Chimeric Antigen Receptor Expressing Natural Killer Cells for the Immunotherapy of Cancer

Rohtesh S. Mehta* and Katayoun Rezvani*

MD Anderson Cancer Center, Houston, TX, United States

Adoptive cell therapy has emerged as a powerful treatment for advanced cancers resistant to conventional agents. Most notable are the remarkable responses seen in patients receiving autologous CD19-redirected chimeric antigen receptor (CAR) T cells for the treatment of B lymphoid malignancies; however, the generation of autologous products for each patient is logistically cumbersome and has restricted widespread clinical use. A banked allogeneic product has the potential to overcome these limitations, yet allogeneic T-cells (even if human leukocyte antigen-matched) carry a major risk of graft-versus-host disease (GVHD). Natural killer (NK) cells are bone marrow-derived innate lymphocytes that can eliminate tumors directly, with their activity governed by the integration of signals from activating and inhibitory receptors and from cytokines including IL-15, IL-12, and IL-18. NK cells do not cause GVHD or other alloimmune or autoimmune toxicities and thus, can provide a potential source of allogeneic “off-the-shelf” cellular therapy, mediating major anti-tumor effects without inducing potentially lethal alloreactivity such as GVHD. Given the multiple unique advantages of NK cells, researchers are now exploring the use of CAR-engineered NK cells for the treatment of various hematological and non-hematological malignancies. Herein, we review preclinical data on the development of CAR-NK cells, advantages, disadvantages, and current obstacles to their clinical use.

Keywords: natural killer cells, chimeric antigen receptor, chimeric antigen receptor, chimeric antigen receptor T, chimeric antigen receptor natural killer, cancer, hematopoietic stem cell transplant

OPEN ACCESS

INTRODUCTION

Chimeric antigen receptor (CAR) T cells have gained enormous clinical recognition with remarkable responses reported in patients receiving autologous CD19 (a B cell-specific antigen)-redirected T cells for the treatment of patients with relapsed or refractory B-cell malignancies (1–9). CAR T cells are genetically engineered to express a single chain variable fragment (scFv) derived from an antibody on their surface, which is coupled to a T-cell signaling domain, thus rendering them highly antigen-specific in a non-human leukocyte antigen (HLA)-restricted manner (2, 10, 11). Thus far, the clinical application of CAR T cells has been largely restricted to CD19-expressing B cell malignancies (1–9); however, ongoing studies are testing its applications in other hematological malignancies such as Hodgkin and non-Hodgkin lymphoma, multiple myeloma, and acute myeloid leukemia (12–14). The US Food and Drug Administration recently approved two autologous CD19 CAR T cell products for the treatment of acute lymphoblastic leukemia and certain types of relapsed or refractory large B-cell lymphoma. However, CAR T-cells have several limitations: (i) it is logistically cumbersome to generate an autologous product from patients; (ii) it takes several weeks before...
CAR T cells are generated—making it impractical for patients with aggressive disease; and (iii) generation of clinically relevant doses of CAR T-cells can be unfeasible from heavily pretreated lymphopenic patients. An alternative approach is to use previously collected T cells from an allogeneic source; however, even if HLA-matched, T cells pose a risk of serious graft-versus-host disease (GVHD) (15). In contrast to the popularity of CAR T cells, the generation and clinical application of CAR natural killer (NK) cells has lagged behind for various reasons, despite the multiple advantages of NK cells. Herein, we describe these barriers and discuss strategies to overcome them.

**Advantages of NK Cells for CAR Therapy**

Among cytolytic lymphocytes, NK cells represent on a per cell basis the most efficient effectors against tumors with a distinct mechanism of action (Figure 1) and provide an attractive source of cells for cancer immunotherapy (16, 17). In contrast to other lymphocytes such as T or B cells, NK cells do not express rearranged, antigen-specific receptors. Instead, NK cells express germline-encoded receptors, which are either activating or inhibitory. Upon interaction with their ligands on target cells, the receptors induce a positive or a negative signal, respectively (18). The balance of these signals ultimately govern NK effector function (16, 19) Among the most heavily studied NK cells receptors are the killer-cell immunoglobulin-like receptors (KIRs) that recognize classical HLA class-I molecules (HLA-A, -B, and -C). Other receptors belong to the C-type lectin family (CD94 and NKG2s, such as NKG2A, -B, -C, -D, -E, and -F) that recognize non-classical HLA class-I molecules (HLA-E and stress-induced MHC-I-related chains—MICA and MICB) [reviewed in Ref. (20–23)]. Healthy cells are protected from NK mediated lysis by the recognition of “self” HLA molecules on their surface by inhibitory NK receptors protects (24–27). On the other hand, tumor or viral infected cells often downregulate or lose their HLA molecules as an escape mechanism against T-cells (28, 29). Loss of HLA class I expression makes them susceptible to lysis by the NK cells due to loss of the inhibitory signal (21, 30–45). Indeed, the clinical significance of NK cell alloreactivity has been demonstrated in multiple studies in the setting of hematopoietic stem cell transplant (HSCT), where patients that received a graft containing alloreactive NK cells had a significantly lower risk of relapse and improved survival (46–55). Adoptive transfer of alloreactive NK cells as a stand-alone therapy (independent of HSCT) also demonstrated encouraging outcomes in a variety of malignancies (56–62). In contrast, several studies of autologous NK cell adoptive therapy showed rather disappointing results (63–71).

Thus, NK cells offer an attractive alternative to T-cells for CAR engineering for a number of reasons: (i) allogeneic NK cells should not cause GVHD, as predicted by observations in murine models (72, 73), as well as clinical studies of haploidentical and cord blood (CB) derived NK cell infusions in patients with hematologic or solid malignancies (56, 59); (ii) mature NK cells have a relatively limited life-span, permitting effective antitumor activity while reducing the probability of long-term adverse events, such as prolonged cytopenias due to on-target/off-tumor toxicity to normal tissues such as B cell aplasia (in the case of CD19 CARs), which can last up to 3 years (74); and (iii) CAR-NK cells retain their intrinsic capacity to recognize and target tumor cells through their native receptors; therefore when compared with the CAR...
T cells, it is theoretically less likely for tumor cells to escape NK immunosurveillance even if they downregulate the CAR target antigen (75). This unique property of NK cells could be further exploited for the generation of NK-CARs by selecting donors based on the donor-recipient KIR-ligand mismatch, or based on donor haplotype B KIR gene content, as both have been shown to be beneficial in the setting of allogeneic HSCT (48, 50, 55, 76). Thus, allogeneic NK cells offer the potential for an off-the-shelf cellular product for immunotherapy that could be readily available for immediate clinical use, in contrast to the current shortage of CAR T-cell products at many centers (77).

