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Opportunities and limitations of NK cells as adoptive therapy for malignant disease

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Abstract

While NK cells can be readily generated for adoptive therapy with current techniques, their optimal application to treat malignant diseases requires an appreciation of the dynamic balance between signals that either synergise with, or antagonise each other. Individuals display wide differences in NK function which determine their therapeutic efficacy. The ability of NK cells to kill target cells or produce cytokines depends on the balance between signals from activating and inhibitory cell-surface receptors. The selection of NK cells with a predominant activating profile is critical for delivering successful antitumor activity. This can be achieved through selection of KIR mismatched NK donors and by using blocking molecules against inhibitory pathways. Optimum NK cytotoxicity may require licensing or priming with tumor cells. Recent discoveries in the molecular and cellular biology of NK cells inform in the design of new strategies, including adjuvant therapies, to maximise the cytotoxic potential of NK cells for adoptive transfer to treat human malignancies.

Biology of natural killer (NK) cells

NK cells are characterised phenotypically by the expression of CD56 and lack of expression of CD3. Around 90% of circulating NK cells are CD56^{dim} and this population plays a key role in mediating cytotoxicity in response to target cell stimulation (1, 2). The remaining NK cells are CD56^{bright} and have a greater capability to secrete and be stimulated by cytokines (3, 4). Unlike B and T cells, NK cells do not undergo antigen-dependent, somatic rearrangement of their receptors and do not possess clonally distributed, antigen-specific receptors comparable to immunoglobulins or T-cell receptors (TCRs). This enables NK cells

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to respond rapidly to specific stress signals, without the need for prior sensitization and clonal expansion. Interestingly, recent data question this dogma and suggest that NK cells possess features of ‘memory’, with limited antigen specificity and the ability to provide anamnestic antigen-specific response upon subsequent antigenic challenge (5).

Although classified as innate immune cells, phylogenetically NK cells appear to have coevolved with T cells rather than antecedent to them (6–8). Resting NK cells share common killing mechanisms with mature CD8⁺ effector T cells; they induce target cell apoptosis through calcium dependent exocytosis of perforin and granzyme, as well as through the Fas and tumour necrosis factor–related apoptosis-inducing ligand (TRAIL) pathways (4, 9). In addition, NK cells secrete cytokines, such as interferon-gamma (IFN γ) and tumour necrosis factor alpha (TNF α), and are involved in regulating the function of other lymphocytes, macrophages, dendritic cells and endothelial cells (10).

Recently micro RNAs, such as miR-150 and miR-181 (11), have been shown to play a key role in the development of NK cells and miR-29 (12) and miR15/6 (13) have been found to modulate cytokine production.

NK recognition of tumor targets

The combination of activating (in particular the natural cytotoxicity receptors [NCR] NKp46, NKp30, NKp44 and the membrane protein NKG2D) and inhibitory cell-surface receptors (notably the killer Ig-like receptors [KIRs] and the heterodimeric C-type lectin receptor NKG2A) determines whether NK cells will or will not kill target cells and produce cytokines during their effector phase of activation (Figure 1 and Table 1) (14).

One of the primary functions of NK cells is the detection and killing of cells under expressing MHC class I, thus preventing viruses and tumours from evading T cell surveillance and this is often termed the ‘missing-self hypothesis’ (15). In humans this phenomenon is predominantly mediated by inhibitory killer cell immunoglobulin-like receptors (KIRs) and CD94/NKG2A, which recognise MHC class I and prevent NK cell mediated killing of cells expressing MHC class I (16).

NK-target cell interactions involve clustering of receptors at the contact area of both cells, termed immune synapses (17). The majority of activating NK receptors share common signalling pathways with B and T cell receptors; using adapter proteins, which contain immunoreceptor tyrosine-based activation motifs (ITAMs). Phosphorylation of ITAMs results in target cell killing through NK cell degranulation in response to increases in intracellular calcium. The majority of inhibitory NK cell receptors also contain a consensus sequence termed the immunoreceptor tyrosine-based inhibitory motif (ITIM), also activated by phosphorylation, which in turn results in dephosphorylation of ITAM motifs and inhibition of calcium signalling.

