Chapter

Natural Killer (NK) Cell Alloreactivities against Leukemic Cells: Functions beyond Defense

Suwit Chaisri and Chanvit Leelayuwat

Abstract

Immunotherapy using adoptive transfer of natural killer (NK) cells has progressively been utilized in hematologic malignancies over the past decade. Presently, NK cell immunotherapy has been promising and feasible in acute leukemia, particularly in acute myeloblastic leukemia (AML). Alloreactive NK cells have been exploited under the killer immunoglobulin-like receptor (KIR)-ligand mismatches between donors and recipients in haploidentical hematopoietic stem cell transplantation (haplo-HSCT) after immunosuppressive chemotherapy. Of interest, alloreactive NK cells killed residual leukemic cells, dendritic cells (DCs) and T cells in acute leukemia patients and led to significantly improved clinical outcomes. Consequently, this chapter provides the *KIR* genetics and the mechanisms of alloreactive NK cells that are shown to be crucial in the successful therapy of acute leukemia (myeloid and lymphoid). Altogether, the donor selection algorithm of haplo-HSCT is discussed to emphasize the importance and give priority to increase the chances of therapy success. These will be useful for students and researchers who work in immunogenetics. Furthermore, the knowledge would be applicable to clinical research and medical sciences.

Keywords: NK cell alloreactivity, *KIR* polymorphisms, KIR-ligands mismatch, acute leukemia, haploidentical HSCT

1. Introduction

Natural killer (NK) cells play a critical role in innate immune responses against infected cells and transformed cells. In the past decades, the molecular mechanisms of NK cell killings have been extensively elucidated as well as employed in clinical applications [1, 2]. The effector functions of NK cells are being investigated in several pathological conditions, particularly in cancers [3, 4]. Many researches highlight on the role of NK cells in hematologic malignancies, particularly in acute leukemia. Acute leukemia is a type of cancer in which the bone marrow produces too many immature white blood cells and they cannot carry out normal functions. In addition, leukemic cells crowd out all blood cell productions in the bone marrow, affecting normal blood functions and leading to serious health problems. The treatment options for acute leukemia include chemotherapy, radiotherapy and bone marrow (hematopoietic stem cell) transplantation. Considering HLA matching between donors and patients for hematopoietic stem cell transplantation (HSCT),

the patient's outcomes are related with a closely matched donor, however, not all patients are able to find a suitable donor. To overcome the limitations of donor availability, a partially matched or haploidentical HSCT (haplo-HSCT) has been established as an alternative expedient and being a mode of curative therapy for hematologic malignancies [5], particularly in acute leukemia patients [6]. In addition, a complication of allogeneic HSCT has improved with graft versus host disease (GvHD) prophylaxis to prevent the effects of donor T cells. Evidently, the role of NK cell alloreactivity can significantly improve clinical outcomes in acute leukemia patients [7]. Among NK cell receptors, killer immunoglobulin-like receptor (KIR) has increasingly been exploited in the aspect of immunotherapy for acute leukemia, which mismatches between KIR on donor NK cells and their cognate ligand HLA class I on receipts lead to alloreactivity of NK cells in haplo-HSCT setting. Alloreactive NK cells exert powerful activity in killing residual leukemic cells, leading to preventing disease relapse and improving survival [7, 8]. With these reasons, NK cell alloreactivities mediated by KIR-ligand mismatches has been increasingly utilized in aspect of immunotherapy for clinical applications. Therefore, this chapter provides *KIR* genetics and KIR-mediated NK cell alloreactivities that have been shown to be crucial in the successful therapy of acute leukemia (myeloid and lymphoid). Additionally, donor selection algorithm of haploidentical HSCT mismatch is discussed to emphasize its role in increasing success rate of these therapies.

