1 | INTRODUCTION

Natural killer (NK) cells are CD56\(^+\), CD3\(^-\) large granular lymphocytes that are a vital component of the innate immune system in killing cancer cells. NK cells were first identified in 1964 when lethally irradiated mice rejected bone marrow allografts without prior sensitization (Cudkowicz & Stimpfling, 1964). Subsequently, NK cells were described in the 1970s as major histocompatibility complex (MHC) unrestricted killers due to their ability to kill tumour targets without prior sensitization to antigens (Herberman, Nunn, Holden, & Lavrin, 1975). Cells may lose MHC class-I molecules under malignant or viral conditions, and this "loss of self" leads to an increased susceptibility to NK cell killing. After the description of the "missing self" hypothesis by Kärre, Ljunggren, Piontek, and Kiessling (1986) to explain this phenomenon, several families of NK cells receptors were identified that recognize MHC and regulate NK-cell activity. Because of the ability of NK cells to lyse cells without previous exposure to tumour antigens, NK cells are of great interest as therapeutic targets to treat cancer and improve the benefits of hematopoietic cell transplantation (HCT) and cancer therapy. Therefore, great strides have been made to manipulate NK cell antitumour properties in immunotherapy. In this review, we discuss the current status of NK cells in immunotherapy (Table 1). We also consider future directions to stimulate endogenous NK cells using bispecific, trispecific and tetraspecific killer engagers, anti-KIR antibodies, and the expansion of NK cells to enhance NK cell activity and specificity in treating cancers.
by the expression of the surface adhesion marker CD56 and lack of the TCR/CD3 complex. NK cells represent 5%–15% of circulating lymphocytes in humans and can be categorized into two distinct phenotypic and functional subsets based on their surface density of CD56. Approximately 2%–10% of NK cells have high surface density expression of CD56 and are designated as CD56bright. This population produces high levels of cytokines, proliferates robustly in response to IL-2, lacks expression of CD16 and killer-immunoglobulin-like receptors (KIRs) and is poor mediators of cytotoxicity (Jacobs et al., 2001). This population is found predominantly in secondary lymphoid tissues (Fehniger et al., 2003). In contrast, 90% of NK cells that are found in peripheral blood and are designated as CD56dim. This subset demonstrates limited proliferation in response to IL-2, expresses CD16 and KIR, and is potently cytotoxic.

NK cells are distinguished from other innate lymphoid cells by their dependence on IL-15 for development and their intrinsic cytotoxic ability. This cytotoxic activity is mediated by a variety of mechanisms. NK cells express a wide variety of germline receptors, such as NKG2D, which bind to stress-induced ligands found on tumour cells (Smyth et al., 2005). Once bound to tumour cells, NK cells degranulate and release granzyme B and perforin to induce apoptosis in the tumour cell (Voskoboinik, Smyth, & Trapani, 2006). NK-cell degranulation can also occur through antigen-dependent cellular cytotoxicity (ADCC) in which the Fc portion of tumour antibodies binds to the low-affinity Fc receptor, CD16, on NK cells. Additionally, NK cells can mediate tumour killing through death-receptor pathways. NK cells express tumour-necrosis factor (TNF) family members FasL or TNF-related apoptosis-inducing ligand (TRAIL) which interacts, respectively, with ligands