The mechanism by which NK cells integrate multiple activating and inhibitory signals is not fully understood and it is likely that multiple mechanisms are involved in the control of NK cell triggering, as in T cells (18). Recent studies suggest that a kinetic segregation model may be involved in NK cell activation (19). In this model, large phosphatases such as CD45

are excluded from the areas of membrane held in close proximity between the NK cell and its target. This leads to phosphorylation by small kinases of the activating and inhibitory NK receptors that are held in the areas of close contact by ligands on the surface of the target cell. This allows NK cell activation to be dependent on the complex summation of multiple activating and inhibitory signals.

The KIR gene family on chromosome 19q13.4 encodes 15 highly polymorphic and rapidly evolving (20) activating and inhibitory receptors that are expressed principally by NK cells but also by subpopulations of T cells (Figure 2). These genes vary with respect to their presence or absence on different KIR haplotypes. They exhibit extensive allelic polymorphism, creating considerable diversity in the number of KIR genotypes observed in the population (21).

Relationship between KIR type and malignant cell targets

A number of solid tumors including melanoma (22), cervical cancer (23, 24) and nasopharyngeal cancer (25) have been associated with certain KIR genotypes. Haematological malignancies and response to therapy have also been linked to KIR genotype through a number of different mechanisms; however, the conclusions drawn from some of these studies should be interpreted with caution in view of the heterogeneity of the study populations and small study groups (see Table 2) (26–31). In the allogeneic stem cell transplant (allo-SCT) setting, receiving a graft from donors with the KIR2DS1 genotype was recently shown to be associated with reduced risk of relapse from acute myeloid leukaemia (AML) following both unrelated (32) and matched sibling donor allo-SCT (33). In addition, KIR genotype predicts response to treatment for haematological malignancy; for instance, the KIR3DS1 genotype predicts for increased relapse rate following autologous stem cell transplantation (auto-SCT) for myeloma, while patients who lack the activating receptor KIR3DS1 have a better outcome (28). The KIR genotype of the patient also appears to determine response to therapy with tyrosine kinase inhibitors (TKIs): in newly diagnosed patients with chronic myeloid leukaemia (CML) treated with imatinib, KIR2DS2 genotype conferred a lower probability of complete cytogenetic remission and reduced overall survival (34), which could be overcome by using dasatinib (35).

Licensing

An important concept in NK cell activation is that NK cells require ‘licencing’ by the appropriate class I ligand before they are able to become activated by the absence of MHC on other cells. A number of experiments in the mouse have established the role of major histocompatibility complex (MHC) class I in rendering NK cells functional (‘licensed’) (36). Conversely, failure to engage inhibitory receptors during development, due to lack of interaction with MHC class I, results in the generation of a subset of anergic or hyporesponsive peripheral NK cells (unlicensed KIRs) (37). Although the molecular mechanisms underlying the interactions between MHC class I and inhibitory receptors conferring NK cell responsiveness remain incompletely defined, the immunoreceptor tyrosine-based inhibitory motif (ITIM) of the inhibitory receptors is essential (37). Similarly, studies in humans have shown that the recognition of self HLA class I by inhibitory KIR is

involved in the licensing of NK cells, endowing them with effector capacity during development and allowing the subsequent recognition of the absence of self MHC class I during interaction with target cells (missing self) (38). KIR2DL2 and KIR2DL3 recognise HLA-C allotypes characterized by Asn80 (grouped as HLA-C1); KIR2DL1, and to a weaker extent some KIR2DL2/3 allotypes, recognize HLA-C allotypes characterized by Lys80 (grouped as HLA-C2); KIR3DL1 recognises HLA-A and HLA-B allotypes with the Bw4 epitope (39). Thus NK licensing is an essential precursor to the acquisition of functionality. Upon encounter with cells with decreased expression of MHC class I molecules, i.e. “missing self” (15), licensed NK cells are no longer subject to inhibitory signals initiated by the engagement of MHC class I-specific receptors, promoting NK cell cytotoxicity and cytokine production. These findings have led to clinical studies that investigate the impact of selecting hematopoietic stem cell transplantation donors with an HLA and KIR type that favours NK-cell activation and thus, could be expected to promote an anti-leukemic effect (40–42). An interesting strategy for enhancing NK effector function is tumor-mediated NK priming (43, 44). Through the interaction of CD2 on NK cells with a ligand on the tumor, NK cells are rendered more cytotoxic, enabling them cells to lyse tumour cells that would normally be impervious to NK mediated destruction. A clinical trial of tumor-primed NK cells in AML was recently completed (43, 44).

