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PROGRESS IN HEMATOLOGY

Advances in immunotherapy for hematological malignancies

NK cell therapy for hematologic malignancies

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Abstract
Natural killer (NK) cells are part of the innate immune system and represent the first line of defense against infections and tumors. In contrast to T cells, NK cells do not require prior antigen sensitization to induce cytotoxicity and do not cause graft-versus-host disease. These, along with other advantages, make NK cells an attractive candidate for adoptive cellular therapy. Herein, we describe the mechanisms of NK cell cytotoxicity, which is governed by an intricate balance between various activating and inhibitory receptors, including the killer cell immunoglobulin-like receptors (KIRs). We illustrate the advantages of NK alloreactivity as demonstrated in various types of hematopoietic stem cell transplants (HSCT), such as haploidentical, human leukocyte antigen-matched related or unrelated donor and umbilical cord blood transplant. We elaborate on different models used to predict NK cell alloreactivity in these studies, which are either based on the absence of the ligands for inhibitory KIRs, presence of activating NK cell receptors and KIR genes content in donors, or a combination of these. We will review clinical studies demonstrating anti-tumor efficacy of NK cells used either as a stand-alone immunotherapy or as an adjunct to HSCT and novel genetic engineering strategies to improve the anti-tumor activity of NK cells.

Keywords NK cells · Natural killer cells · Adoptive immunotherapy · Immunotherapy · Haploidentical · Cord blood · Hematopoietic stem cell transplant · Stem cell transplant · KIR · KIR mismatch · HLA mismatch

Introduction

Natural killer (NK) cells are very potent effector lymphocytes that can induce cytotoxicity against a vast array of tumors without the need for antigen specificity. They are the first subset of lymphocytes to reconstitute after hematopoietic stem cell transplant (HSCT) [1], and likely play an important role in offering protection against relapse in the early months after transplant. In contrast to T cells, NK cells do not cause graft-versus-host disease (GVHD) in the allogeneic setting; indeed, a number of preclinical studies suggest that they may even protect against GVHD by targeting the recipient's dendritic cells [2–5]. Owing to these unique properties, multiple studies are exploring the role of NK cells in the context of HSCT or as adoptive cellular therapy (ACT). There are multiple potential source of NK cells for adoptive cellular therapy, including bone marrow (BM), peripheral blood (PB), readily available cryopreserved umbilical cord blood (CB), various cells lines, such as the NK-92, KHYG-1 [6, 7], or human embryonic stem cells (hESCs) [8] and induced pluripotent stem cells (iPSCs) [8, 9].

Mechanism of action of NK cells

NK cells are $CD3^-$ and $CD56^+/CD16^+$ large granular lymphocytes that can be classified into two broad subsets—the naïve $CD56^{bright}CD16^{dim}$ cells and the mature $CD56^{dim}CD16^{bright}$ cells which are highly cytotoxic [10]. Contrary to T cells, NK cells do not require prior antigen sensitization or antigen presentation by the major histocompatibility complex (MHC) class I molecules to recognize their targets [11–13]. Their cytotoxicity rather depends upon complex interactions between their various germline-encoded activating and inhibitory receptors and ligands on the surface of target cells. Among these, the NK cell killer immunoglobulin (Ig)-like receptors (KIRs) are most extensively studied. The inhibitory NK cell receptors include various

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KIRs (KIR2DL1, KIR2DL2/L3, KIR2DL4, KIR2DL5A, KIR2DL5B, KIR3DL1, KIR3DL2 and KIR3DL3) [14] and a C-type lectin receptor—NKG2A. Known ligands for some of the inhibitory KIRs are HLA-C1-related alleles (Cw2, Cw5, and Cw6) for KIR2DL2 and KIR2DL3; HLA-C2-related alleles (Cwl, Cw3, Cw7, and Cw8, C*02/04/05/06/12:42/15/16:02/17) for KIR2DL1 and HLA-Bw4 alleles for KIR3DL1 [15–18]. Various activating receptors on NK cells include the natural cytotoxicity receptors (NCRs—NKp30, NKp44 and NKp46), C-type lectin receptors (NKG2C and NKG2D), the DNAX accessory molecule-1 (DNAM-1 or CD226) and the activating KIRs (KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5) [14, 19–27]. In contrast to the inhibitory KIRs, the ligands for activating KIRs are less well understood, except KIR2DS1 and KIR2DS5, both of which recognize HLA-C2-related alleles, albeit with a lower affinity than their inhibitory counterparts [28–31]. KIR2DS2 was initially reported to recognize HLA-A*11—the functional significance of which remains unknown [32], and more recently shown to interact with an as yet unknown β_2-microglobulin-independent ligand on cancer cells [33]. Other NK cell-activating receptors such as NKG2D recognize stress-induced molecules including the MHC class I-related genes (MICA and MICB) and UL16-binding proteins (ULBP) [19, 20, 34].