TABLE 1  Clinical studies of NK cells in cancer immunotherapy

| Therapy                              | Stage            | Disease                                      | Interpretation                                                                 |
|--------------------------------------|------------------|----------------------------------------------|--------------------------------------------------------------------------------|
| Adoptive transfer                    |                  |                                              |                                                                                 |
| Autologous NK cells                  | Clinical trials  | Haematologic malignancies                    | Safe but limited clinical efficacy                                            |
| Allogeneic NK cells                  | Clinical trials  | Haematologic malignancies                    | Promising data in AML but overcoming host immunity is a challenge             |
| K562 NK-cell expansion               | Clinical trial   | AML                                          | Easy to expand; safe; trials ongoing                                          |
| iPSC-NK cells                        | Preclinical      | Many cancers                                 | Clonal starting cell lines; easy to expand; easy to genetically modify; unlimited capacity; off-the-shelf |
| CAR-NK cells                         | Clinical trial   | B-cell malignancies                          | Use against a specific tumour antigen; many trials planned using this approach|
|                                      |                  |                                              |                                                                                 |
| Cytokines                            |                  |                                              |                                                                                 |
| IL-2                                 | FDA approved     | Melanoma and kidney cancer                   | Repeated injections well tolerated but high treatment limited by vascular leak and limited efficacy |
| rhIL-15                              | Clinical trial   | Many tumours                                 | Activates NK cells without activating Treg cells; tested daily or five times per week |
| IL-15 complexes (ALT-803)            | Clinical trial   | Many tumours                                 | Activates NK cells without activating Treg cells; can be given weekly and is safe; combination testing in progress |
| Depletion of Treg cells (IL2DT)      | Clinical trial   | AML                                          | Improved efficacy of haploidentical NK-cell transfer; safe;                    |
| Induction of adaptive NK cells       |                  |                                              |                                                                                 |
| FATE-100 (GSK3 inhibitor)            | Clinical trial   | AML, ovarian cancer and other solid tumours  | Enhances NK-cell killing; clinical testing in progress                        |
| Agonists of NK cell activating receptors |                |                                              |                                                                                 |
| Tumour-targeting mAbs                | FDA approved     | Many tumours                                 | Use against a specific tumour antigen to promote ADCC                         |
| Immune engagers                      | Preclinical studies | AML and solid tumours                      | Use against a specific tumour antigen; many be developed with different structures |
| ADAM17 inhibitor                     | Clinical trial   | B-cell lymphoma                              | The goal is to enhances ADCC by preventing CD16 clipping; in clinical testing; debate as to whether inhibitor will prevent release of NK cells to mediate serial killing |
| Checkpoint inhibitors                |                  |                                              |                                                                                 |
| anti-KIR mAbs (IPH2101)              | Clinical trials  | AML, Multiple myeloma                        | Safe; can be used in any patient without KIR genotyping; combination testing also being tested |
| anti-NKG2A mAbs (IPH2201-monalizumab)| Clinical trials  | Lymphoma, multiple myeloma                   | In clinical testing                                                           |
Las and TRAIL-receptor expressed on tumour cells (Screpanti, Wallin, Ljunggren, & Grandien, 2001). In addition, NK cells secrete proinflammatory cytokines and chemokines that can have antitumour activity. This antitumour activity is regulated by receptors found on the NK cells.

3 | NK-CELL RECEPTORS

Therapeutic strategies using allogeneic NK cells in treating cancer are based on our understanding of the signalling pathways that regulate the antitumour activity of NK cells.

NK-cell activity is mediated through a balance of activating and inhibitory receptors. The combination of receptors that an individual has ultimately determines the cytotoxic activity of the NK cell. Therefore, certain human tumours are more amenable to NK cell activity than others. Inhibitory receptors prevent NK cells from killing "self" expressing normal tissue, protecting against autoimmune diseases. Major histocompatibility class-I (MHC-I) molecules provide an inhibitory signal to the NK cell when ligated to an inhibitory receptor, preventing degranulation and cytokine production. These inhibitory receptors include the following: (a) killer-immunoglobulin-like receptors (KIRs); (b) NKG2A/CD94; and (c) leucocyte immunoglobulin-like receptors (LILRs; Lanier, 2008). In addition to inhibitory receptors, NK cells express germline encoded activating receptors, including CD94/NKG2C, the SLAM family receptors and the low-affinity Fc receptor CD16 which mediates antibody-dependent cellular cytotoxicity (ADCC). Great reviews have been published on these activating receptors, and they will not be discussed further (Lanier, 2015; Raulet, Gasser, Gowen, Deng, & Jung, 2013).

Of these receptors, most studies have focused on ways to manipulate NK cells to lessen interactions between inhibitory KIRs and MHC-I ligands on target cells. Inhibitory KIRs are transmembrane molecules that are found on chromosome 19 and interact with HLA-A, HLA-B and HLA-C allotypes. KIR genes are highly polymorphic, and these polymorphisms determine their binding affinities to MHC-I molecules (Hilton & Parham, 2017). In general, KIR genes can be divided into two broad haplotypes, KIR-A and KIR-B. KIR-A contains only one activating receptor, whereas KIR-B contains two or more (Pyo et al., 2010). The effectiveness of NK cell therapy is based on the variations in ligands among individuals.