Immune escape from NK cell control

A limitation to successful NK therapy is the capacity of a variety of malignancies to evade NK control. Several mechanisms have been identified:

1. Upregulation of MHC class I, Such a phenomenon has been described in myeloma where in early stage disease plasma cells express low levels of MHC class I and are recognised by, and susceptible to, NK cell-mediated killing. However, plasma cells derived from myelomatous pleural effusions, which only occur in advanced and aggressive disease, have high levels of MHC class I expression, allowing them to evade control by NK cells (45). Down regulation or release of NKG2D ligands, including MHC class I chain-related proteins A and B (MICA/B) and unique long 16 (UL16) binding proteins 1–6 (ULBP1-6)(46–48), have also been reported as potential immune evasion strategies in a number of haematological malignancies (49–53). In a c-myc transgenic mouse model of spontaneously arising lymphoma, NKG2D ligands were over-expressed on the surface of tumour cells at early stages of the disease, with progressive loss of expression, associated with escape from NK cell surveillance, in later stages of disease (54).
2. Abnormal overexpression of MICA/B has also been observed on CML CD34+ cells. However, in vivo, CML cells avoid NK-cell-mediated immune destruction by shedding MICA/B, thereby blocking NKG2D on CD56+ cells, thus preventing NK-cell activation by the target (55). Interestingly, different subpopulations of CML cells appear to exhibit different sensitivities to NK-mediated killing. In a recent report, quiescent CML CD34+ cells were found to be less susceptible to NK cell cytotoxicity than cycling CD34+ and CD34– cells (56, 57). The mechanisms behind the greater resistance of quiescent, primitive, leukemic cells to cell-mediated cytotoxicity are not well understood but may relate to the inherent

metabolic inactivity of this population of cells. The observation that quiescent CD34+ cells express a higher surface level of the death receptors, TRAIL DR4 and DR5, suggests that TRAIL-enhancing agents such as bortezomib can be used to augment the sensitivity of quiescent CD34+ CML cells to NK cell mediated killing (56).

3. Down-regulation of activating receptors on the surface of NK cells. We showed that co-incubation with AML blasts impaired NK effector function. The mechanism leading to altered NK function is unclear. Several cytokines, including transforming growth factor- β (TGF- β), inhibit NK cell activation. A recent study suggests abnormal expression of the immunosuppressive tyrosine converting enzyme indoleamine 2, 3-dioxygenase (IDO) as a mechanism for reduced NK cytotoxicity in melanoma patients (58), as well as impaired T cell function in AML (59). Our own data support a role for IL-10 in AML-mediated NK cell dysfunction (60).

Cell therapy with NK cells

Cellular therapy with NK cells has been accomplished either in isolation or in the context of an allogeneic transplant. A number of different strategies have been tested to provide pure populations of autologous or allogeneic NK cells, including purified NK cells from the peripheral blood of donors, unrelated cord blood units or in vitro propagated NK cell lines. Some of these strategies are discussed in more details below.

NK cell transfer and production by stem cell allografts

NK cells are usually the earliest blood lymphocytes to return after haematopoietic stem cell transplantation (61). The behaviour of NK cells following transplantation can be altered by the manipulation of the graft and post transplant immunosuppression. In particular, T cells in the graft are thought to impair NK responses post transplant, since KIR expression is augmented in T-cell depleted transplants (62).

The pioneering work of Velardi and coworkers (40) showing that donor-versus-recipient NK-cell alloreactivity could eliminate leukaemia relapse without increasing the risk of graft versus host disease (GVHD) in HLA mismatched recipients resulted in a new era in the exploitation of NK cells for cancer immunotherapy. In these studies the authors compared the outcome of patients based on the NK-alloreactivity of the donors (Figure 3). In patients with AML, transplantation from NK-alloreactive donors was associated with significantly lower relapse rate compared to non-NK-alloreactive donor (5 year relapse rate 0% vs. 75%; $P=0.0008$). These data led to several retrospective analyses of KIR ligand mismatch effect on outcome in the HLA-matched unrelated and matched sibling transplant settings. Although a number of large registry studies failed to show an increased graft-versus-leukaemia (GvL) effect (63–65), others showed a GvL effect associated with a survival advantage (41, 66–68). The conflicting data may be related to differences in the transplant conditioning protocols, patient populations, underlying diseases, graft composition, and post-transplant immunosuppressive regimens (69). Subsequent studies have shown that the accuracy of the prediction of relapse could be improved by taking into consideration the

presence of inhibitory KIRs on the donor's NK cells and the absence of corresponding KIR ligand in the recipient's HLA repertoire (a receptor-ligand model) (70).