**SOURCE OF NK CELLS FOR ADAPTIVE IMMUNOTHERAPY**

Functional NK cells can be generated from numerous sources. Although autologous NK cells can be utilized for adoptive therapy, their efficacy against autologous cancerous cells is rather limited (63–71, 78, 79), which we have shown may not be easily overcome by CAR engineering (80). Allogeneic NK cell sources include peripheral blood (PB), bone marrow (BM), human embryonic stem cells (hESCs), induced pluripotent stem cells (iPSCs) (81–83), umbilical CB, or readily available NK cell lines (84). Obtaining NK cells from the PB by apheresis or from BM by harvesting are both cumbersome and are associated with potential risks to the healthy donors (85–87). NK cell derivation from hESCs or iPSCs (81–83) is a complex process and the field is still evolving. In contrast, NK cell lines such as NK-92 (88–93), KHYG-1 (94), NKL, NKG, and YT, to name a few, provide an easily accessible and homogeneous source of cells for the generation of large numbers of CAR-transduced NK cells. NK-92 is a highly cytotoxic NK cell line that was derived from a patient with NK lymphoma (95) and is characterized as CD56brightCD16neg/lowNK- CD7 positive leukemia or AML and CD19 CD19 positive leukemia or B cell malignancies. NK-92 cells have a number of disadvantages that need to be taken into account. First and foremost, NK-92 cells are derived from a patient with NK lymphoma (95) and thus have the potential for tumor engraftment following infusion. Moreover, they are EBV-positive and carry multiple cytogenetic abnormalities resembling those of NK lymphoma (116). Thus, as a safety measure, NK-92 cells must be irradiated before infusion into patients to prevent permanent engraftment. This can negatively impact their in vivo proliferation and persistence, both factors shown to be crucial for the success of cellular therapy in studies with infusion of tumor-infiltrating lymphocytes (117–119) as well as CAR-T cells (3). Indeed, in a study of NK-92 cells engineered with ErbB2/HER2-CAR, while irradiation had no effect on the in vitro cytotoxicity of CAR-transduced NK92 cells, it negatively impacted their in vivo replication and persistence, with the cells no longer detectable within 7 days of adoptive infusion (109). Of note, NK-92 cells are CD16 (FCRIIIa) negative and cannot mediate antibody-dependent cell cytotoxicity (ADCC), unless genetically modified to express CD16 (120).

Cord blood, on the other hand, is a readily available source of allogeneic NK cells with distinct benefits over related or unrelated adult donors, including the speed of availability (especially since it is available as an off-the-shelf frozen product) and tolerance of HLA mismatches, the latter of which expands the donor pool. The frequencies of NK cells in CB (~15–20%) are similar to PB (~10–15%) (121–124). However, until recently the small volume of blood in a CB unit made it challenging to obtain adequate numbers of NK cells for clinical use. Moreover, resting CB NK cells are phenotypically and functionally immature, with higher expression of the inhibitory receptor NKG2A and lower expression of activating and maturation receptors such as NKP46, NKG2C, DNAM-1 (124), and CD57 (124–127). To overcome these limitations, our group has developed a Good Manufacturing Practice (GMP)-compliant procedure, using GMP-grade K562-based artificial antigen-presenting cells (aAPCs) expressing membrane bound IL-21 and 4-1BB ligand, which reliably generates clinically relevant doses of GMP-grade NK cells from a CB unit for adoptive immunotherapy (128). Following ex vivo activation and expansion, CB-derived NK cells display the full array of activating and inhibitory receptors, strongly express eomesoderm (Eomes) and T-bet, two factors necessary for NK cell maturation, and exert similar cytotoxicity to PB-NK cells (129, 130). Taken together, these studies support the use of NK cells as a source of cellular therapy in cancer.

**Constituents of CAR**

A CAR construct consists of three components: an extracellular antigen-recognition part, a transmembrane domain and an intracellular signaling domain. The extracellular domain is the antigen-recognition site and is generally composed of an scFv derived from the variable regions of both the heavy and light chains of a monoclonal antibody, fused together via a flexible linker. Most scFvs studied to date are of murine origin, with the potential to induce a human antimouse antibody (HAMA) or an anti-idiotype immune response. A number of investigators are exploring strategies to humanize scFVs (131–136) to circumvent induction of HAMA; however, this approach will

---

**TABLE 1 | Clinical trials with NK CAR.**

| Clinical trial identifier | NK cell source | Target antigen | Disease | Study location |
|--------------------------|----------------|----------------|---------|---------------|
| NCT029441462             | NK-92 cell line | CD33           | AML     | China         |
| NCT02892695              | NK-92 cell line | CD19           | CD19 positive B cell malignancies | China |
| NCT02742727              | NK-92 cell line | CD7            | CD7 positive leukemia or lymphoma | China |
| NCT02839954              | NK-92 cell line | MUC1           | MUC1 positive solid tumors (colorectal, gastric, pancreatic, NSCLC, breast, glioma) | China |
| NCT03056339              | Cord blood     | CD19           | CD19 positive leukemia or lymphoma | MDACC, USA |

AML, acute myeloid leukemia; MDACC, MD Anderson Cancer Center; NK, natural killer; NSCLC, non-small cell lung cancer.
not prevent the development of anti-idiotype antibodies. The antigen binding domain of a CAR is linked to a “hinge” which imparts flexibility for adequate orientation and binding to the antigen. The hinge binds the extracellular component to a transmembrane domain, which is the link to the intracellular signaling component (137–139). The size of the hinge region has been shown to affect CAR-T cell function, with some studies reporting superior anti-tumor activity of CAR T-cells expressing a shorter hinge (140, 141). The transmembrane domain lies between the hinge and the signaling endodomains. Different types of transmembrane domains have been studied, including the CD3-ζ chain of the T-cell receptor, CD4, CD8, or CD28. The type of transmembrane domain has also been shown to affect the function and stability of the CAR molecule in T cells (142). The endodomain then transmits activation signals to T cells. The “first-generation” CARs used a single intracellular signaling domain (CD3-ζ chain alone) while the second- and third-generation CARs incorporate one or more additional costimulatory signaling domains, such as CD28, CD137, or OX40 to render them more potent (143, 144). CD3ζ is critical for signaling and activation of both T and NK cells (145). In NK cells, CD3ζ homodimer transmits signals from FcγRIII (CD16), thus aiding in ADCC (146). Although CD28 is one of the most commonly employed costimulatory domains in CAR T cells, except in certain cell lines (147), its role in NK cell function is less clearly defined (148). Nonetheless, its addition to CD3ζ in a second generation ErbB2-specific NK-92 CAR led to improved function compared to a CD3ζ construct alone and was similar to that of CD137-CD3ζ CAR against ErbB2-expressing tumor cells (109). Another study showed that NK-92 cells transduced with a CD19-CAR expressing CD28-CD3ζ had superior cytotoxicity against CD19-positive targets compared to cells expressing a CD137-CD3ζ containing CAR (88). DNAX-activation protein 12 (DAP12) is transmembrane protein involved in signal transduction of several NK cell activating receptors including NGK2c, NKp44, and the activating KIRs (149). One study tested if a CAR against prostate stem cell antigen (PSCA) that used DAP12 as an intracellular signaling domain can provide sufficient signaling to induce NK cell activation when compared to a CD3ζ-containing CAR. The authors transduced YTS-NK cells and primary NK cells with PSCA-DAP12 CAR and noted superior cytotoxicity when compared to NK cells expressing a CD3ζ-based CAR (150). While the importance of incorporating costimulatory molecules in the CAR construct has been clearly shown for CAR T cells (4, 5, 151), additional studies are needed to define the optimal costimulatory molecule and signaling endodomain for NK cells.

**CAR Transduction**

The incorporation of a foreign gene into a cell requires the use of a vector, which can be based on viral or non-viral systems. The most commonly used tools for CAR gene delivery include genetically engineered retroviruses [lentiviral (152) and gamma-retroviral (153) vectors]. Lentiviruses have the advantage that they are capable of infecting both dividing and non-dividing cells, while retroviruses only infect dividing cells. Therefore, lentiviral vectors can be used for transduction of a wider variety of cell types including quiescent stem cells (154–156). In addition, lentiviral vectors can accommodate larger transgenes when compared with retroviral vectors (157). Insertional mutagenesis, although extremely rare, remains a concern with viral vectors although its likelihood is influenced by a number of factors such as the specific type of vector used and the site of integration (158). For instance, in earlier trials of gene therapy with CD34+ hematopoietic cells for X-linked severe combined immunodeficiency (SCID), 2 of the 10 treated children developed acute leukemia in one study (159) and 1 of the 4 children in another study (160). However, none of the subsequent trials of gene-modified hematopoietic stem cells in SCID children (161) or studies of adoptive immunotherapy with CAR T-cells (2, 3, 5, 9, 162) have witnessed adverse events related to insertional mutagenesis to date. Yet, to mitigate any theoretical concerns, various non-viral techniques such as the transposon/transposase system (82, 163) or mRNA transfection (113, 115) have also been tested. The transduction efficiency using these techniques varies remarkably from study to study (82, 113, 115, 163, 164) and depends on a number of factors, including the cell source (82, 113, 115, 163–167). In general, the transduction efficiency for CAR T cells is about 50% but can range up to 90% or higher (152, 164, 168).