The interaction of the inhibitory receptors with their ligands (self MCH class I molecules) inhibits NK cell activity. According to the "missing self" hypothesis, if this inhibitory signal is lost or dampened [11, 35, 36], as is the case on tumor cells or virally infected cells, NK cells become predestined to kill [37, 38]. Although "missing self" is critical, it is not sufficient to trigger NK cytotoxicity, which also requires stimulatory signals generated by the ligation of activating receptors [39–41]. Further, the activity of NK cells is also uniquely augmented by the expression of CD16, which is a transmembrane receptor that binds to the Fc portion of IgG on target cells leading to antibody-dependent cellular cytotoxicity (ADCC) [42, 43].

Autologous NK cell immunotherapy

Many early clinical trials initially explored the possibility of expanding and enhancing the anti-tumor activity of the native lymphocytes of patients in vivo simply by giving patients high-dose interleukin-2 (IL-2) [44–50]. The use of high-dose IL-2 led to enormous expansion of NK cells in vivo—up to 2500% or more over baseline [46–50] and enhanced in vitro lytic activity against NK-resistant cell lines [47–49]. The foundation of ACT was laid by the National Cancer Institute group led by Rosenburg et al. in the early 1980s using autologous "lymphokine-activated killer" (LAK) cells generated ex vivo by incubating human PB lymphocytes with IL-2 [51, 52]. The clinical responses to LAK cells infused along with high-dose IL-2 were less than optimal and produced unacceptable IL-2 related toxicities. Nonetheless, responses in some of the end-stage metastatic cancer patients, with some complete remissions (CR), were exciting and generated further interest in the field [51–54]. Thereafter, many studies utilized ex vivo activated/expanded autologous NK cells along with intravenous or subcutaneous low-dose IL-2 [46, 55–57]. Although low-dose IL-2 was better tolerated, responses remained suboptimal, likely due to IL-2-induced expansion of regulatory T cells (T_{regs}) which inhibit NK cell proliferation and function [58], and/or due to the inhibition of autologous NK cells by the self-HLA molecules on the tumor cells. Due to these limitations, the use of allogeneic NK cells was the next logical step for investigators to explore.

Allogeneic NK cell immunotherapy in the setting of HSCT

Allogeneic HSCT creates a unique condition for NK cell alloreactivity by virtue of the "missing-self" phenomenon. As the KIR genes (chromosome 19q13.4) and the HLA genes (chromosome 6p21) segregate independently, a donor–recipient pair can be HLA-matched and KIR-mismatched simultaneously [59]. In fact, only about 25% of the HLA-matched sibling donor/recipient pairs are KIR identical, while the probability of an HLA-matched unrelated donor (MUD)/recipient pair to be KIR identical is virtually zero [60]. A KIR ligand mismatch in HSCT can be predicted using an online calculator (https://www.ebi.ac.uk/ipd/kir/ligand.html) by entering the HLA types of the donor and the recipient.

Donor–recipient KIR ligand mismatch in the setting of haploidentical HSCT

The potent anti-tumor efficacy of allogeneic NK cells was first clinically demonstrated in the context of HSCT by Ruggeri et al. [2] in a study of patients with acute myeloid leukemia (AML) or acute lymphoblastic leukemia (ALL) who underwent T cell-depleted PB haploidentical transplant. As compared to the patients who received KIR ligand-matched HSCT, patients undergoing a KIR ligand-mismatched HSCT had a significantly lower risk of relapse (75 vs. 0% at 5 years, respectively) and improved overall survival (OS; 5 vs. 60%, respectively). For unclear reasons, the beneficial effect of KIR ligand mismatch was noticeable only in AML patients. Similar findings were observed in a subsequent study including a larger cohort of AML patients [61].