More recently, specific activating *KIR* genes in donors were found to predict the risk of relapse following allogeneic transplantation for AML. Patients with AML who received allografts from donors who were positive for *KIR2DS1* had a lower risk of relapse (26.5%) than those with allografts from donors who were negative for *KIR2DS1* (32.5%); $P=0.02$ (32). The *KIR2DS1*-associated reduction in the rate of AML relapse was restricted to donors with *HLA-C1/C1* or *C1/C2*, in whom *KIR2DS1* expressing NK cells are presumed to be licensed, and the benefit was eliminated in transplants from donors with *HLA-C2/C2*, where *KIR2DS1*-expressing NK cells are expected to be tolerized in the setting of self *HLA-C2* and unlikely to mediate leukemic cytotoxicity. Similar results have also been reported in the setting of matched sibling donor transplants; Patients with AML who received allografts from donors who were positive for *KIR2DS1* had 4 times lower risk of relapse than those who received a graft from *KIR2DS1* negative donors (33).

A limitation of many of these studies is that they do not take into account the effects of KIR licensing on NK function following allogeneic stem cell transplantation. Although it is presumed that the acquisition of NK cell function is influenced by the inhibitory input from the donor HLA, murine studies suggest a greater degree of plasticity and indicate that NK cell responsiveness can also be influenced by the inhibitory input from the recipient (71–73). Outcome following allogeneic stem cell transplantation may also be influenced in other ways by NK cells and KIR genotype, for example there is evidence that the rates of CMV reactivation are modulated by activating KIR receptor genotype (74, 75).

Adoptive NK cell therapy

Attempts to use adoptive NK cell therapy for the treatment of malignant disorders date back more than 25 years. In one of the pioneering studies by Rosenberg and colleagues, autologous 'lymphokine activated killer cells' combined with interleukin-2 (IL-2) were given to patients with metastatic renal cell cancer and melanoma (76). This resulted in a response in around 20% of patients. Disappointingly, in further studies, no consistent efficacy of autologous NK-cell therapy was found in cancer patients when compared with cohorts of matched controls treated with IL-2 alone (77).

The last decade has seen the development of a number of strategies for the ex vivo selection and expansion of clinical grade NK cells. These include magnetic selection using CD56 Miltenyi beads (+/- CD3 depletion) to directly select NK cells from apheresis products (Table 3). Protocols for ex vivo expansion of NK cells using EBV-LCL feeder cell lines, or artificial antigen presenting cells engineered to express co-stimulatory molecules and membrane-bound cytokines such as IL-15 or IL-21 (78, 79) have also been shown to yield large numbers of highly activated NK cells under good manufacturing practice (GMP) conditions.

A number of studies have reported encouraging results of NK adoptive infusion in patients with AML. NK-donor lymphocyte infusions (NK-DLI) are generally well tolerated and GVHD has not been a major problem in the trials to date. In the NKAML pilot study,

children with high risk AML who achieved in first complete remission following conventional chemotherapy received infusions of haploidentical NK cells, preceded by conditioning with cyclophosphamide and fludarabine. This was found to be both feasible and safe, and at 2 years there was 100% event free survival (80). In another study 5 of 19 adults with advanced AML achieved a complete haematological response following haploidentical NK-DLI and conditioning with low-dose total body irradiation or a combination of fludarabine and cyclophosphamide (42). Haploidentical NK cells have also been used with some success in concert with autologous transplantation for multiple myeloma (81, 82) and non Hodgkins lymphoma (83). Generally, without prior conditioning, allogeneic NK cells are only transiently detectable. Attempts have been made to augment NK responses and persistence using a variety of agents such as IL-2 (84) and bortezomib (85) with some success. A number of investigators have also studied the role of adoptive immunotherapy with the NK lymphoma derived cell line NK-92 (86). Although reported to be safe, limited data exist on the efficacy of this approach. Our group is exploring the use of off-the-shelf cord blood derived NK cells in the treatment of haematological malignancies.