**CHALLENGES**

Despite the many advantages of NK cells, there are several impediments to the successful generation of CAR NK cells for clinical use. Until recently, the genetic engineering of NK cells, even with viral methods, had proved challenging, with reports of <10% transduction efficiency for primary CB or PB derived NK cells (113, 165). However, recent optimization in protocols for viral transduction and electroporation (166, 167) has revived enthusiasm for the genetic engineering of NK cells. While viral methods appear to be largely ineffective for inducing CAR expression in freshly isolated PB NK cells, significantly better transduction efficiency can be achieved when NK cells from PB (12–73%) (113) or CB are activated and expanded (median 69%; range 43–93%) (169) in one study and 80% (range 67–96%) (170) in another study. In contrast to studies with primary NK cells, NK92 cells are easier to transduce with mRNA electroporation (113, 115), with efficiencies averaging from 25 to 50% (171). However, as the mRNA transcript is not incorporated into the genome, expression of the CAR molecule is often short-lived and detectable for only a few days, which may negatively impact the efficacy of the engineered cells following adoptive transfer (115, 166, 167).

Another concern with using allogeneic NK cells is the possibility of infusing contaminating T or B cells in the expanded NK cell product, which can theoretically cause GVHD or posttransplant lymphoproliferative disease, respectively. As some degree of HLA-mismatch after CB transplantation is well tolerated, the risk of clinically significant GVHD may be less with CB-derived CAR-NK cells compared to PB. Plus, with the exception of one study reporting GVHD following adoptive transfer of donor-derived IL-15/4-1BBL-activated NK cells in recipients...
of HLA-matched, T-cell-depleted PB HSCT (72), clinical studies of haploidentical and CB NK cell infusions in hundreds of patients with both hematologic and solid malignancies have not reported a higher risk of GVHD (56, 58, 128, 172–174). Rather, experimental evidence obtained in mice have reported reduced risk of GVHD with NK cells via multiple mechanisms, including depletion of host antigen-presenting cells and activated alloreactive T cells (46, 72, 175, 176). Another potential limitation of NK cells for immunotherapy is that in contrast to T cells, they are highly sensitive to the freeze and thaw process and they lose activity after thawing. A number of groups are exploring strategies to optimally cryopreserve NK cells and have shown that the activity of frozen NK cells can be restored by overnight incubation with cytokines such as IL-2 (177–182). It is not yet known if a similar strategy can be used to restore function of frozen CAR NK cells for adoptive therapy.

Another characteristic of NK is that they do not persist after adoptive transfer without cytokine support (183). While the shorter life-span of NK cells may be advantageous, allowing for antitumor activity while reducing the probability of long-term adverse events such as prolonged cytopenias caused by off-target/on-tumor toxicity to normal tissues, it may also limit their efficacy. For in vivo survival and proliferation, NK cells require continuous cytokine support, without which they are detectable in the circulation for only 1–2 weeks (183). The two most commonly used cytokines to support the persistence of adoptively transferred NK cells are IL-2 and IL-15 (184, 185). The infusion of IL-2 has substantial side effects including fevers, chills, myalgias and capillary leak syndrome (186), and can promote expansion of regulatory T cells (Treg) which are suppressive to NK cells (187).

Another important aspect of NK cells is that they do not persist after adoptive transfer without cytokine support (183). While the shorter life-span of NK cells may be advantageous, allowing for antitumor activity while reducing the probability of long-term adverse events such as prolonged cytopenias caused by on-target/off-tumor toxicity to normal tissues, it may also limit their efficacy. For in vivo survival and proliferation, NK cells require continuous cytokine support, without which they are detectable in the circulation for only 1–2 weeks (183). The two most commonly used cytokines to support the persistence of adoptively transferred NK cells are IL-2 and IL-15 (184, 185). The infusion of IL-2 has substantial side effects including fevers, chills, myalgias and capillary leak syndrome (186), and can promote expansion of regulatory T cells (Treg) which are suppressive to NK cells (187). IL-15, on the other hand, does not support Treg (188) expansion but when administered as an exogenous bolus to patients with metastatic melanoma and renal carcinoma can result in dose-dependent toxicity, including neutropenia (189). An alternative approach to exogenous administration of cytokines is to treat patients with lymphodepleting chemotherapy such as cyclophosphamide and fludarabine prior to infusion of NK cells, which provides a favorable environment for NK cell expansion by depleting mature lymphocytes (which consume IL-15), resulting in a marked increase in endogenous IL-15 levels (56). Another novel technique is to incorporate genes for IL-2 (104, 190–192) or IL-15 (80, 193–195) within the CAR construct to constantly provide cytokine support to the CAR-transduced cells. We recently showed the feasibility and efficacy of this approach in a mouse model of Raji lymphoma. Although a single infusion of 1 × 10^7 CAR.19+ (without IL15) or CAR.19/IL15+ CB-NK cells both improved tumor control and prolonged survival compared to non-transduced CB-NK, CAR.19/IL15+ CB-NK cells controlled tumor expansion and prolonged survival significantly better than the CAR.CD19 construct lacking the IL-15 gene, which underscores the critical influence of IL-15 in enhancing antitumor activity in vivo (80).

### Suicide Genes

Given the recent safety concerns such as cytokine release syndrome and neurotoxicity associated with infusion of CAR-modified T cells (196, 197) (and possibly NK cells), careful consideration of whether a suicide system should be incorporated into the construct as a safety measure is needed. One of the most extensively tested safety switches include the herpes simplex virus thymidine kinase gene (198, 199). While a number of studies have tested this approach, the highly immunogenic virus-derived protein can lead to the rejection of cells expressing it, plus it requires administration of ganciclovir—which takes several days to work and leads to cytopenias (200–202). Because of these disadvantages, inducible caspase-9 (iCas9) has emerged as one of the most commonly used suicide genes in adoptive cell therapy trials (80, 193, 203–206). When exposed to a synthetic bionoert small-molecule dimerizing drug, the iCas9 becomes activated and leads to rapid apoptosis of cells expressing it. Another suicide gene under investigation is the truncated epidermal growth factor receptor (EGFR), which lacks intracellular tyrosine kinase activity while expressing an intact binding epitope that can be targeted with the anti-EGFR monoclonal antibody cetuximab for the rapid elimination of the transgenic cells (207, 208).

### Preclinical Studies of CAR-NK Cells

Building on the knowledge gained with CAR T-cells, a multitude of preclinical studies have tested the efficacy of CAR NK cells against a variety of target antigens for hematological malignancies such as CD19 (167, 169), CD20 (209, 210), CD138 (211), CS1 (111), CD3 (212), CD5 (101), CD123 (213), as well as solid tumors such as HER-2/Erb-2 (109, 214–216), GD2 (114), EpCAM (195), EGFR and mutant EGFRVIII (89), WT1 (217), and ROR-1 (218) to name a few. An alternative and non-antigen specific approach to engineering NK cells was tested by Chang et al. (170), where the authors induced supra-physiologic expression of NGK2D, a key receptor for NK cell activation and signal transduction via a synthetic bioinert small-molecule dimerizing drug, the iCas9 becomes activated and leads to rapid apoptosis of cells expressing it. Another suicide gene under investigation is the truncated epidermal growth factor receptor (EGFR), which lacks intracellular tyrosine kinase activity while expressing an intact binding epitope that can be targeted with the anti-EGFR monoclonal antibody cetuximab for the rapid elimination of the transgenic cells (207, 208).