KIRs became a major focus for NK-cell immunotherapy when the Perugia group showed that KIR ligand mismatch between donors and patients was associated with improved outcomes in myeloid leukaemia after T-cell deplete haploidentical transplantation (Ruggeri et al., 2002). Since then, KIR ligand incompatibility in hematopoietic cell transplantation (HCT) has been highly studied and much progress has been made in understanding NK-cell function and the immunogenetics of NK-cell receptors. The presence of activating KIR2DS1 has been shown to prevent relapse in AML patients (Venstrom et al., 2012). Additionally, studies have emerged showing that donors with KIR-B haplotypes can protect against relapse in both AML and non-Hodgkin lymphoma in unrelated donor transplants (Bachanova et al., 2016; Cooley et al., 2010, 2014). In addition to relapse protection, recent studies have shown that donor-recipient matching for KIR genotypes can protect against chronic GVHD and that missing inhibitory KIR ligands reduced relapse after unrelated donor transplantation (Faridi et al., 2016). Selection of donors with favourable KIR-B haplotypes is important in HCT, and formal prospective clinical testing is in progress. Strategies for donor selection based on allele level KIR typing are also being contemplated as higher resolution typing methods become available.

4 | NK-CELL EDUCATION

Inhibitory receptors that recognize MHC also serve to educate or license NK cells to acquire function to respond to MHC-I-deficient cells. The process by which NK cells acquire function is known as NK-cell licensing or NK-cell education. Simply put, the expression of inhibitor receptors on NK cells during development is crucial for functional competence. NK cells that do not express inhibitory receptors, such as KIR and NKG2A, lack the signalling to undergo education and are therefore hyporesponsive. For example, NK cells that are KIR3DL+ from an HLA-Bw6 individual are less responsive compared with KIR3DL+ NK cells from an HLA-Bw4 individual (because Bw4 is the ligand for KIR3DL1; Anfossi et al., 2006; Cooley et al., 2007). Therefore, it has been proposed that NK cells can be tuned by the strength of their class I recognizing inhibitory signals.

5 | DISCOVERY OF VIRAL-INDUCED ADAPTIVE NK CELLS

NK cells were traditionally seen as short-lived lymphocytes that lack antigen specificity and are considered part of the innate immune system. Over the past several years, this view has been challenged as studies have provided evidence that in response to stress, NK cells do possess some adaptive immune traits as a result of their ability to be educated. Recently, the novel concept of NK-cell memory emerged with the identification of NK cells in mice that expand in response to cytomegalovirus (CMV) infection (Sun, Beilke, & Lanier, 2009). In humans, we and others have discovered that NKG2C+ NK cells specifically expand in response to human CMV. These NKG2C+ NK cells express an inhibitory receptor for self-HLA and progressively acquire CD57, a marker of maturation. (Foley, Cooley, Verneris, Curtsinger, et al., 2012; Foley, Cooley, Verneris, Pitt, et al., 2012; Lopez-Vergès et al., 2011). Whether these NK cells possess all of the attributes ascribed to classical memory T and B cells or whether they are "memory-like" is still a matter of debate and terminology. Regardless, the NKG2C+ CD57+ NK cells are referred to as "adaptive" NK cells as they exhibit characteristics of immunological memory such as viral antigen specificity, clonal-like expansion, and persistent and rapid recall response. These adaptive NK cells are transplantable from CMV-seropositive donors which result in enhanced function of NK cells when latent CMV is encountered in a CMV-seropositive patient (Foley, Cooley, Verneris, Curtsinger, et al., 2012; Foley, Cooley, Verneris, Pitt, et al., 2012).
We believe that these adaptive NK cells are induced by CMV specifically and are not induced by other pathogens. Although others have suggested that NKG2C+ NK cells are associated with the hantavirus in Sweden and the chikungunya virus in Africa, all of the patients participating in these studies were also co-exposed to CMV, suggesting that the NKG2C+ NK cells were caused by CMV infection rather than infection with other viruses (Braun et al., 2014; Petitdemange et al., 2016). To further highlight the specificity of CMV-induced adaptive NK cells, recent studies have shown that CD57+ NKG2C+ NK cells are not expanded in response to Epstein–Barr Virus (EBV) and that human HSV-2 infection did not induce adaptive NK cells (Björkström, Svensson, Malmberg, Eriksson, & Ljunggren, 2011; Hendricks et al., 2014). Even though it is not fully known whether other pathogens can induce adaptive NK cells, studies conducted thus far point to CMV-specific induction of adaptive NK cells.