Augmenting function of NK cells by adjuvant therapies

Modulation of the host immune system represents a promising therapeutic approach against cancer. NK cells are also thought to be involved in mediating the effects of many therapies used to treat haematological malignancies through a wide variety of mechanisms. In myeloma, for example, treatment with drugs such as melphalan, etoposide and doxorubicin activates DNA damage responses, resulting in up-regulation of the NK activating receptors, DNAX accessory molecule-1 (DNAM-1) and NKG2D ligands, leading to increased NK-mediated cell cytotoxicity against tumour cells (87). Similarly heat shock 90 inhibitors upregulate the expression of the NKG2D receptor ligands MICA and MICB, rendering myeloma cells more susceptible to NK cell degranulation (88). The histone deacetylase (HDAC) inhibitor valproic acid has also been shown to upregulate the expression of NKG2D ligands on the surface of AML blasts, thereby increasing their sensitivity to NK cell mediated destruction (89).

Novel drugs with immunomodulatory properties are increasingly being used to treat hematological malignancies. Most appear to impact NK cell activity or target susceptibility to NK lysis through a number of mechanisms (summarised in Table 2). Thalidomide increases the number of peripheral blood NK cells with enhanced in vitro cytotoxicity against patient myeloma cells (90). Lenalidomide activates NK function by promoting IL-2 release from bystander CD4+ helper T-cells (91–93), downregulating the expression of the programmed death receptor-1 ligand (PD-L1), the ligand for the inhibitory receptor programmed death receptor-1 (PD-1) expressed on both T and NK cells (94), and upregulation of Fas expression and costimulatory molecules on myeloma cells leading to greater Fas-mediated apoptosis. The proteasome inhibitor bortezomib has also been shown to modulate NK function by reducing the amount of peptide available to bind to MHC class I and consequently, expression of MHC class I on the cell surface, thereby increasing NK mediated lysis of myeloma cells (95) and CML progenitors (56).

NK cell effector functions can be exploited for the treatment of a number of malignancies through their ability to mediate antibody-dependent cellular cytotoxicity (ADCC) (23). A number of monoclonal antibodies such as rituximab bind to CD16 (the FCR γ III receptor) on the surface of NK cells; NK cell recognition of an antibody-coated target cell in turn results in rapid NK cell activation and degranulation (96, 97). Polymorphisms in the FCR γ III receptor have been shown to determine clinical response to rituximab (96); homozygosity for Fc γ RIIIa-158V is associated with higher Fc γ RIIIa membrane expression on NK cells (98) and increased affinity of binding to human IgG1 and IgG3 (99).

Future directions

The therapeutic potential of NK cells is likely to be harnessed in a number different ways in the future; ranging from improved donor selection in the context of allogeneic transplant, to novel strategies to augment NK cell tumour killing (100) and prevent tumour escape. As has been the case with T-cells (101), genetic engineering of NKs might also become an important method by which their therapeutic potential is manipulated and enhanced. Strategies to combine the adoptive infusion of ex vivo activated autologous or allogeneic NK cells with conventional chemotherapy or with novel immunomodulatory agents, are being tested in a number of clinical trials worldwide.

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Figure 1. NK cell activation by a kinetic segregation model

Activating NK receptors generally use adapter proteins such as Fc $\epsilon\gamma$, and DAP12, which contain ITAM consensus sequences. These trigger a rise in intracellular Ca²⁺, and degranulation following phosphorylation by tyrosine kinases such as Syk and ZAP70. Similarly the majority of inhibitory NK cell receptors contain an ITIM consensus motif, phosphorylation of which results in recruitment of tyrosine specific phosphatases such as SHP-1, SH2-containing protein tyrosine phosphatase-1 (SHP-1) and SH2-containing inositol polyphosphate 5-phosphatase (SHIP). These act by dephosphorylating ITAM motifs and SHIP degrades phosphatidylinositol-3,4,5-trisphosphate, leading to inhibition of sustained calcium signalling. The kinetic segregation model of NK cell activation (19) proposes that the normal balance between phosphorylation and dephosphorylation of these receptors is disturbed by physical extrusion of large phosphatases such as CD45 and CD148 from areas of close contact between the NK cell and its target. This leads to phosphorylation by small kinases of both the activating and inhibitory receptors that diffuse into, and are held at the immune synapse, and allows NK cell activation to be dependent on the balance between the number of activating and inhibitory ligands on the target cell. Several important receptor subtypes are not depicted in this diagram (e.g. the activating receptor CD16, which uses the ITAM containing adapter protein Fc $\epsilon\gamma$, see Table 1). In addition non-ITAM mediated activation also occurs, particularly by the NKG2D-DAP10 complex (102).