Despite these impressive preclinical data, there are currently only five registered clinical trials testing the safety and efficacy of CAR-NK cells in cancer patients (Table 1). Four of these trials are being conducted in China using the CAR-engineered NK92 cells. The only trial using primary NK cells (CB NK cells) is being conducted in the United States by our group at the MD Anderson Cancer Center (NCT03056339). Patients with relapsed or refractory CD19+ B cell lymphoid malignancies are eligible for this trial. All patients receive lymphodepleting chemotherapy with fludarabine and cyclophosphamide, followed by the infusion of allogeneic CB-derived NK cells that are genetically modified with a retroviral vector, iC9-2A-CAR.CD19-CD28-CD3ζ-2A-OHIL-15 (iC9/CAR.19/IL15) (80), which (i) includes CAR.19 gene to redirect specificity to CD19; (ii) produces IL-15 ectopically—a cytokine crucial for NK cell survival and proliferation (219); and (iii) incorporates inducible caspase-9 (iCas9)—a suicide gene, which can be activated pharmacologically to eliminate CAR cells, as needed (203, 219).
CONCLUSION

We are in an exciting era in the field of cellular therapy. NK cells hold great promise and offer the potential for an off-the-shelf cellular product for immunotherapy that could be readily available for immediate clinical use. Yet, before NK cells can be extended to larger cohorts of patients a number of scientific questions and regulatory hurdles must be addressed. What is the ideal vector, signaling endodomain and costimulatory molecule for NK cells—one with the best response and safety profile? Will CAR NK cells have a different safety and efficacy profile to CAR T cells, given their distinct mechanism of action? Will “off-the-shelf” CAR NK cells be able to sustain the clinical demand, give the shortage of CAR-T cells at many centers and the uncertainty regarding the health economics of this treatment? Can CAR NK cells lead to durable responses, considering their limited in vivo life-span or will they be used as a “bridge” to more aggressive treatment such as HSCT? Based on the results of haploidentical and unrelated donor HSCT, should NK cells for CAR modification be selected based on KIR-KIR ligand mismatch or KIR haplotype to harness their native NK cell activity and will this approach reduce the risk of disease escape through downregulation of the CAR target antigen? With the plethora of preclinical studies and clinical research that are underway, it is expected that engineered NK cells will make a significant contribution to the recent paradigm shift in cancer treatment.

AUTHOR CONTRIBUTIONS

RM and KR reviewed the literature, analyzed data, and wrote the manuscript.