Although these results are fascinating, T cell-depleted haploidentical HSCT is rarely performed nowadays due to

higher risk of relapse and non-relapse mortality as compared with an unmanipulated (T-cell replete) HSCT [62–64]. Moreover, the introduction of novel GVHD prophylaxis regimens, such as the use of post-transplant cyclophosphamide (PT-Cy) has led to a universal increase in the numbers of unmanipulated haploidentical HSCT [65–67]. The impact of PT-Cy on NK cell alloreactivity in patients undergoing myeloablative unmanipulated haploidentical HSCT was recently explored by Russo et al. in 99 patients with hematological malignancies (60% with AML) [68]. Within the first few days of HSCT, mature graft-derived NK cells proliferated to a greater extent than T cells, but these proliferating cells, including the potentially alloreactive single-KIR^+ NK cells, were rapidly eliminated by cyclophosphamide (administered on days +3 and +4 post HSCT). By day 15, a second wave of NK cells emerged with an immature $CD56^{bright}NKG2A^+CD62^+$ KIR^- phenotype. Although NK cells remained the dominant lymphocytes for the first 3 months post HSCT, full reconstitution of a mature population took about 6–12 months. Consequently, patients with predicted NK alloreactivity ($n = 41$) had similar outcomes [GVHD, relapse and progression free survival (PFS)] as those without NK alloreactivity ($n = 58$) [68].

In contrast, two other studies in unmanipulated haploidentical HSCT and PT-Cy showed conflicting effects of NK alloreactivity on outcomes. The larger of these included 144 patients with various hematological (65% lymphoid) malignancies who received primarily non-myeloablative conditioning (65%). In this study, the benefit of KIR ligand mismatch (lower relapse risk and improved PFS) was restricted to patients who had active disease at the time of HSCT, but not in those who were in CR [69]. Yet another study ($n = 34$) showed significantly higher risk of acute GVHD (13/17 vs. 6/17, $p = 0.001$) and lower relapse (2/17 vs. 7/17, $p = 0.05$) in patients with KIR ligand mismatch than those with no mismatch [70]. Variability in the conditioning regimens, patient populations and methods to assess NK cell alloreactivity in these studies could account for the differences in outcomes. Nevertheless, these studies suggest that the donor–recipient KIR ligand mismatch should not be used a prime determinant while selecting a donor for patients undergoing T-cell replete haploidentical HSCT with PT-Cy.

Can the potentially detrimental effect of PT-Cy on NK cell alloreactivity be compensated for by supplemental infusions of NK cells in the peri-transplant setting? This question was assessed in a study by Ciurea et al. [71] who administered multiple infusions of ex vivo expanded NK cells to patients with myeloid malignancies ($n = 13$) undergoing unmanipulated haploidentical HSCT. Patients received reduced intensity conditioning (RIC) with fludarabine and melphalan, followed by PT-Cy on days +3 and +4. NK cells were infused on days −2, +7 and +28. Five of the 13 patients were KIR ligand mismatched with their donors. As compared to historical controls ($n = 45$) treated with the same conditioning regimen but without the supplemental NK cell infusions, patients who received NK cells had higher frequencies of TNF-α and IFN-γ secreting NK cells at day 28 and had a higher proportion of mature/potentially alloreactive single-KIR^+ NK cells expressing CD16 and NKG2C. Yet, the authors did not observe any differences in the incidence of acute or chronic GVHD, relapse or PFS; NK cells, however, appeared to be protective against cytomegalovirus reactivation (31% in the NK group vs. 70%—in the non-NK controls, $p = 0.01$).

Donor–recipient KIR ligand mismatch in HLA-matched related or unrelated donor HSCT

The impact of KIR ligand mismatch on HSCT outcomes has also been studied extensively in the setting of HLA-matched related [72–76] or unrelated [73, 76–83] donor transplants using T-cell replete [73, 75, 76, 78–80, 82] or T-cell deplete [72, 74, 77, 79–81] grafts with conflicting results. A common factor that surfaces from these heterogeneous studies is that a T-lymphopenic environment, created using in vivo or ex vivo T-cell depletion, is critical to harness the benefits of NK cells. This echoes findings from studies showing that T cells can dominate and dampen NK cell alloreactivity [84, 85].