Figure 2. KIR genotype and receptor function
Simplified schema of the KIR gene locus (modified from the UCSC genome browser <http://genome.ucsc.edu/>, hg19 (2009)). At least 37 haplotypes have been identified thus far; however, the KIR genotype can broadly be divided into two groups, depending on whether the person is homozygous for genotype A (AA), in which case they only express one activating KIR (KIR2DS4) or whether they have at least one B haplotype (Bx). The B haplotype is defined by the presence of a variable number of additional activating and inhibitory KIRs (KIR2DL5, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS5 and KIR3DS1).

Figure 3. KIR ligand mismatch

KIR Ligand mismatch is largely dictated by the ligands of the three inhibitory KIR receptors KIR2DL1, KIR3DL1 and KIR2DL2, which are highly prevalent (97%, 90% and 95% respectively). This model predicts that when the transplant recipient is missing at least one of the three major classes of HLA ligands for inhibitory KIR, the donor NK cells will recognise the missing ligand in the host, resulting in an augmented graft versus leukaemia effect. Theoretically this can occur in the opposite direction (where the ligand is missing from the donor) but there is little evidence that this leads to worse rates of graft rejection.

Table 1

NK cell receptors

KIR	KIRs are part of the immunoglobulin superfamily. KIRs either possess two (KIR2D) or three (KIR3D) extracellular Ig-like domains (103–105) and can either have an activating short (S) cytoplasmic domain or inhibitory long (L) domain (16). KIR receptors are able to bind four of the six types of class I in the human MHC (HLA-A, HLA-B, HLA-C and HLA-G). The details of the interaction between KIR3DL1 and HLA were recently demonstrated by crystallography (106). The target antigen within the MHC and the degree of specificity vary depending on the KIR receptor. For example, KIR2DL4 binds to HLA-G with broad specificity, whilst KIR3DL1 has a very narrow specificity and only binds to HLA Bw4. NK receptors are expressed stochastically on the surface of NK cells and these complex expression patterns allow individual NK cells to recognise cells missing one or more MHC class I molecules (6).
NKG2 (NKG2A, - C, -E and – F) and CD94 receptors	These molecules are part of the C-type lectin-like family and tend to be expressed on the cell surface as heterodimers between CD94 and NKG2A or NKG2C, which act as inhibitory or activating receptors respectively (107). CD94 can also be expressed as a homodimer, although it is not believed to have a role in signalling because it lacks a cytoplasmic domain. Unlike KIR expression, CD94/NKG2 receptor expression can be induced by a number of cytokines including IL-15 (108) and IL-12 (109). Both the inhibitory receptor CD94/NKG2A and the activating receptor CD94/NKG2C bind HLA-E, with high and low affinity respectively. Interestingly, the most abundant peptides presented by this MHC class Ib molecule are peptides derived from the leader segments of other class I molecules, allowing NK cells to monitor the MHC class I proteins in a cell. Peptides from heat shock protein 60 (hsp60) are also presented on HLA-E and there is evidence that the inhibitory CD94/NKG2A receptors fail to bind HLA-E molecules containing hsp60 peptides, providing a mechanism for NK cells to potentially eliminate “stressed” cells (110).
NKG2D	This is a key receptor in NK cell activation, although it is also found on virtually all $\gamma\delta$ and CD8+ T cells. The NKG2D receptor is encoded by a single gene that has little sequence homology with the other NKG2 genes and it acts through DAP10, using an ITAM independent mechanism (102). Unlike many of the other NK cell receptor genes, there is very little polymorphism in NKG2D in humans (111, 112). It is a member of the lectin-like receptor family and recognises the MHC class I related molecules MICA and B (113, 114). Ligands of the NKG2D receptor may be upregulated as part of the DNA damage response induced by chemotherapy and radiotherapy and it is likely that NK cells play a key role in mediating tumour cell apoptosis following these cytotoxic treatments (115). Intriguingly, polymorphisms in the NKG2D target MICA are associated with T-cell large granular lymphocyte leukaemia (116).
Natural cytotoxicity receptors	The natural cytotoxicity receptors (NCRs) include NKP30, NKP44 (117) and NKP46 (118). Activation of these receptors generally results in strong enhancement of NK-mediated cytolytic activity. NKP46 can bind viral haemagglutinins and activate cell lysis (119) but is also involved in mediating tumour cell destruction in myeloma (120). To add a further layer of complexity, NKP30 has splice variants, which have different functions, (121) with NKP30a and NKP30b being stimulatory and NKP30c inhibitory. These are coexpressed but the balance of the expression is predominantly genetically determined, giving rise to either activating or inhibitory profiles. The expression pattern has prognostic implications in gastrointestinal stromal tumours, with the inhibitory profile (NKP30c predominance) conferring a shortened survival (121).
CD16	NK cells are also important mediators of antibody dependent cell-mediated cytotoxicity (ADCC). The CD56 ^{dim} subset in particular mediates this effect through antibody mediated binding of the low affinity Fc receptor (Fc γ RIII / CD16). This binds to the IgG1 and IgG3 subclasses and enables NK cells to mediate ADCC. Through this pathway, NK cells are involved in eliciting the effects of monoclonal antibody therapies such as rituximab and gemtuzumab (96, 97).
LAIR-1 (CD305)	Leukocyte associated immunoglobulin-like receptor 1 (LAIR-1) is another inhibitory receptor that is linked to the SHP pathway (122).
SLAM 2B4 (CD244) NTB-A (CD352)	The signalling lymphocyte activation molecules (SLAM) are a family of receptors that include the 2B4 receptor and the NK, T and B cell antigen-A (NTB-A) (CD150). 2B4 is interesting in that it can have both activating (123) and inhibitory (124) properties and it interacts with CD48, which is widely expressed on haematopoietic cells.
Others	There are a large number of other activating and inhibitory receptors also expressed by NK cells including: leukocyte immunoglobulin-like receptor B1 (LILRB1, CD85j); CD2-like receptor activating cytokine cells (CRACC); and DNAX accessory molecule-1 (DNAM-1/CD266).