REFERENCES

1. Kochenderfer JN, Wilson WH, Janik JE, Dudley ME, Stetler-Stevenson M, Feldman SA, et al. Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologous T cells genetically engineered to recognize CD19. Blood (2010) 116(20):4099–102. doi:10.1182/blood-2010-04-281931
2. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. N Engl J Med (2011) 365(8):725–33. doi:10.1056/NEJMoa1103849
3. Porter DL, Hwang WT, Frey N, Lacey SF, Shaw PA, Loren AW, et al. Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia. Sci Transl Med (2015) 7(303):303ra139. doi:10.1126/scitranslmed.aac5415
4. Lee DW, Kochenderfer JN, Stetler-Stevenson M, Cui YK, Delbrook C, Feldman SA, et al. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukemia in children and young adults: a phase 1 dose-escalation trial. Lancet (2015) 385(9967):517–28. doi:10.1016/S0140-6736(14)61403-3
5. Maude SL, Frey N, Shaw PA, Aplenc R, Barrett AJ, Cutler C, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. N Engl J Med (2014) 371(16):1507–17. doi:10.1056/NEJMoa1407222
6. Davila ML, Riviere I, Wang X, Bartido S, Park J, Curran K, et al. Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. Sci Transl Med (2014) 6(224):224ra225. doi:10.1126/scitranslmed.3008226
7. Li Z, Chen L, Rubinstein MP. Cancer immunotherapy: are we there yet? Exp Hematol Oncol (2013) 2(1):33. doi:10.1186/2162-3619-2-33
8. Brentjens RJ, Davila ML, Riviere I, Park J, Wang X, Cowell LG, et al. CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. Sci Transl Med (2013) 5(177):177ra38. doi:10.1126/scitranslmed.3005930
9. Grupp SA, Kalos M, Barrett D, Aplenc R, Porter DL, Rheingold SR, et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. N Engl J Med (2013) 368(16):1569–18. doi:10.1056/NEJMoai1215134
10. Irving BA, Weiss A. The cytoplasmic domain of the T cell receptor zeta chain is sufficient to couple to receptor-associated signal transduction pathways. Cell (1991) 64(5):891–901. doi:10.1016/0092-8674(91)90314-O
11. Gross G, Waks T, Eshhar Z. Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity. Proc Natl Acad Sci U S A (1989) 86(24):10024–8. doi:10.1073/pnas.86.24.10024
12. Luo Y, Chang L-J, Hu Y, Dong L, Wei G, Huang H. First-in-man CD123-specific chimeric antigen receptor-modified T cells for the treatment of refractory acute myeloid leukemia. Blood (2015) 126:3778.
13. Ritchie DS, Neson PJ, Khot A, Peinert S, Tai T, Tainton K, et al. Persistence and efficacy of second-generation CAR T cell against the LeY antigen in acute myeloid leukemia. Mol Ther (2013) 21(11):2122–9. doi:10.1038/mt.2013.154
14. Wang QS, Wang Y, Lv HY, Han QW, Fan H, Guo B, et al. Treatment of CD33-directed chimeric antigen receptor-modified T cells in one patient with relapsed and refractory acute myeloid leukemia. Mol Ther (2015) 23(1):184–91. doi:10.1038/mt.2014.164
15. Goulmy E. Human minor histocompatibility antigens: new concepts for marrow transplantation and adoptive immunotherapy. Immunol Rev (1997) 157:125–40. doi:10.1111/j.1600-065X.1997.tb00978.x
16. Davies JOI, Stringaris K, Barrett AJ, Rezvani K. Opportunities and limitations of natural killer cells as adoptive therapy for malignant disease. Cytotherapy (2014) 16(11):1453–66. doi:10.1016/j.jcyt.2014.03.009
17. Vivier E, Ugolini S, Blaise D, Chabannon C, Brossay L. Targeting natural killer cells and natural killer T cells in cancer. Nat Rev Immunol (2012) 12(4):239–52. doi:10.1038/nri3174
18. Lanier LL. NK cell receptors. Annu Rev Immunol (1998) 16:359–93. doi:10.1146/annurev.immunol.16.1.359
19. Caligiuri MA. Human natural killer cells. Blood (2008) 112(3):461–9. doi:10.1182/blood-2007-09-077438
20. Parham P. MHC class I molecules and KIRs in human history, health and survival. Nat Rev Immunol (2005) 5(3):201–14. doi:10.1038/nri1570
21. Moretta L, Moretta A. Killer immunoglobulin-like receptors. Curr Opin Immunol (2004) 16(5):626–33. doi:10.1016/j.coi.2004.07.010
22. Farag SS, Fehninger TA, Ruggeri L, Velardi A, Caligiuri MA. Natural killer cell receptors: new biology and insights into the graft-versus-leukemia effect. Blood (2002) 100(6):1935–47. doi:10.1182/blood-2002-02-0350
23. Moretta L, Bottino C, Vitale M, Pende D, Canavese C, Migliari MC, et al. Activating receptors and coreceptors involved in human natural killer cell-mediated cytosisis. Annu Rev Immunol (2001) 19:197–223. doi:10.1146/annurev.immunol.19.11.1997
24. Lanier LL. Face off – the interplay between activating and inhibitory immune receptors. Curr Opin Immunol (2001) 13(3):326–31. doi:10.1006/cioi.2000.0222-3
25. Yokoyama WM. Natural killer cell receptors. Curr Opin Immunol (1998) 10(3):298–305. doi:10.1016/S0952-7915(98)80164-4
26. Ljunggren HG, Karre K. In search of the ‘missing self ‘: MHC molecules and NK cell recognition. Immunol Today (1990) 11(7):237–44. doi:10.1016/0167-5699(90)90097-S
27. Harel-Bellan A, Quillet A, Marchiol C, DeMars R, Turz S, Fradelizi D. Natural killer susceptibility of human cells may be regulated by genes in the HLA region on chromosome 6. Proc Natl Acad Sci U S A (1998) 95(15):8588–92. doi:10.1073/pnas.95.15.8588
28. Algarra I, Garcia-Lora A, Cabezas T, Ruiz-Cabello F, Garrido F. The selection of tumor variants with altered expression of classical and nonclassical MHC class I molecules: implications for tumor immune escape. Cancer Immunol Immunother (2004) 53(10):904–10. doi:10.1007/s00262-004-0517-9
29. Costello RT, Ghaust JA, Olive D. Tumor escape from immune surveillance. Arch Immunol Ther Exp (Warsz) (2004) 52(6):567–77. doi:10.1678/aite.2004.52.6.567
30. Robertson MJ, Ritz J. Biology and clinical relevance of human natural killer cells. Blood (1990) 76(12):2421–38.
31. Cooper MA, Fehniger TA, Caligiuri MA. The biology of human natural-killer-cell subsets. *Trends Immunol* (2001) 22(11):633–40. doi:10.1016/S1471-490X(01)02028-9
32. Fehniger TA, Cooper MA, Nuovo GJ, Cella M, Facchetti F, Colonna M, et al. CD56bright natural killer cells are present in human lymph nodes and are activated by T cell-derived IL-2: a potential new link between adaptive and innate immunity. *Blood* (2003) 101(8):3052–7. doi:10.1182/blood-2002-09-2876
33. Yokoyama WM. Natural killer cell receptors. *Curr Opin Immunol* (1995) 7(1):110–20. doi:10.1016/0952-7915(95)80036-0
34. Brandt CS, Baratin M, Yi EC, Kennedy J, Gao Z, Fox B, et al. The B7 family member B7-H6 is a tumor cell ligand for the activating natural killer cell receptor NKP30 in humans. *J Exp Med* (2009) 206(7):1495–503. doi:10.1084/jem.20090681
35. Poggé von Strandmann E, Simhadri VR, von Tresckow B, Sasse S, Reiners KS, Hansen HP, et al. Human leukocyte antigen-β-associated transcript 3 is released from tumor cells and engages the NKP30 receptor on natural killer cells. *Immunity* (2007) 27(6):965–74. doi:10.1016/j.immuni.2007.10.010
36. Bloushtain N, Qimron U, Bar-Ilan A, Hershkovitz O, Gazit R, Fima E, et al. Membrane-associated heparan sulfate proteoglycans are involved in the recognition of cellular targets by NKP3 and NKP46. *J Immunol* (2004) 173(4):2392–401. doi:10.4049/jimmunol.173.4.2392
37. Jinushi M, Takehara T, Tatsuki T, Kanto T, Groh V, Spies T, et al. Expression and role of MICA and MICB in human hepatocellular carcinoma and their regulation by retinoic acid. *Int J Cancer* (2003) 104(3):534–61. doi:10.1002/ijc.10966
38. Boyington JC, Brooks AG, Sun PD. The structure of killer cell immunoglobulin-like receptors. *Mol Immunol* (2002) 38(14):1007–21. doi:10.1016/S1381-1359(02)00030-5
39. Boyington JC, Brooks AG, Sun PD. Structure of killer cell immunoglobulin-like receptors and their recognition of the class I MHC molecules. *Immunol Rev* (2001) 181:66–78. doi:10.1034/j.1600-065X.2001.1810105.x
40. Steiner A, Li P, Morris DL, Groh V, Morris DL, Groh V, et al. Interactions of human ULK2 and its ligands MICA, MICB, and homologs of the mouse RAE-1 protein family. *Immunogenetics* (2001) 53(4):279–87. doi:10.1007/s002510030325
41. Das H, Groh V, Kujil C, Sugita M, Morita CT, Spies T, et al. MICA engagement by human Vgamma2Vdelta2 T cells enhances their antigen-dependent effector function. *Immunity* (2001) 15(1):83–93. doi:10.1016/S1074-7613(01)00168-6
42. Cosman D, Müllberg J, Sutherland CL, Chin W, Armitage R, Fanslow W, et al. ULBP genes, a novel MHC class I-related molecules, bind to CMV glycoprotein UL16 and stimulate NK cytotoxicity through the NKG2D receptor. *Immunity* (1999) 12(3):233–43. doi:10.1016/S1074-7613(00)00995-4
43. Bauer S, Groh V, Wu J, Steine A, Phillips JH, Lanier LL, et al. Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. *J Immunol* (1999) 285(4528):727–9. doi:10.4049/jimmunol.285.4528.727
44. Gumperz JE, Barber LD, Valiante NM, Percival L, Phillips JH, Lanier LL, et al. Selective modulation of human natural killer cells in vivo after pro-therapy with recombinant interleukin 2. *Science* (1999) 285(5428):727–9. doi:10.1126/science.285.4528.727
45. Gumperz JE, Barber LD, Valiante NM, Percival L, Phillips JH, Lanier LL, et al. Conservation and variable residues within the Bw4 motif of HLA-B make separable contributions to recognition by the NKB1 killer cell-inhibitory receptor. *J Immunol* (1997) 158(11):5237–41.
46. Mandelboim O, Reyburn HT, Sheu EG, Vales-Gomez M, Davis DM, Pazmany L, et al. The binding site of NK receptors on HLA-C molecules. *Immunity* (1997) 6(3):341–50. doi:10.1016/S1074-7613(97)00363-2
47. Ruggeri L, Capani M, Urbanelli E, Puccio K, Slomchik WD, Tosti A, et al. Effectiveness of donor normal killer cell alloactivity in mismatched hematopoietic transplants. *Science* (2002) 295(5562):2097–100. doi:10.1126/science.1068440
48. Ruggeri L, Capani M, Casucci M, Volpi I, Tosti A, Puccio K, et al. Role of normal killer cell alloactivity in HLA-mismatched hematopoietic stem cell transplantation. *Blood* (1999) 94(1):333–9.
49. Cooley S, Trachtenberg E, Bergemann TL, Saetern K, Klein J, Le CT, et al. Donors with group B KIR haplotypes improve relapse-free survival after unrelated hematopoietic cell transplantation for acute myelogenous leukemia. *Blood* (2009) 113(3):726–32. doi:10.1182/blood-2008-07-171926
50. Oevermann L, Michaelis S, Mezger M, Lang P, Toporski J, Bertaina A, et al. KIR B haplotype donors confer a reduced risk for relapse after haploidentical transplantation in children with ALL. *Blood* (2014) 124(17):2744–7. doi:10.1182/blood-2014-03-565069
66. Lister J, Rybka WB, Donnenberg AD, deMagalhaes-Silverman M, Pincus SM, Bloom EJ, et al. Autologous peripheral blood stem cell transplantation and adoptive immunotherapy with activated natural killer cells in the immediate posttransplant period. Clin Cancer Res (1995) 2(4):697–701.