The unique characteristics of adaptive NK cells make them of great interest in the quest for cancer immunotherapies. Adaptive NK cells do not express FcR, SYK, EAT-2 and PLZF and have an epigenetic methylation signature similar to that of CD8+ T cells (Schlums et al., 2015). We have found that adaptive NK cells produce significantly more IFN-γ and TNF in response to CD16. Degranulation was similar between adaptive and conventional NK cell subsets, reflecting the known lower activation threshold required for degranulation relative to cytokine production in NK cells. Thus, adaptive NK cells appear to be specialized for enhanced target recognition through CD16 for not only CMV infection, but also against tumour targets (Zhang, Scott, Hwang, & Kim, 2013). We think these cells are optimally primed by CMV to change the repertoire of NK cells to fight cancer. To this end, we have shown that higher numbers of CMV-induced CD57+ NKG2C+ adaptive NK cells (compared to conventional NK cells) after hematopoietic cell transplantation for haematologic malignancy correlates with relapse protection. These adaptive NK cells also persist in the body for at least a year in post-HCT recipients (Cichocki et al., 2016; Foley, Cooley, Verneris, Curtsinger, et al., 2012; Foley, Cooley, Verneris, Pitt, et al., 2012). Thus, we have shown a correlation between adaptive NK cells and clinical outcomes.

Despite the potential clinical significance of mature adaptive NK cell subsets that express CD57, relatively little is known about how CMV drives the expansion of NK cells and the signals that drive late-stage NK cell maturation. Recently, we discovered that the addition of glycogen synthase kinase 3 (GSK3) inhibitor CHIR99021 during ex vivo NK cell expansion with IL-15 significantly enhanced CD57 acquisition and maturation. GSK inhibition also leads to increased expansion of several transcription factors associated with late-stage NK cell maturation, such as T-BET and BLIMP-1, without affecting NK-cell viability. These NK cells expanded in the presence of CHIR99021 produced significantly more IFN-γ and TNF in response to CD16 and had greater ADCC against tumour targets compared with conventional NK cells (Figure 1). Additionally, in a xenograft model of ovarian cancer, NK cells expanded with CHIR99021 showed greater antitumour efficacy (Cichocki et al., 2017). These findings have immediate clinical applications and the first-in-human clinical trial using NK cells that are expanded with CHIR99021 and IL-15 is underway.

Recently, we have developed FATE-NK100, a GMP product that is enriched for adaptive NK cells. The main challenge in enriching for CD57+ adaptive NK cells is that they are cocultured with IL-15 which preferentially expands less mature NK cell subsets that fail to terminally differentiate in culture. In the FATE-NK100 protocol, PBMCs from a CMV seropositive donor are depleted of CD3+ T cells and CD19+ B cells and are cultured for 7 days with IL-15 and a glycosynthes kinase 3-beta inhibitor; these adaptive NK cells are then infused into patients. We currently have a clinical trial in progress using FATE-NK100 in combination with IL-2 in patients with refractory or relapsed AML, ovarian cancer and other solid tumours.

6 | ADOPTIVE NK-CELL THERAPY

The first studies in NK cell adoptive transfer used autologous NK cells to treat cancer by stimulating them with cytokines and type-I interferons to further activate NK cells and improve their antitumour responses. While an increase in NK-cell activity against malignant cells was seen with this method, limited success was observed in patients treated with cytokines such as IL-2. Recently, we have learned that IL-2 therapy results in activation of regulatory T cells (Tregs) which inhibits NK cell function and limits their antitumour activity (Ghiringhelli et al., 2005; Verneris, 2013). Subsequently, we and others are exploring the possibility of using allogeneic NK cells from related donors in adoptive transfer over autologous NK cells.