The conflicting results can also be explained by differences in the definitions of KIR ligand mismatch among the studies, significant polymorphisms in the KIR genes and the stochastic surface expression of specific KIRs on individual NK cells. For instance, not all KIR ligand mismatches have equal "strength." The inhibitory potential of KIR2DL1–HLA-C2 interaction is much stronger than that of KIR2DL2/3–HLA-C1 [16, 86, 87]. Moreover, even within a specific KIR–HLA ligand combination, the strengths of their interactions can vary among individuals. In case of HLA-Bw4–KIR3DL1, the interaction can be strong (isoleucine; Bw4-80I) or weak (threonine; Bw4-80T), depending upon the HLA-Bw4 amino acid residue at position 80 and whether the surface expression of KIR3DL1 is high ($3DL1^{high}$) or low ($3DL1^{low}$) [86, 88, 89]. $KIR3DL1^{high}$ has a higher affinity for, and hence induces a greater inhibitory signal, with Bw4-80I than Bw4-80T, while the opposite is true for $KIR3DL1^{low}$, which has a higher affinity for Bw4-80T [86, 90]. The clinical implications of these findings were illustrated by Boudreau et al. [90] in a study of 1328 patients with AML who underwent 9/10- or 10/10-MUD HSCT. The authors categorized patients into having either weak/non-inhibitory or strong inhibitory KIR3DL1/HLA-Bw4 interactions. Patients with weak/no inhibition of KIR3DL1 had a significantly lower risk of relapse (HR, 0.72; $p = 0.004$) and improved PFS (64 vs. 39%, $p = 0.05$) compared to those with strong inhibitory combinations. This protective effect

was independent of and additive to the presence of donor activating KIR2DS1 [90].

Impact of activating NK-cell receptors and KIR haplotypes on HSCT outcomes

In contrast to the KIR ligand mismatch model described above, which primarily focuses on the degree of mismatch between the inhibitory KIRs and their ligands, a different perspective of NK alloreactivity focuses on their activating KIR profile. Based on the composition of activating and inhibitory KIRs, the KIR repertoire can be classified broadly into two KIR haplotypes [14]. Haplotype A is characterized by a predominance of inhibitory genes—specifically, 5 inhibitory KIR genes and a single activating gene (KIR2DS4) [14]. Conversely, haplotype B is characterized by a predominance of activating genes (KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS5, KIR3DS1) and only one inhibitory gene (KIR2DL5) [14]. Furthermore, each KIR haplotype is a combination of a centromeric and a telomeric KIR gene motif [91]. Genes encoding the inhibitory receptors for the HLA-C1 and C2 epitopes are located in the centromeric region, while the telomeric region contains genes encoding the activating receptor for HLA-C2 (KIR2DS1) and the inhibitory receptors for the HLA-Bw4 and HLA-A3/11 epitopes [91]. This specific organization and content of KIR genes carry important clinical significance.

The impact of KIR haplotype on HSCT outcomes was evaluated by Cooley et al. in a study of 448 AML patients who received T-cell replete unrelated donor transplant [92]. There was no impact of recipient KIR haplotype on survival; however, HSCT from a KIR B/x donor as compared with KIR A/A donor was associated with a significantly improved PFS (28 vs. 17% at 3 years, $p = 0.003$) and OS (31 vs. 20% at 3 years, $p = 0.007$). This effect was noted in patients who received KIR ligand-matched (HLA-matched or -mismatched) HSCT, but not in HLA-mismatched/KIR ligand-mismatched HSCT. In an attempt to explore the underlying mechanism for this protective effect, the authors noted that two donor KIR genes—KIR2DL2 and KIR2DS2, which are in strong linkage disequilibrium with each other, had independent effects on survival, but definitive analyses were restricted due to limited power. This was investigated in their subsequent study with a larger cohort of patients with AML ($n = 1086$) or ALL ($n = 323$) who received myeloablative, T-cell replete unrelated donor HSCT. Similar to the prior study, donor KIR B/x haplotype was associated with superior outcomes; however, the advantage was most pronounced if the KIR B genes were homozygous and located in the centromeric region (Cen-B/B). This group (Cen-B/B) had a significantly lower probability of relapse than those with either Cen-A/A or Cen-A/B donor [93]. Moreover, the relapse protection offered by the donor KIR B genes appeared to be more specific for C1/x recipients than C2/C2 recipients [94].

An independent role for specific activating genes on outcomes, particularly donor KIR2DS1 and KIR3DS1, has also been reported [95–97]. In a study of 1277 patients with AML who had received a MUD HSCT, Venstrom et al. showed that receipt of an allograft from a KIR2DS1-positive donor was associated with a 24% lower risk of relapse than from a KIR2DS1-negative donor [97]. However, this protection was dependent upon both donor and recipient HLA-C. The benefit was noted only for C1/x donors and recipients but not for HLA-C2/C2 donors or recipients. Also, donor KIR3DS1 was associated with 17% lower risk of mortality with no impact on relapse [97].