67. Metrop NJ, Porter M, Blumenson LE, Lindemann MJ, Perez RP, Vaickus L, et al. Daily subcutaneous injection of low-dose interleukin 2 expands natural killer cells in vivo without significant toxicity. Clin Cancer Res (1996) 2(4):697–701.

68. Blaise D, Attal M, Pico JL, Reiffers J, Stoppa AM, Bellanger C, et al. Use of a sequential high dose recombinant interleukin 2 regimen after autologous bone marrow transplantation does not improve the disease free survival of patients with acute leukemia transplanted in first complete remission. Leuk Lymphoma (1997) 25(5–6):649–678. doi:10.3109/10428199709039034.

69. Miller JS, Tessmer-Tuck J, Pierson BA, Weisdorf D, McGlave P, Blazar BR, et al. Low dose subcutaneous interleukin-2 after autologous transplantation generates sustained in vivo natural killer cell activity. Biol Blood Marrow Transplant (1997) 3(1):34–44.

70. deMagalhaes-Silverman M, Donnenberg A, Lembersky B, Elder E, Lister J, Rybka W, et al. Posttransplant adoptive immunotherapy with activated natural killer cells in posttransplantation period. Clin Cancer Res (1995) 16(6):607–14.

71. Burns LJ, Weisdorf DJ, DeFor TE, Vesole DH, Repka TL, Blazar BR, et al. IL-2-based immunotherapy after autologous transplantation for lymphoma and breast cancer induces immune activation and cytokine release: a phase I/II trial. Bone Marrow Transplant (2003) 32(2):177–86. doi:10.1038/sj.bmt.3700486.

72. Olson JA, Leveson-Gower DB, Gill S, Baker J, Beilhack A, Negrin RS. NK cells. Blood (2009) 114(17):3551–60. doi:10.1182/blood-2009-05-22190.

73. Genssler S, Burger MC, Zhang C, Oelsner S, Mildenberger J, Wagner M, et al. Dual targeting of glioblastoma with chimeric antigen receptor-engineered natural killer cells overcomes heterogeneity of target antigen expression and enhances antitumor activity and survival. Oncotarget (2016) 7(4):41193–41204. doi:10.18632/oncotarget.7842.

74. Vaughan BV, Perelman M, Genssler S, Korvick JA, Mildenberger J, Wagner M, et al. Tumor-directed redirected lysis of glioblastoma cells by natural killer cells engineered against EGFRvIII. Cytotherapy (2019) 21(2):132–41. doi:10.1080/14653240802301872.
103. Hsich YT, Aggarwal P, Cirelli D, Gu L, Surowy T, Mozier NM. Characterization of FcgammaRIIIA effector cells used in in vitro ADCC bioassay: comparison of primary NK cells with engineered NK-92 and Jurkat T cells. J Immunol Methods (2017) 441:56–66. doi:10.1016/j.jim.2016.12.002

104. Jochema C, Hodge JW, Fantini M, Fujii R, Morillon YM II, Greiner JW, et al. An NK cell line (hANK) expressing high levels of granzyme and engineered to express the high affinity CD16 allele. Oncotarget (2016) 7(52):86359–73. doi:10.18632/oncotarget.13411

105. Samara P, Skopeliti M, Tsatias ML, Georgaki S, Gouloumis C, Voelter W, et al. A cytokine cocktail augments the efficacy of adoptive NK-92 cell therapy against mouse xenografts of human cancer. Anticancer Res (2016) 36(7):3737–82.

106. Allkins R, Burgess A, Kerbel R, Wels WS, Hynynen K. Early treatment of HER2-amplified brain tumors with targeted NK-92 cells and focused ultrasound improves survival. Neuro Oncol (2016) 18(7):974–81. doi:10.1093/neuonc/nox318

107. Clémenceau B, Valsesia-Wittmann S, Jallas AC, Vivien R, Rousseau R, Boissel L, Betancur M, Wels WS, Tuncer H, Klingemann H. Transfection with a human CD16 or with a chimeric antigen receptor reveals potential off-target interactions due to the IgG2 CH2-CH3 CAR-spacer. Front Immunol (2015) 2015:482089. doi:10.1155/2015/482089

108. Han J, Chu J, Keung Chan W, Zhang J, Wang Y, Cohen JB, et al. CAR-mediated NK cells targeting wild-type EGFR and EGFRVIII enhance killing of glioblastoma and patient-derived glioblastoma stem cells. Sci Rep (2015) 5:11483. doi:10.1038/srep11483

109. Schönfeld K, Sahm C, Zhang C, Naundorf S, Brendel C, Odendahl M, et al. Selective inhibition of tumor growth by clonal NK cells expressing an ErbB2-HER2-specific chimeric antigen receptor. Mol Ther (2015) 23(2):330–8. doi:10.1038/mt.2014.219

110. Boissel L, Betancur-Boissel M, Lu W, Krause DS, Van Etten RA, et al. A cytokine cocktail augments the efficacy of adoptive NK-92 cell therapy against mouse xenografts of human cancer. Oncotarget (2016) 18(7):974–81. doi:10.18632/oncotarget.13411

111. Chu J, Deng Y, Benson DM, He S, Hughes T, Zhang J, et al. CS1-specific chimeric antigen receptor (CAR)-engineered natural killer cells enhance in vitro and in vivo antitumor activity against human multiple myeloma. Leukemia (2014) 28(4):917–27. doi:10.1038/leu.2013.279

112. Li J, Liu H, Li L, Wu H, Wang C, Yan Z, et al. The combination of an oxygen-dependent degradation domain-regulated adenosineexpressing the chemokine RANTES/CC15 and NK-92 cells exerts enhanced antitumor activity in hepatocellular carcinoma. Oncol Rep (2013) 29(3):895–902. doi:10.3892/or.2012.2217

113. Boissel L, Betancur M, Lu W, Wels WS, Marino T, Van Etten RA, et al. Comparison of mRNA and lentiviral based transfection of natural killer cells with chimeric antigen receptors recognizing lymphoid antigens. Leuk Lymphoma (2012) 53(5):958–65. doi:10.1080/10428194.2011.634084

114. Esser R, Müller T, Stefes D, Kloess S, Seidel D, Gillies SD, et al. NK cells engineered to express a GD2-specific antigen receptor display built-in ADCC-like activity against tumour cells of neuroectodermal origin. J Cell Mol Med (2012) 16(3):569–81. doi:10.1111/j.1582-4934.2011.01343.x

115. Boissel L, Betancur M, Wels WS, Tuncer H, Klingenmann H. Transfection with mRNA for CD19 specific chimeric antigen receptor restores NK cell mediated killing of CLL cells. Leuk Res (2009) 33(9):1255–9. doi:10.1016/j.leukres.2008.11.024

116. MacLeod RA, Nagel S, Kaufmann M, Greulich-Bode K, Dreger HG. Multicolor-FISH analysis of a natural killer cell line (NK-92). Leuk Res (2002) 26(11):1027–33. doi:10.1016/S0145-2126(02)00053-3

117. Rosenberg SA, Dudley ME. Adoptive cell therapy for the treatment of patients with metastatic melanoma. Curr Opin Immunol (2009) 21(2):233–40. doi:10.1016/j.coi.2009.03.002

118. Besser MJ, Shapira-Frommer R, Treves AJ, Zippel D, Itzhaki O, Hershkovitz L, et al. Clinical responses in a phase II study using adoptive transfer of short-term cultured tumor infiltration lymphocytes in metastatic melanoma patients. J Immunother (2010) 33(4):563–74. doi:10.1097/jim.0b013e3181e00447

119. Call ME, Schnell JR, Xu C, Lutz RA, Chou JJ, Wucherpfennig KW. The structure of the zetazeta transmembrane dimer reveals features essential for its assembly with the T cell receptor. Cell (2006) 127(2):355–68. doi:10.1016/j.cell.2006.08.044
Efficient Tumaini B, Lee DW, Lin T, Castiello L, Stroncek DF, Mackall C, et al.
Savoldo B, Ramos CA, Liu E, Mims MP, Keating MJ, Carrum G, et al.
Yamasaki S, Ishikawa E, Kohno M, Saito T. The quantity and duration of FcRgmma signals determine mast cell degranulation and survival. Blood (2004) 103(8):3093–101. doi:10.1182/blood-2003-08-2944
Hudecek M, Lupo-Stanghellini MT, Kossash PL, Sommerner D, Jensen MC, Rader C, et al. Receptor affinity and extracellular domain modifications affect tumor recognition by ROR1-specific chimeric antigen receptor T cells.
Clin Cancer Res (2013) 19(12):3153–64. doi:10.1158/1078-0432.CCR-13-0300.