2. Natural killer (NK) cells

NK cells are considered a part of lymphocytes that account for approximately 10% of blood lymphocytes. NK cells are characterized by expression of CD56 surface antigen and a lack of CD3 antigen. Based on the density of CD56 expression, human NK cells are phenotypically divided into two groups: $CD56^{bright}$ and $CD56^{dim}$. Of these NK cell populations, $CD56^{dim}$ NK cells represent up to 90% of NK cells in human peripheral blood mononuclear cells (PBMCs) and are considered the most cytotoxic subset, while $CD\ 56^{bright}$ NK cells comprise approximately 10% of NK cells in PBMCs and are known as the cytokine-producing subset. NK cells play important role of the first line of defense to infected cells and transformed cells without prior sensitization [9, 10]. Several receptors present on NK cells are currently identified, however, they are classified into two groups depended on signal transductions derived from those receptors, namely activating and inhibitory receptors [11] (**Table 1**). Importantly, the dynamic equilibrium of signals obtained by these receptors is important to determine whether NK cells are activated to kill target cells [12, 13]. The missing self-hypothesis has been proposed to explain whether NK cells discriminate target cells form healthy "self" cells by their various receptors [14]. Normally, engagement of inhibitory receptors by self MHC class I molecule leads to transmission of an inhibitory signal to switch off the NK cell functions, while down-regulated MHC molecules on target cells by viral infection or malignant transformation is recognized and attacked by NK cells. Cytotoxicity and cytokine secretion of NK cells depended on the interaction between their receptors and their corresponding ligands. Activated NK cells usually exert cytotoxic activity through three main pathways. Firstly, the perforin/granzymes pathway, activated NK cells release these molecules to intracellular space. The perforin directly forms a transmembrane channel on the target cell, leading to increased permeability of the target cell membrane and causing osmotic lyses of target cells. In addition, granzymes enter the cytoplasm of target cells through transmembrane pores to promote target cells apoptosis [15]. Secondly, the Fas/FasL pathway, when Fas on NK cells binds to FasL on the target cells, Fas derivers a death signal to the target cell

Type of receptors	Ligands
Activating receptors	
CD94-NKG2C/E	HLA-E
NKG2D	MIC-A/-B, ULBP1–6
KIR-S	HLA-C
NKp30	B7H6, BAT3
NKp44	Proteoglycans
NKp46	Heparin
CD16	IgG
Inhibitory receptors	
KIR-L	HLA-A, -B, -C
LAIR-1	Collagen
LILRB1	HLA-A, -B, -C
NKR	LLT-1
KLRG1	Cadherins
SIGLEC3, 7,9	Sialic acid
CD94-NKG2A	HLA-E
Activating or inhibitory receptors	
KIR2DL4	HLA-G?

KIR-S: killer cell immunoglobulin-like receptor with short cytoplasmic tail; KIR-L: killer cell immunoglobulin-like receptor with long cytoplasmic tail; LAIR-1: leukocyte-associated immunoglobulin-like receptor 1; LILRB1: leukocyte immunoglobulin-like receptor B1; KLR: killer cell lectin-like receptor; NKR: NK cell receptor; SIGLEC: sialic acid-binding immunoglobulin-type lectins; HLA: human leukocyte antigen; LLT: lectin-like transcript 1. IgG: immunoglobulin G; BAT3: leukocyte antigen-B-associated transcript 3; MIC: MHC class I-related chain family; ULBP: UL16-binding proteins; HLA-G?: It is controversial whether HLA-G is a lignad of KIR2DL4.

Table 1.
NK cell receptors and their cognate ligands.

and they undergo apoptosis [16, 17]. Lastly, the cytokine pathway, NK cells secrete *various* cytokines, such as IFN-γ, TNF-α, GM-CSF and IL-10. These cytokines play an important role in immune responses of NK cells. For example, TNF-α alters the stability of lysosome in target cells, resulting in leakage of various hydrolases, effect on metabolism of cell membrane phospholipid and degradation of genomic DNA by endonuclease [18]. With these mechanisms, applications of NK cells have currently been established in tumor immunotherapy as chimeric antigen receptor-modified NK cells (CAR-NK cells) and adoptive immunotherapy.