In contrast, KIR2DS4, which is the only activating gene within the KIR A haplotype, appears to impart detrimental outcomes after HSCT, especially if it is fully expressed on the cell membrane. KIR2DS4 has two allelic variants—full-length ($KIR2DS4^{full}$—2DS4*00101) or deleted ($KIR2DS4^{del}$—including 2DS4*003, S4*004 and S4*006) [98–100]. The latter yields a truncated KIR2DS4 protein, which is not bound to the cell membrane but is rather secreted in a soluble form [99]. The impact of donor KIR2DS4 on HSCT outcomes was assessed in 111 Croatian patients with a variety of hematological malignancies who underwent T-cell replete HLA-matched related or unrelated donor HSCT. Among related donor HSCT recipients, patients whose donors had 1–2 $KIR2DS4^{full}$ alleles had a significantly lower OS as compared to patients whose donors had a $KIR2DS4^{del}$ allele or no KIR2DS4 allele (HR = 7.9; $p = 0.016$). In the MUD group, HSCT from a $KIR2DS4^{full}$ donor was associated with a higher risk for GVHD (HR = 8.2; $p = 0.012$) and non-relapse mortality (NRM) than from a donor with $KIR2DS4^{del}$ allele or no KIR2DS4 allele [101]. Another study in 75 Chinese patients who underwent T-cell deplete MUD HSCT showed significantly higher risk of acute GVHD (RR 9.0, $p = 0.01$) in patients whose donors were homozygous for $KIR2DS4^{full}$ allele [102]. It remains to be explored if these effects are restricted to particular racial and ethnic groups, as KIR diversity varies dramatically among different populations.

Role of NK cells in cord blood transplant

Umbilical cord blood transplant (CBT) generates a multifaceted NK cell alloreactive environment, especially in patients who receive double unit CBT, where three-way interactions between two CB units and the host make it even more complex to fully comprehend the role of NK cells than in other types of HSCT. This has been investigated in a handful of studies that generated controversial findings. The beneficial effect of KIR ligand mismatch in CBT was demonstrated in

only one study in patients with acute leukemia ($n = 218$), in which the authors reported a lower risk of relapse and improved OS in the KIR ligand-mismatched group. The majority of patients in this study received in vivo T-cell depletion (82%) and myeloablative conditioning (83%) and all patients received a single CB unit [103]. Subsequent studies in heterogeneous populations that included either single or double unit CBT, with or without lymphodepletion and a blend of conditioning regimens displayed inconsistent outcomes [104–107]. Three of these studies [105–107] showed no impact of KIR ligand mismatch on outcomes after CBT, while one study [104] revealed rather unfavorable outcomes in patients receiving KIR ligand-mismatched RIC-CBT, with a higher risk of acute GVHD and NRM and lower OS.

More recently, a study by our group assessed whether specific combinations of donor–recipient KIR–HLA genotypes could improve outcomes after CBT [108]. This study included 110 patients with myeloid or lymphoid malignancies who received predominantly double unit (95%) CBT following myeloablative (72%) or RIC regimens. HLA-C1/x patients had significantly better outcomes (lower relapse and superior survival) than homozygous HLA-C2 patients, related to the observation that the HLA-C1-specific KIR2DL2/L3/S2-expressing NK cells appeared significantly earlier and in greater numbers after CBT than the HLA C2-specific KIR2DL1/S1-expressing NK cells. Among HLA C1/x patients, those who received a graft with a combined HLA-C1-KIR2DL2/L3/S2 genotype (where donor NK cells were licensed for KIR2DL2 or 2DL3 and expressed activating KIR2DS2) had a significantly lower risk of relapse and improved OS than those with CB grafts lacking KIR2DS2 or HLA-C1 (i.e., where donor NK cells were either not licensed or the activating KIR2DS2 gene was absent). Similarly, HLA-C2/C2 patients had a lower risk of relapse and improved survival if they received a graft with the combined HLA-C2–KIR2DL1/S1 genotype (i.e., where CB NK cells were licensed for KIR2DL1 and the activating KIR2DS1 gene was present) [108]. Based on these findings, we initiated a clinical trial of personalized CBT in patients with hematological malignancies, where we select a CB unit with the best probability of eliciting NK alloreactive responses. For instance, for C1/x patients, we select at least one CB unit that is positive for licensed KIR2DL2/L3 and the activating KIR gene KIR2DS2. For C2/C2 patients, we infuse activated mature CB NK cells expressing the C2-specific NK receptor KIR2DL1 (expanded from the dominant CB unit determined at the time of engraftment by chimerism analysis) between 1 and 3 months post-transplant to reduce the risk of relapse [NCT02727803]. In addition, given the unique advantages of CB-derived NK cells (reviewed in [109]), we are also conducting other clinical trials where ex vivo expanded CB NK cells are infused along with high-dose chemotherapy in the setting of HSCT in patients with lymphoma [NCT03019640], multiple myeloma [NCT01729091] or leukemia [NCT01619761].