Hombach A, Heuser C, Gerken M, Fischer B, Lewalter K, Diehl V, et al. T cell activation by recombiant FcεRIlong gamma chain immune receptors: an extracellular spacer domain impairs antigen-dependent T cell activation but not antigen recognition. Gene Ther (2000) 7(12):1067–75. doi:10.1038/sj.gt.3301195.

Dotti G, Gottschalk S, Savolbo B, Brenner MK. Design and development of therapies using chimeric antigen receptor-expressing T cells.
Immunol Rev (2014) 257(1):107–26. doi:10.1111/imr.12131.

Finnh YM, Akbar AN, Lawson AD. Activation of resting human T cells with chimeric receptors: costimulation from CD28, inducible costimulator, CD134, and CD137 in series with signals from the TCR zeta chain. J Immunol (2004) 172(1):104–13. doi:10.4049/jimmunol.172.1.104.
Klumper HM, Bebbington CR, Weir AN. Chimeric receptors providing both primary and costimulatory signaling in T cells from a single gene product. J Immunol (1998) 161(6):2791–7.

Moinoeng P, Lucchin JL, McConkey DJ, Letourneur F, Malissen B, Kochan J, et al. CD3 eta dependence of the CD2 pathway of activation in T lymphocytes and natural killer cells. Proc Natl Acad Sci U S A (1992) 89(4):1492–6. doi:10.1073/pnas.89.4.1492.

Vivier E, Ackerly M, Rotch N, Anderson P. Structure and function of the gene product.
Eur J Immunol (2007) 37(2):408–15. doi:10.1002/eji.200533797.

Shimasaki N, Fujisaki H, Cho D, Masselli M, Lockey T, Eldridge P, et al. A serious adverse event after successful gene therapy for SCID-X1. Science (2003) 300(5644):415–9. doi:10.1126/science.1085847.

Hacein-Bey-Abina S, von Kalle C, Le Deist F, Wulfraat N, McIntyre E, et al. A serious adverse event after successful gene therapy for X-linked severe combined immunodeficiency. N Engl J Med (2003) 348(3):255–6. doi:10.1056/NEJM200301163480314.

Anurathapan U, Chan RC, Hindi HF, Mucharla R, Baquian P, Hayes BC, et al. Kinetics of tumor destruction by chimeric antigen receptor-modified T cells. Mol Ther (2014) 22(3):623–33. doi:10.1038/mt.2013.262.

Imai C, Iwamoto S, Campagna D. Genetic modification of primary natural killer cells overcomes inhibitory signals and induces specific killing of leukemic cells. Blood (2005) 106(1):376–83. doi:10.1182/blood-2004-12-4797.

Johnson LA, June CH. Driving gene-engineered T cell immunotherapy of cancer. Cell Res (2017) 27(1):38–58. doi:10.1038/cr.2016.154.

Sutlif T, Nystrom S, Gilliam M, Stellan B, Applequist J, Alci E. Inhibition of intracellular antiviral defense mechanisms augments lentiviral transduction of human natural killer cells: implications for gene therapy. Hum Gene Ther (2012) 23(10):1090–100. doi:10.1089/hum.2012.080.

Li L, Liu LN, Feller S, Allen C, Shivakumar R, Fratantoni J, et al. Expression of chimeric antigen receptors in natural killer cells with a regulatory-compliant non-viral method. Cancer Gene Ther (2010) 17(3):147–54. doi:10.1038/cgt.2009.61.

Shimasaki N, Fujisaki H, Cho D, Masselli M, Lockey T, Eldridge P, et al. A clinically adaptable method to enhance the cytotoxicity of natural killer cells against B-cell malignancies. Cyttherapy (2012) 14(7):830–40. doi:10.3109/14653249.2012.761519.

Tassev DV, Cheng M, Cheung NK. Retargeting NK92 cells using an HLA-A2-restricted, EBNA3C-specific chimeric antigen receptor. Cancer Gene Ther (2012) 19(2):84–100. doi:10.1038/cgt.2011.66.

Locatelli F, Moretta F, Bresca L, Merli P. Natural killer cells in the treatment of high-risk leukemia. Semin Immunol (2014) 26(2):173–9. doi:10.1016/j.smim.2014.02.004.

Yoon SR, Lee YS, Yang SH, Ahn KH, Lee JH, Lee JH, et al. Generation of donor natural killer cells from CD34(+)-progenitor cells and subsequent infusion after HLA-mismatched allogeneic hematopoietic cell transplantation: a feasibility study. Bone Marrow Transplant (2010) 45(6):1038–46. doi:10.1038/bmt.2009.304.

Rezvani K, Rouce RH. The application of natural killer cell immunotherapy for the treatment of cancer. Front Immunol (2015) 6:578. doi:10.3389/fimmu.2015.00578.

Asai O, Longo DL, Tian ZG, Hornung RL, Taub DB, Russetti FW, et al. Suppression of graft-versus-host disease and amplification of graft-versus-tumor effects by activated natural killer cells after allogeneic bone marrow transplantation. J Clin Invest (1998) 101(9):1835–42. doi:10.1172/JCI12568.