A second method to deliver allogeneic NK cells to the patient involved adoptive transfer of donor NK cells enriched ex vivo and infused into the patient. NK cells from healthy, related donors have the advantage of being fully functional and educated in a nonimmunosuppressive environment. Additionally, allogeneic NK cells may have some degree of receptor/HLA mismatch mimicking missing self. The first trial involving adoptive transfer using allogeneic NK cells was conducted at the University of Minnesota in 2005 (Miller et al., 2005). This study included 43 patients with metastatic melanoma, metastatic renal cell carcinoma or poor prognosis acute myeloid leukaemia (AML). PBMCs were collected from haploidentical-related donors and then depleted of CD3 (now CD3 and CD19) cells overnight before being incubated with IL-2. Prior to NK-cell infusion, patients underwent a regimen that included one of three chemotherapy preps: (a) high cyclophosphamide and fludarabine (Hi-Cy [60 mg/kg × 2 days]/Flu [25 mg/m² × 5 days]); (b) low cyclophosphamide and methylprednisolone (Lo-Cy [750 mg/m²]/mPred [1,000 mg/m²]); and (c) fludarabine alone (25 mg/m² × 5 days). Following infusion of NK cells, patients received IL-2 daily (1.75 × 10⁶ U/m²) for 14 days. NK-cell expansion was only observed in patients receiving the preparatory regimen of Hi-Cy/Flu. Successful expansion of NK cells was defined by a detection of >100 NK cells/μl of blood 12–16 days after infusion. There was a clear association between NK-cell expansion and clinical outcomes; 30% of poor prognosis AML patients achieved complete remission. These results suggest that NK cells themselves play a role in the antileukaemia response over the Hi-Cy/Flu chemotherapy regimen.
Some trials have shown that KIR ligand mismatching between donor NK cells and recipient correlated with better NK cell therapy. Shi et al. (2008) infused KIR–mismatched NK cells into 10 patients with relapsed multiple myeloma and followed 14 days later with an autologous stem cell graft; half of the patients achieved near complete remission. In paediatric AML patients, KIR–HLA mismatched NK cells prolonged disease-free survival and OS (Rubnitz et al., 2010). Additionally, it has been shown that KIR–mismatched NK cells could be transplanted into elderly patients with high-risk AML who are not candidates for stem cell transplantation and was not associated with toxicity (Curti et al., 2011). After NK cell transplantation, leukaemia-associated transcripts disappeared in elderly patients. Most recently, it was shown that haploidentical NK cells induce remissions in non-Hodgkin’s lymphoma patients (Bachanova et al., 2018). These studies point to the utility of allogeneic NK-cell transplants in the antitumour response in a variety of cancers.

7  USE OF CYTOKINES TO ENHANCE AND EXPAND NK CELLS IN ADOPTIVE THERAPY

Overcoming the immunosuppressive tumour environment is an attractive therapeutic option for improving NK cell antitumour activity. Thus, approaches to enhance NK-cell antitumour functionality before adoptive transfer are being explored. The use of IL-2 to expand NK cells showed no clinical advantage as Tregs express a high affinity IL-2 receptor (CD25) which competes for IL-2 and dampens NK-cell proliferation (Ghiringhelli et al., 2005). We have since overcome this challenge with the use of IL-2 diphtheria toxic fusions (IL2DT) to deplete Tregs (Bachanova et al., 2014). IL2DT is a recombinant fusion protein that includes sequences for diphtheria toxin followed by truncated amino acid sequences for IL-2 that will deplete all cells expressing IL-2 receptors, including Tregs. After patients received the Hi-Cy/Flu chemotherapy regimen, 15 patients also received IL2DT and NK-cell expansion was assessed. In refractory AML patients, IL2DT led to improved rates of in vivo NK-cell expansion and AML remission. This study shows the importance of interrupting the immunosuppressive environment in order to expand NK cells.