Allogeneic NK cells as a stand-alone therapy

The findings generated from the studies of KIR ligand-mismatched haploidentical HCT suggested safety and potential efficacy of using allogeneic NK cells as stand-alone therapy. This was first explored in the non-transplant setting by Miller et al. [110] in 43 patients with solid tumors, Hodgkin disease and relapsed/refractory AML who were given infusions of haploidentical NK cells followed by exogenous IL-2. All patients received one of three lymphodepleting preparative regimens to prevent rejection of donor cells, including (a) low-dose cyclophosphamide (750 mg/m^2) and methylprednisolone, (b) fludarabine (25 mg/m^2 for 5 days) or (c) high-dose cyclophosphamide (60 mg/kg for 1 or 2 doses) and fludarabine (25 mg/m^2 for 5 days) ("Hi-Cy/Flu"). Of these, the most potent lymphodepleting Hi-Cy/Flu regimen induced massive T-cell lymphopenia resulting in high endogenous concentrations of IL-15 and the best in vivo expansion of NK cells. No patient developed GVHD and 5 of the 19 AML patients attained CR, with higher responses seen in those with a KIR ligand-mismatched donor [110]. In a subsequent study [111], the authors incorporated a recombinant IL-2 diphtheria fusion protein (IL2DT) into the Hi-Cy/Flu conditioning regimen ($n = 15$) to deplete Tregs and noted even higher expansion of NK cells, which translated into higher rates of CR at day 28 (53 vs. 21%; $p = 0.02$) and PFS at 6 months (33 vs. 5%; $p < 0.01$) compared to those who did not receive IL2DT ($n = 42$) [111].

Future directions: next generation engineered NK cells for immunotherapy of cancer

Allogeneic NK cells have proven to be safe with modest efficacy in clinical trials as detailed above. However, there are still some limitations that need to be overcome for NK cellular therapy to have a larger clinical impact. Some of these obstacles include limited in vivo persistence, restricted homing to tumor sites, hampered function due to the immunosuppressive tumor microenvironment and lack of antigen specificity. The field of genetic engineering has seen tremendous advances in the past few years and has proven to be a powerful approach to improve the efficacy of immune effector cells for adoptive therapy. The most notable advances were seen with T-cell therapy, and have led to the Food and Drug Administration (FDA) approval of CD19-redirected chimeric antigen receptor (CAR) engineered T cells for relapsed B-ALL in children and young adults [112]

and for patients with relapsed NHL [113]. These advances have also applied to the field of NK cellular therapy where CAR engineering is being explored in multiple preclinical studies (reviewed in [114–116]). Our group is leading the first in-human clinical trial to test the safety and efficacy of off-the-shelf CB-NK cells engineered to express a CAR against CD19, ectopically produce IL-15 to support NK cell proliferation and persistence in vivo, and express a suicide gene, inducible caspase 9, to address any potential safety concerns for the treatment of refractory lymphoid malignancies [NCT03056339]. Genetic engineering can be employed to enhance the effectiveness of adoptively transferred NK cells by increasing their in vivo persistence and cytotoxicity, improve their trafficking and homing to tumor sites and enhance their ability to circumvent the immunosuppressive tumor microenvironment [reviewed in [115, 116]. In addition to CAR engineering, other strategies, including bi-specific killer cell engagers (BiKEs) and tri-specific killer cell engagers (TriKEs) are also being employed to enhance the efficacy of NK cellular therapy (reviewed in [117–119]).

Conclusion

The field of NK cell immunotherapy has advanced remarkably over the last decade. Although initial studies with autologous NK cells were discouraging, the use of allogeneic NK cells has resulted in favorable outcomes both in the transplant and non-transplant settings. Our increasing understanding of the biology of NK cells along with the advancements in the field of ex vivo manipulation and genetic engineering is laying the foundation for readily available universal, yet customizable, NK cells for the adoptive immunotherapy of cancer.

Compliance with ethical standards

Conflict of interest The authors have no conflict of interest with the information provided in this article.

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