expansion with lymphocyte[35] or APC feeders.[36,37] However, it is not known how these cells will compare with fresh activated NK cells tested by our group. To address this issue, we have developed a xenogeneic model of human NK cell transfer in which 10^6 NK cells are given IV to NSG mice after 250 cGy radiation with or without 6 doses of IL-2 or IL-15 intraperitoneally. Ex vivo–expanded NK cells (from Baylor University, made with GMP mIL-15 K562 feeders) were compared directly with fresh activated NK cells (from University of Minnesota, made with IL-2- or IL-15–activated $CD3^-/CD19^-$–enriched NK cells). The kinetics and homing differences of these 2 cell products were striking. We found that ex vivo NK cell expansion changed in vivo persistence early and late after adoptive transfer with a pattern consistent with cytokine addiction (ie, rapid elimination after cytokines were stopped). Specifically, ex vivo NK cells decreased by 90% 1 week after cytokine administration was discontinued, compared with a 45% decrease when fresh activated NK cells were used. It is possible that longer term culture with exposure to higher dose cytokines ex vivo may make cells more sensitive to apoptosis in vivo when cytokine concentrations greatly diminish. This relative dependence on cytokines leads to the concept of "cytokine addiction." Which cell product and what characteristics are needed in vivo to correlate with antitumor efficacy needs to be tested and may be different for different tumor types. Irrespective of the cell product manipulation, in vivo expansion was dependent on cytokines and IL-15 was found to be superior to IL-2 for both cell products. A phase 1 clinical trial using human IL-15 to promote fresh activated donor NK cells is in progress and robust in vivo NK cell expansion has been seen in some patients.

The future: enhancing NK cell function and specificity to eliminate tumors

Despite their discovery more than 40 years ago, new and exciting areas of NK cell biology continue to emerge. The effective use of NK cells to treat cancer will only increase as we further our understanding of how NK cells gain function, how the multitude of receptors expressed by NK cells control function, and how we can exploit this to eliminate tumors. Several tumor-targeted antibody strategies have been proposed to enhance NK cell activity or targeting. These are intended to interrupt NK cell inhibition, provide costimulation, or enhance targeting through CD16. Each of these strategies has the potential to enhance the therapeutic benefit of NK cells and to broaden the impact of their use beyond hematologic malignancies.

NK cells recognize antibody-coated targets through CD16 and mediate ADCC. Rituximab, a monoclonal antibody directed against CD20 on mature B cells, has been used to treat non-Hodgkin lymphoma. Allelic polymorphisms within the CD16 gene have been shown to influence NK cell–mediated ADCC. One such polymorphism is at position 158, a region of the receptor that interacts with the hinge region of IgG antibodies, has either a phenylalanine (F) or valine (V) at this position, and alters NK cell binding.[38] The 158V polymorphism results in higher CD16 binding to IgG. Cartron et al demonstrated that the 158V polymorphism was associated with higher responses to rituximab therapy in patients with follicular non-Hodgkin lymphoma,[39] supporting proof of concept that NK cells are involved with the therapeutic response. Other monoclonal antibodies have been developed that also mediate NK cell ADCC, including trastuzumab (Her2 on breast cancer), alemtuzumab (CD52 on CLL), and cetuximab (EGFR on colorectal cancer).[40] CD16 polymorphisms have also been associated with the efficacy of using other monoclonal antibodies to mediate ADCC.

Because inhibitory KIRs bind to self-HLA class I and inhibit NK cell function, antibodies directed against the inhibitory KIRs may have therapeutic potential. Romagne et al generated a human monoclonal antibody called 1-7F9 that recognizes the inhibitory KIRs KIR2DL1, KIR2DL2, and KIR2DL3, but not KIR3DL1.[41] During preclinical characterization, blocking KIRs with 1-7F9 increased lysis of primary AML blasts. In transgenic mice engineered to express KIR2DL3, HLA-$Cw3^+$ splenocytes were rejected after adding 1-7F9, and in NOD-SCID mice, NK cells lysed autologous tumors when 1-7F9 was added. Current clinical trials are under way to investigate the efficacy of this anti-KIR therapy in humans.

CD137 or 4-1BB is a costimulatory molecule of the TNF receptor family. On resting NK cells, its expression is low and CD16 activation can induce its expression.[42] CD137 can be activated by binding to its natural ligand or can be triggered with a monoclonal antibody against it. Anti-CD137 antibodies have been used in combination with other monoclonal antibodies to increase NK cell activation. Anti-CD137 antibodies in combination with rituximab have been shown to increase degranulation and IFNγ production.[43] Upon engagement of CD16 with rituximab-coated lymphoma cells, CD137 is up-regulated on the NK cell and the addition of an agonist against CD137 increased NK cell–mediated ADCC. A similar effect was also observed using a combination of anti-CD137 and trastuzumab to eliminate breast cancer cells. Other drugs such as lenalidomide, which is currently used in the treatment of multiple myeloma, has also been shown to enhance NK cell–mediated ADCC in combination with rituximab.[44] An alternative to combining drug therapy is to combine NK-stimulating cytokines. IL-2, IL-12, IL-15, and IL-21 have all been shown to enhance NK cell–mediated ADCC.