Another way to circumvent this challenge is to use cytokines that activate NK cells but not Tregs. Currently, IL-15 seems to hold the most promise. IL-15 is necessary for homoeostasis of T cells and NK cells and has been shown to stimulate NK-cell proliferation in a similar but not identical manner to that of IL-2 (Huntington et al., 2009). Several IL-15 products are in clinical development. In 2015, the first clinical trial was published in which recombinant IL-15 delivered to patients with metastatic cancer led to expansion of NK cells...
and the clearance of lung lesions in patients (Conlon et al., 2015). Other studies have shown that preactivation with IL-12, IL-18 and IL-15 differentiates NK cells in adaptive NK cells, suggesting promise as an immunotherapy strategy (Romee et al., 2016). It is believed that endogenous IL-15 in serum binds to IL-15Ra to form a natural complex. This natural complex interacts with IL-2Rβ on NK cells and CD8+ T cells through a process called IL-15 trans-presentation. This process is thought to overcome the Treg stimulatory effects of IL-2 (Cooper et al., 2002; Oh, Perera, Burke, Waldmann, & Berzofsky, 2004; Wong, Jeng, & Rhodes, 2013). The recently designed IL-15 superagonist/IL-15Ra-Sushi-Fc fusion complex (ALT 803) exhibits greater activity than that of native IL-15 in various malignancies (Felices et al., 2017; Kim et al., 2016). The design included a mutant IL-15, the addition of a sushi domain to inhibit complement activation, increased avidity of the molecule to IL-2Rβ on NK cells and increased half-life and stability by inclusion of the Fc domain (Xu et al., 2013). An additional advantage to the IL-15 complexes, like ALT-803, is that they stabilize IL-15 so it can be given in fewer doses and allow for better homing to lymphoid tissue. The first-in-human studies of ALT-803 show that ALT-803 is well tolerated by patients. Additionally, ALT-803 was shown to promote NK and CD8+ T-cell expansion in vivo without stimulating Treg cells. IL-15 complexes hold much promise in cancer immunotherapy, and the future will include combining IL-15 with other methods to activate NK cells.

8 | STRATEGIES TO EXPAND NK CELLS FOR ADOPTIVE TRANSFER

In addition to cytokines, other avenues are being explored to expand NK cells. One popular approach is to expand NK cells from PBMCs using feeder cells, such as K562 cells modified with membrane-bound IL-15 or IL-21. Fujisaki et al. (2009) showed that the leukaemia cell line K562 was able to be modified with 41BB ligand (K562-mb15-41BBL) to generate human NK cells with enhanced antitumour activity. Coculture of the 41BB ligand induced a 21.6-fold expansion of NK cells from PBMCs in patients undergoing acute leukaemia; these NK cells are still highly function. Recently, Denman et al. (2012) developed a method to expand NK cells ex vivo using K562 feeder cells expressing membrane-bound IL-21 (mblL21). This method expands NK cells up to 35,000-fold in 3 weeks, and these NK cells still maintain antitumour activity. A Phase I clinical trial using mblL21 ex vivo to expand NK cells showed that these expanded NK cells can be safely infused posthaplo transplant in patients with leukaemia and that infusion of NK cells was associated with improved NK cell antitumour function and low relapse (Ciurea et al., 2017). These results represent a new way to expand NK cells that can be used to treat various cancers.

Although the use of haploidentical NK cells has shown promise form a variety of cancers, limitations still exist. One of the limitations to using NK cells isolated from peripheral blood that are CD3 and CD19 depleted is that the cellular produce still contains a mixture of cells, with only about 30%–50% of the infused cells being NK cells (Koepsell et al., 2013). To produce a homogenous NK cell product, studies have used NK cell derived from induced pluripotent stem cells (iPSCs). These iPSC-NK cells are able to be expanded in vivo and have been shown to be effective against leukaemia and ovarian cancer in xenograft models (Hermanson et al., 2016; Woll, Martin, Miller, & Kaufman, 2005). Recently, a good-manufacturing practice-compatible iPSC source and industry-friendly protocol to produce NK cells from iPSCs has been developed. This protocol makes large-scale quantities of "universal" NK cells that express no KIRs, making them unrestricted by HLA genotypes. This overcomes selection of NK cell donors for a particular patient (Zeng, Tang, Toh, & Wang, 2017) and offers a truly off-the-shelf advantage. These iPSCs show promise to be used NK-cell immunotherapy, but there is still much to be learned about their efficacy and viability in clinical trials.