Table 2

Association of KIR genotypes and haematological malignancies

KIR genotype and associations with haematological malignancies				
KIR gene	Disease	Effect	No patients in study	References
Risk of disease				
KIR-2DS1 KIR-2DS5	Aplastic anaemia / Paroxysmal nocturnal haemoglobinuria	Reduced frequency in patient population	55	(125)
KIR2DS3	AML/ALL	Reduced frequency in patient population	40/32	(126)
Activating KIR genes, especially KIR2DS2	Pre-B ALL	Reduced frequency in patient population	102	(127)
KIR3DS1 and KIR2DS1	Hodgkin's Lymphoma	Reduced frequency in patients	84 families	(128)
KIR2DL2 and KIR2DS2	CML	Reduced frequency in patients	52	(129)
KIR2DL2 and KIR2DL3 in combination with their HLA-Cw1 ligand	CML/AML	Increased frequency in patients	19/29	(31)
KIR2DL5a and 2DL5b	NK type lymphoma	Increased frequency in patients	35	(26)
KIR3DL1 and the HLA-Bw4- ligand	CLL	Increased frequency in patients in the presence of Bw4 and reduced risk when Bw4 absent.	31	(31)
Response to treatment				
KIR2DS1	CML	Reduced response to imatinib and worse overall survival. Dasatinib therapy overcomes the poor prognostic impact of KIR2DS1.	166 + validated a further 174 on SPIRIT2	(34) (35)
KIR3DS1	Myeloma	Worse DFS following autologous stem cell transplant.	182	(28)
Donor KIR B/B homozygotes	AML	Reduced risk of relapse following unrelated allogeneic transplant (relapse rate 15.4% B/B v 36.5% A/A)	1086	(130)
Donor KIR 2DS1 (plus coinherited 3DS1 and 2DL5a)	AML	Reduced relapse rates following HLA-identical sibling allogeneic stem cell transplant (relapse rate 13% with SD1+ve donor v 57% if -ve)	248 patients: 68 with AML	(33)
Donor KIR 2DS1	AML	Reduced relapse rates following HLA-identical unrelated donor allogeneic stem cell transplant (relapse rate 26.5% with 2DS1+ donor v 32.5% 2DS1-ve)	1277	(32)
Donor KIR haplotype B	Myeloma	Improved progression free survival (30 v 14%)	118	(67)