We have recently identified a novel inhibitory mechanism that dampens CD16 signaling. Cytokine activation and target cell stimulation through activating receptors, including CD16, led to rapid (detected after overnight incubation) and marked decreases in CD16 expression through a shedding mechanism presumed to decrease subsequent ADCC. A disintegrin and metalloprotease-17 (ADAM17) is expressed by NK cells and its selective inhibition abrogated CD16 shedding and led to enhanced IFNγ production, especially when triggering was delivered through CD16.[45] Fc-induced production of cytokines by NK cells exposed to rituximab-coated B-cell targets was further enhanced by ADAM17 inhibition. This supports an important role for targeting ADAM17 to prevent CD16 shedding and to improve the efficacy of therapeutic antibodies. Our findings demonstrate that overactivation of ADAM17 in NK cells may be detrimental to their effector functions by down-regulating surface expression of CD16.

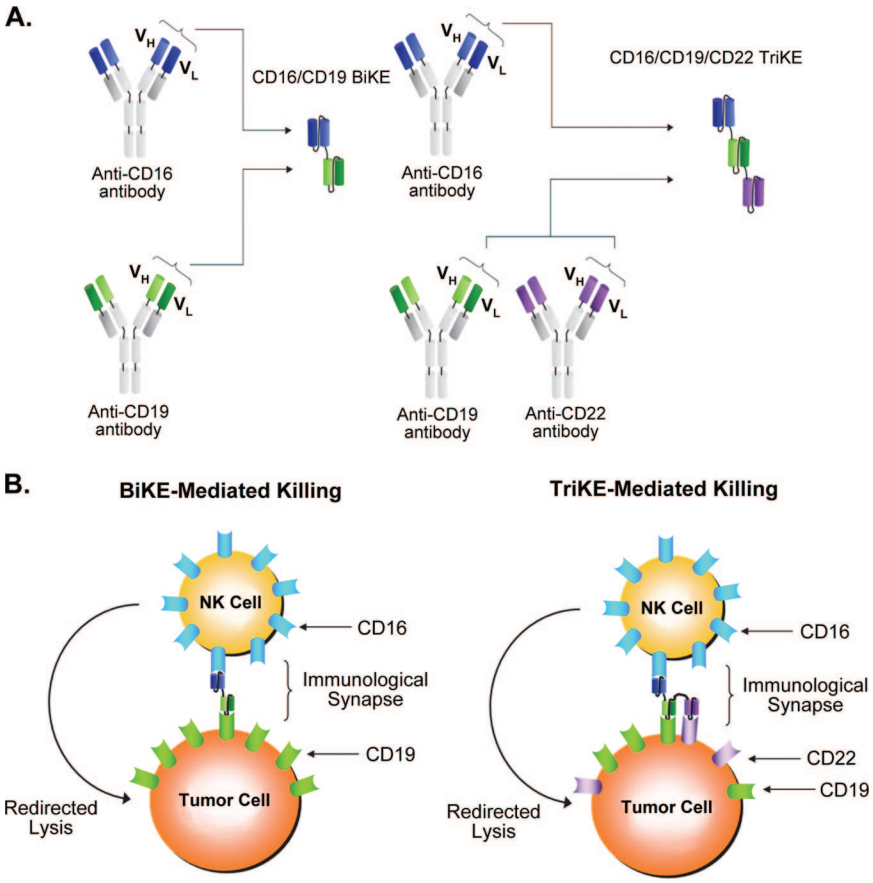

Figure 1. BiKE- and TriKE-mediated NK cell targeting to tumor-associated antigens. (A) BiKEs or TriKEs are generated from the variable single-chain region of a specific monoclonal antibody containing the component capable of recognizing specific antigen. The anti-CD16 component recognizing NK cells can be combined with the single-chain component of 1 or 2 tumor-specific regions to create BiKE- or TriKE-targeted agents, respectively. (B) These drugs specifically enhance the immunologic synapse by specific recognition of tumor-associated antigens and delivery of a potent activating signal through CD16 on NK cells.

In addition to monoclonal antibodies, we 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 (Figure 1). Using CD16x19 BiKEs and a trispecific CD16x19x22 (TriKEs), 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.[46] 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 AML, we found that CD16x33 BiKE overcomes inhibitory KIR signaling, leading to potent killing and production of cytokines by NK cells.[47] Interestingly, ADAM17 inhibition enhances CD16x33 BiKE responses against primary AML targets. When evaluating scenarios in which BiKEs can be used in a minimal residual disease setting, we found that CD16 targeting can be amplified most potently on NK cells that exhibit enhanced maturation by CMV activation early after transplantation, again supporting an important role of CMV in setting the stage for NK cell antitumor activity.

Finally, several agents, such as proteasome inhibitors and histone deacetylase (HDAC), may enhance NK cell recognition of targets. Bortezomib, a proteasome inhibitor, can up-regulate death receptors such as Fas and TRAIL-R2/DR5[48] and induce target apoptosis by NK cells through Fas/FasL and TRAIL/DR5 interactions. Depsipeptide, an HDAC inhibitor, has also been shown to sensitize tumors to NK cell–mediated apoptosis through the up-regulation of death receptors.[49] Another HDAC inhibitor, valproic acid, has been shown to induce expression of ULBP1, MICA, and MICB on AML blasts, increasing NK cell killing.[50] Other targeted therapies will likely have off-target effects to sensitize targets to NK cell killing and should be explored. Overall, the future is exciting for the use of NK cells in cancer therapy, going beyond hematologic malignancy to solid tumors as well. Ultimately, the challenge will be to optimally activate NK cells endogenously without the use of a cell infusion. The novel strategies proposed in this section and a homeostatic drive by IL-15 holds promise to achieve this goal.

Disclosures

Conflict-of-interest disclosure: The author has been on advisory committees for Coronado Biosciences and Celgene. Off-label drug use: IL-2.

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