9 | DESIGN OF CD16 TARGET AGENTS TO ENHANCE ADOPTIVE TRANSFER THERAPIES

Targeted immunotherapies are currently a subject of great clinical potential. Recently, a great deal of interest has been placed on genetic manipulations geared towards maximizing NK-cell function, such as the generation of chimeric antigen receptor-expressing NK cells. Although these approaches show great potential for improving NK-cell adoptive therapy, they require a personalized approach that is expensive, time-consuming and difficult to apply on a large scale. Therefore, we have focused on two strategies to augment the ADCC in NK-cell adoptive transfers—improving maintenance of the CD16 receptor and using bispecific, trispecific and tetraspecific killer engagers to improve targeting to tumours (BiKEs, TRiKEs and TetraKEs, respectively; Figure 1).

One strategy to enhance NK cell function is to exploit the ability of NK cells to recognize antibody-coated targets through CD16 and kill tumour targets through ADCC. Recently, we discovered that NK-cell activation leads to a decrease in CD16 expression. Loss of CD16 expression is problematic because, without CD16, ADCC will not occur. This decrease in CD16 expression is due to disintegrin and metalloproteinase-17 (ADAM17) mediated clipping of the CD16 receptor, which leads to CD16 shedding (Gryzwacz, Kataria, & Verneris, 2007; Zhou, Gil-Krzewska, Peruzzi, & Borrego, 2013). To counter ADAM17 clipping and maintain CD16 and ADCC, a highly selective ADAM17 inhibitor is being used and has shown to increase IFN-α after CD16 cross-linking (Romee et al., 2013). Following upon the success of in vitro studies, clinical studies are being conducted using ADAM17 inhibitors in combination with rituximab after HCT in patients with in diffuse large B-cell lymphoma (NCT02141451).

In addition to monoclonal antibodies, we have focused on the generation of BiKEs and TRiKEs, which target NK cells to the tumour synapse and induce their activation at that site. BiKEs and TRiKEs are small molecules constructed with a single-chain Fv against CD16 and one or two (BiKEs and TRiKEs, respectively) variable portions from other antibodies against tumour-associated antigens.
antigens. We have shown that BiKEs and TRiKEs specific to CD16 and CD19/22 elicit direct killing of tumour targets and NK cell-mediated responses and that these molecules elicit better CD107a responses against acute lymphoblastic leukaemia compared to rituximab (Gleason et al., 2012). We have recently shown that anti-CD16x33 BiKE activation overcomes inhibitory signalling via class-I HLA to potently kill primary cancer targets, as well as targeting CD33+ myeloid-derived suppressor cells (MDSC) in patients with myelodysplastic syndrome. (Gleason et al., 2014; Schmohl, Felices, et al., 2016; Schmohl, Gleason, Dougherty, Miller, & Valleria, 2016). This BiKE has important therapeutic potential due to its ability to target a drug-resistant cell population that is CD33+ in many cancers. Additionally, we have now incorporated a novel human IL-15 cross-linker into the CD16x33 BiKE to produce a 161,533 TRIKE. When compared to the CD16x33 BiKE, the addition of the IL-15 platform induced superior NK-cell cytotoxicity, degranulation, NK-cell expansion and prolonged NK-cell survival postadoptive transfer. In a leukaemia xenograft model, this TRIKE showed greater tumour control as compared to the BiKE without IL-15 (Vallera et al., 2016). Additionally, improved results have been seen when combining this TRIKE with an ADAM17 inhibitor (Wiernik et al., 2013). Most recently, we have engineered a 1615EpCAM133 TetraKE which engages EPCAM, a tumour antigen present on solid tumour targets and CD133 which is expressed on drug-resistant cancer stem cells. This TetraKE is highly specific to EpCAM and CD133 and, similar to the 161,533 TRIKE, mediates NK-cell expansion and ADCC (Schmohl, Felices, et al., 2016; Schmohl, Gleason, et al., 2016). As shown by these studies, the BiKE and TRIKE platforms are highly flexible, making it possible to target any tumour-specific antigen. One major advantage of the anti-CD16 in this platform is that BiKEs and TRIKEs bind Fc at high affinity compared with the low-affinity binding of Fc portions of whole antibodies. These are continuing to revolutionize NK cell-targeted immunotherapy.