Shlomchik WD, Couzens MS, Tang CB, McNiff J, Robert ME, Liu J, et al. Prevention of graft versus host disease by inactivation of host antigen-presenting cells. Science (1999) 285(5426):412–5. doi:10.1126/science.285.5426.412.
177. Fujiwara S, Akiyama M, Yamakido M, Seyama T, Kobuke K, Hakoda M, et al. Cryopreservation of human lymphocytes for assessment of lymphocyte subsets and natural killer cytotoxicity. J Immunol Methods (1986) 90(2):265–73. doi:10.1016/0022-1759(86)90084-0
178. Dominguez E, Lowdell MW, Perez-Cruz I, Madrigal A, Cohen SB. Natural killer cell function is altered by freezing in DMSO. Biochem Soc Trans (1997) 25(2):175S. doi:10.1042/bst025175s
179. Yoshol H, Dullens HF, Den Otter W, Vliegenthart JF. Human natural killer cells: a convenient purification procedure and the influence of cryopreservation on cytotoxic activity. J Immunol Methods (1993) 165(1):21–30. doi:10.1016/0022-1759(93)90102-D
180. Domogala A, Madrigal JA, Saudemont A. Cryopreservation has no effect on function of natural killer cells differentiated in vitro from umbilical cord blood CD34+ cells. Cytoterapy (2016) 18(6):754–9. doi:10.1016/j.jcyt.2016.02.008
181. Koehl U, Brehm C, Huenecke S, Zimmermann SY, Kloeß S, Brehm M, et al. Clinical grade purification and expansion of NK cell products for an optimized manufacturing protocol. Front Oncol (2013) 3:118. doi:10.3389/fonc.2013.00118
182. Lapteva N, Durett AG, Sun J, Rollins LA, Huye LL, Fang J, et al. Large-scale rapid enrichment and selective cytotoxicity of gene-modified effectors that target CD19 and expressing IL-15 have long term persistence and exert potent anti-leukemia activity. Blood (2017) 130(21):2295–306. doi:10.1182/blood-2017-06-793141
183. Liu E, Tong Y, Dotti G, Savoldo B, Muftuoglu M, Kondo K, et al. Cord blood-derived natural killer cells engineered with a chimeric antigen receptor targeting CD19 and expressing membrane-bound interleukin-15. Blood (2013) 122(7):1081–8. doi:10.1182/blood-2014-02-556837
184. Sahin C, Schonfeld K, Wels WS. Expression of IL-15 in NK cells results in rapid enrichment and selective cytotoxicity of gene-modified effectors that carry a tumor-specific antigen receptor. Cancer Immunol Immunother (2012) 61(9):1451–61. doi:10.1007/s00262-012-1212-x
185. Anthony GK, Dudek AZ. Interleukin 2 in cancer therapy. Curr Med Chem (2005) 12(29):3297–302. doi:10.2174/092986705773176410
186. Becknell B, Caligiuri MA. Interleukin-2, interleukin-15, and their roles in the treatment of aggressive T cell malignancies. Curr Med Chem Anti-Cancer Agents (2005) 5(2):159–64. doi:10.2174/092986705350176410
187. Fujisaki H, Kakuda H, Shimasaki N, Imai C, Ma J, Lockey T, et al. Expansion of genetically altered, interleukin 2-independent natural killer cell lines suitable for adoptive cellular immunotherapy. J Immunol (1999) 162(8):4010–7. doi:10.4049/jimmunol.162.8.4010
188. Waldmann TA, Lugli E, Roederer M, Perera LP, Smedley JV, Macallister RP, et al. Autonomous growth and increased cytotoxicity of natural killer cells differentiated in vitro from umbilical cord blood. J Immunol (1998) 91(10):3850–61. doi:10.4049/jimmunol.91.10.3850
189. Tam YK, Maki G, Miyagawa B, Hennemann B, Tonn T, Klingemann HG. Characterization of genetically altered, interleukin-2 independent natural killer cell lines suitable for adoptive cellular immunotherapy. Hum Gene Ther (1999) 10(8):1359–73. doi:10.1089/10430349950018030
190. Conlon KC, Lugli E, Wilkes HC, Rosenberg SA, Fojo AT, Morris JC, et al. Reduction of hyperproliferation, activation and death of natural killer cells and CDR T cells, and cytokine production during first in-human clinical trial of recombinant human interleukin-15 in patients with cancer. J Clin Oncol (2015) 33(1):74–82. doi:10.1200/JCO.2014.57.3329
191. Mukherjee D, Cai L, Chakraborty P, Conlon KC, Lugli E, Wilkes HC, et al. A transgene-encoded cell surface polypeptide for selection, in vivo tracking, and ablation of engineered cells. Front Immunol (2016) 7:75. doi:10.3389/fimmu.2016.00075
192. Hoey V, Savolbo B, Quintarelli C, Mahendravada A, Zhang M, Vera J, et al. Engineering CD19-specific T lymphocytes with interleukin-15 and a suicide gene to enhance their anti-lymphoma/leukemia effects and safety. Leukemia (2010) 24(6):1165–70. doi:10.1038/leu.2010.75
193. Straathof KC, Pulé MA, Yostna P, Dotti G, Vanin EF, Brenner MK, et al. An inducible caspase 9 safety switch for adoptive cell therapy. Blood (2005) 105(15):4247–54. doi:10.1182/blood-2004-11-4564
194. Vanin EF, Yostna P, Chu Y, Hochberg J, Yahr A, Ayello J, et al. Targeting CD20+ aggressive B-cell non-Hodgkin lymphoma by anti-CD20 CAR mRNA-Modified expanded natural killer cells in vitro and in NSG mice. Cancer Immunol Res (2015) 3(4):333–44. doi:10.1158/2326-6066.CIR-14-0114
195. Wang X, Chang WC, Wong CW, Colcher D, Sherman M, Ostberg JR, et al. Expression of a CD20-specific chimeric antigen receptor enhances cytotoxic activity of NK cells and overcoming NK-resistant of lymphoma and leukemia cells. Cancer Immunol Immunother (2008) 57(3):411–23. doi:10.1007/s00262-007-0383-3
196. Liu H, Wada M, Firor AE, Pinz KG, Jares A, Chen KH, et al. Novel anti-CD3 targeted CAR for highly differentiated aggressive T cell malignancies. Oncotarget (2016) 7(35):56219–32. doi:10.18632/oncotarget.11019
197. Konishi K, Yoda K, Yozu N, Gotoda T, Kato K, et al. Transfection of chimeric anti-CD138 specific gene enhances natural killer cell activation and killing of myeloma cells. Mol Oncol (2014) 8(2):297–310. doi:10.1016/j.molonc.2013.12.001
198. van der Velden GR, Mijnheer J, van den Berg M, et al. Novel anti-CD3 chimeric antigen receptor targeting of aggressive T cell malignancies. Mol Cancer Ther (2016) 15(7):1907–13. doi:10.1158/1535-7163.MCT-15-0803
199. Liu H, Wada M, Firor AE, Pinz KG, Jares A, Chen KH, et al. Novel anti-CD3 chimeric antigen receptor targeting of aggressive T cell malignancies. Oncotarget (2016) 7(35):56219–32. doi:10.18632/oncotarget.11019
200. Sinha S, Athar K, Sanford J, Holloway R, et al. Optimization of the CD123 CAR construct to improve ADCC activity. Mol Ther (2017) 25(2):269–78. doi:10.1016/j.ymthe.2016.12.023

Frontiers in Immunology | www.frontiersin.org February 2018 Volume 9 Article 283 11
214. Kruschinski A, Moosmann A, Poschke I, Norell H, Chmielewski M, Seliger B, et al. Engineering antigen-specific primary human NK cells against HER-2-positive carcinomas. *Proc Natl Acad Sci U S A* (2008) 105(45):17481–6. doi:10.1073/pnas.0804788105

215. Liu H, Yang B, Sun T, Lin L, Hu Y, Deng M, et al. Specific growth inhibition of ErbB2-expressing human breast cancer cells by genetically modified NK92 cells. *Oncol Rep* (2015) 33(1):95–102.

216. Uherek C, Tomn T, Uhreke B, Becker S, Schnierle B, Klingemann HG, et al. Retargeting of natural killer-cell cytolytic activity to ErbB2-expressing cancer cells results in efficient and selective tumor cell destruction. *Blood* (2002) 100(4):1265–73.

217. Zhao Q, Ahmed M, Tassev DV, Hasan A, Kuo TY, Guo HF, et al. Affinity maturation of T-cell receptor-like antibodies for Wilms tumor 1 peptide greatly enhances therapeutic potential. *Leukemia* (2015) 29(11):2238–47. doi:10.1038/leu.2015.125

218. Park H, Awasthi A, Ayello J, Chu Y, Riddell S, Rosenblum J, et al. ROR1-specific chimeric antigen receptor (CAR) NK cell immunotherapy for high risk neuroblastomas and sarcomas. *Biol Blood Marrow Transplant* (2017) 23(3 Suppl):S136–7. doi:10.1016/j.bbmt.2017.01.056

219. Tagaya Y, Bamford RN, DeFilippis AP, Waldmann TA. IL-15: a pleiotropic cytokine with diverse receptor/signaling pathways whose expression is controlled at multiple levels. *Immunity* (1996) 4(4):329–36. doi:10.1016/S1074-7613(00)80246-0

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 Mehta and Rezvani. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.