Table 3

Trials of Adoptive NK cell therapy

Disease	Setting	Method of preparation	Cell number	Concomitant medication	n	Effect on patient outcome	
AML	NK-DLI for patients in first CR following chemotherapy	Apheresis followed by CD3 depletion and subsequent CD56 selection	$2.9 \times 10^7/\text{kg}$ (range $0.5\text{--}8 \times 10^7/\text{kg}$)	Cyclophosphamide (day -7) Fludarabine (Day -6 to -2)	10	100% 2 year event free survival	NKAML study (80)
AML	NK-DLI for poor prognosis AML	Apheresis followed by CD3 depletion and ex-vivo stimulation with IL-2	$1 \times 10^5 - 2 \times 10^7$	Low dose cyclophosphamide/melphalan vs. High dose cyclophosphamide and fludarabine. IL-2 given to all patients	19	Complete haematological remission in 5 of 19 patients	(42)
Myeloma	Haploidentical KIR ligand mismatched NK-DLI followed by delayed autologous stem cell transplant	Apheresis followed by CD3 depletion	$2.4 \times 10^7/\text{kg}$	Melphalan and fludarabine conditioning	10	Near CR in 50% of patients	(81)
CD20+ NHL	NK-DLI for refractory disease (more than 2 salvage therapies)	Apheresis followed by CD3 depletion	$2.1 \times 10^7/\text{kg}$ (range $0.2\text{--}40 \times 10^7/\text{kg}$)	Fludarabine (25mg/m2) Cyclophosphamide (60mg/m2)	6	4 objective remissions 2 complete remissions	(83)
Not stated	Allogeneic stem cell transplant	Apheresis followed by CD3 depletion and subsequent CD56 selection	$1.2 \times 10^7/\text{kg}$	Not stated	14	NK infusions well tolerated, aside one case of grade IV	(131)
High risk myeloid malignancies	Graft failure/mixed donor chimerism following allogeneic (haploidentical) stem cell transplant	Apheresis followed by CD3 depletion and subsequent CD56 selection	$1.61 \times 10^7/\text{kg}$ (range $0.21\text{--}2.2$)	None	5	Reversal of mixed donor chimerism in 2/5	(132)
Not stated	Following 3-6/6 HLA matched T cell-depleted nonmyeloablative allogeneic transplant	Apheresis followed by CD3 depletion and CD56 selection	$1.06 \times 10^7/\text{kg}$	Infusion performed 6-8 weeks following fludarabine conditioned allogeneic transplant with alemtuzumab	30	Improved T cell recovery and duration of remission	(133)
Breast and ovarian carcinoma	NK DLI for patients with relapsed disease	Apheresis followed by CD3 depletion	$2.17 \times 10^7/\text{kg}$	Fludarabine and cyclophosphamide Total body irradiation in 7 patients IL-2 x6 doses s.c. post DLI	20	TBI improved longevity of NK engraftment	(134)
Colorectal carcinoma, hepatocellular carcinoma, renal cell carcinoma and B-CLL	Previous allogeneic BMT with donor derived NK cells	Apheresis followed by ex vivo expansion with IL-2	0.1×10^7 followed by two doses mean 3.3×10^7	Variable; some patients received sc IL-2	5	Minor responses in 2 patients	(135)
Advanced non-small cell lung cancer	NK-DLI after chemotherapy	CD56 selection followed by culture with IL-15	$0.46 \times 10^7/\text{kg}$ (range $0.2\text{--}2.9$)	2-4 doses of NK DLIs given 2 days after chemotherapy (carboplatin-paclitaxel most commonly used)	16	Trend to better OS in subgroup receiving 4 doses of NK cells	(136)

Disease	Setting	Method of preparation	Cell number	Concomitant medication	n	Effect on patient outcome	
Advanced renal cell carcinoma and malignant melanoma	NK-92 cell line infusion	Expansion of NK lymphoma cell line	Up to $3 \times 10^9/\text{m}^2$	Cell infusion alone	12	Infusion well tolerated. Possible response in 2 patients	(86)