10 | CAR-NK CELLS IN CANCER IMMUNOTHERAPY

Recently, Chimeric antigen receptors (CARs) on NK cells have been used to direct NK-cell antitumour activity. In a recent study, cord-blood-derived NK cells were transduced with a retroviral vector incorporating CAR-CD19, IL-15 and inducible caspase-9 suicide (iC9) gene (Liu et al., 2017). This CAR-NK cell incorporates CD19 to redirect NK-cell specificity, IL-15 so that they can sustain their growth, and iC9 so that the CAR-NK cell can be eliminated. This CAR-NK cell proliferated rapidly in vivo in a mouse model of Raji lymphoma and exhibited potent antitumour responses. Clinical trials for CAR-NK cells against B-cell malignancies are currently underway (NCT03056339). Despite this promise, however, it may be difficult to manufacture and export large numbers of CAR-NK cells to other centres; this will need to be overcome if CAR-NK cells are to be clinically useful.

11 | CHECKPOINT BLOCKADE IN CANCER IMMUNOTHERAPY

A major strategy being in cancer immunotherapy is checkpoint blockade. The principle of checkpoint blockade is blocking an inhibitory receptor to unleash better cell function. One type of checkpoint blockade being explored in NK cells is driving KIR:HLA mismatch to increase the graft versus leukaemia effect (GVL) during adoptive transfer. Because inhibitory KIRs bind to MHC-I and inhibit NK-cell function, antibodies that bind to KIR and prevent inhibitory signal may have therapeutic potential. IPH2101 (previously I-7F9) is an IgG4 monoclonal antibody that recognizes inhibitory KIRs. KIR2DL1, KIR2DL2 and KIR2DL3 blocks inhibitory KIR signalling (Romagné et al., 2009; Figure 1). Phase I clinical trials showed that this antibody is tolerated by AML and MM patients and that it led to enhanced cytotoxic activity (Benson et al., 2011, 2015; Vey et al., 2012). Despite the promise that IPH2101 held, Phase II trials failed to show any clinical efficacy in patients with smouldering multiple myeloma (Carsten et al., 2016; Korde et al., 2014). In a recent commentary, we discussed the potential that IPH2101 dampens NK-cell education though monocyte-drive trogocytosis, a process that would not have been identified when studying IPH2101 outside of the larger context of the immune system (Felices & Miller, 2016). Despite this drawback as a single agent, IPH2101 may be useful in combination with other agents in targeted immunotherapy. These findings suggest that KIR inhibition is context-dependent and that investigating a single immune component out of context may result in misleading information and faulty conclusions.

Similarly, studies blocking CD94-NKG2A, the inhibitory receptor for HLA-E is also being explored. The humanized anti-NKG2A antibody (IPH2201-monalizumab) converted NKG2A+ NK cells in effector NK cells that had the ability to kill HLA-E+ lymphoma cells and multiple myeloma cells (Ruggeri et al., 2016). The anti-NKG2A antibody is currently in Phase I/II clinical trials for a variety of tumour types, either alone or as combination therapy.

12 | CONCLUDING REMARKS

Over 60 years of study have led to a vastly increased understanding of NK-cell development and how their innate properties can be honed to improve cancer immunotherapy. The current approaches in NK cell-targeted immunotherapy interrupt NK-cell inhibition, further stimulate NK cells through cytokines or enhance targeting through CD16. Current studies are focused on ways to expand NK cells and identify how adaptive NK cells can be used in the clinic. While acknowledging the importance of these developments, much more work is needed to fully understand and utilize NK cells in cancer immunotherapy. Relapse remains a major problem after HCT. Strategies to exploit favourable donor immunogenetics, NK-cell expansion ex vivo from blood and the potential of adaptive NK cells into successful clinical applications require further study.
A major challenge will be finding ways to activate NK cells endogenously without NK-cell infusion or with the use of off-the-shelf NK-cell products in development. The future of NK-cell immunotherapy lies in using combination therapies. Combining expansion of CMV-induced adaptive NK cells with enhanced CD16 signalling and activation by IL-15/IL-15Ra complexes and creation of NK-cell antigen-specific BiKEs and TRIKes and CAR-NK cells offer great promise for the success of NK-cell immunotherapies.

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CONFLICT OF INTEREST

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