3. *Killer immunoglobulin-like receptor (KIR)* polymorphisms mediated-heterogeneity of NK cell responses

Killer immunoglobulin-like receptors (KIRs) are cell surface receptors expressed on NK cells and subpopulation of T cells. Similar to HLA class I, KIR ligands, *KIRs* are highly polymorphic genes, including allelic polymorphisms, genes content and copy number variations [19]. Genetic variations of *KIRs* and *HLA* among individuals generate heterogeneity of immune responses of NK cells [20]. Interestingly, current evidences demonstrate the impact of *KIR* gene variations on disease susceptibility or resistance in several pathological conditions, such as infection, autoimmune/inflammatory disorder, implantation and particularly in hematopoietic stem cell

transplantation [21]. Here, to better understand the role of NK cell alloreactivities, we thoroughly describe the basis of KIRs as well as its role in the immune system.

3.1 Killer immunoglobulin-like receptors (KIRs, CD158)

Killer immunoglobulin-like receptors are type I transmembrane glycoprotein expressed on the plasma membrane of NK cells, subpopulations of memory T cells and most of $CD8^+$ T cells [22, 23]. The KIR family consists of 15 functional genes (*KIR2DL1–4*, *2DL5A*, *2DL5B*, *2DS1–5*, *3DL1–3*, *3DS1*) and 2 pseudogenes (*2DP1* and *3DP1*) encoded within a 150 kb region of the leukocyte receptor complex (LRC) located on chromosome 19 (19q13.4) [24, 25] . The KIR proteins have either two or three extracellular immunoglobulin domains (KIR2D or KIR3D) and cytoplasmic (CYT) tails with long (L) or short (S) tails. Based on structural feature, a long *cytoplasmic tail* (KIR2DL or KIR3DL) contains immunoreceptor tyrosine-based inhibitory motif (ITIM) that functions to inhibit NK cell responses [26]. In contrast, a short *cytoplasmic tail* (KIR2DS or KIR3DS) has a positive charge amino acid residue in the transmembrane region to associate with DAP12 containing an immunoreceptor tyrosine-based activating motif (ITAM) that turns on NK cell functions [27]. Uniquely, KIR2DL4, a long cytoplasmic tail containing ITIM and linking with an adaptor molecule FcεRI, can deliver both activating and inhibitory signals to control NK cell responses [28, 29]. However, the mechanism by which KIR2DL4 delivers activating or inhibitory signals to NK cells is not established. Two pseudogenes, *KIR2DP1* and *KIR3DP1,* are not expressed on NK cells.

3.2 Diversity of *KIRs*

The extensive variation of *KIR* loci is achieved through allelic polymorphisms, a combination of gene content (absence/presence polymorphisms) and copy number variations. Firstly, allelic polymorphisms of *KIR* have been documented in the Immuno Polymorphism Database (IPD) showing each *KIR* locus contains 16–164 alleles of different genes [30]. Later, a combination of genes content generates distinct *KIR* genotypes, showing 625 different *KIR* genotypes reported in 171 populations worldwide [31]. Lastly, copy number variation (CNV) of *KIR* is currently studied and demonstrated that equal and unequal crossing over generates individual *KIR* gene duplication, deletion and hybridization [32]. Moreover, stochastic and variegated *KIR* expressions on NK cells by epigenetic regulation facilitate a diverse repertoire of NK cell clones within an individual [33] (**Figure 1**). As a consequence, the influence of *KIR* diversity on immune responses has been reported in several diseases [21, 34–36]. On the basis of gene content, *KIRs* are classified into group A and B haplotypes [37]. Both group A and B haplotypes are conserved with four framework genes (*KIR3DL3-3DP1-2DL4-3DL2*). Group A haplotype consists of the four framework genes and *2DL3*, *2DP1*, *2DL1*, *3DL1* or *2DS4*, whereas group B haplotype has variable gene content. Distinctly, group A haplotype shows predominantly inhibitory *KIR* genes except *KIR2DS4*, but group B haplotype contains dominantly activating *KIR* genes. The distributions of *KIR* haplotypes have been studied, showing variations of A and B are found among populations [38–43].

3.3 *KIR* and their cognate ligands

Both inhibitory and activating KIRs on NK cells recognize HLA class I molecules of target cells. Most KIR ligands have recently characterized as shown in **Table 2**, whereas some KIR ligands are still unknown [44–47]. The affinity of KIR and HLA interaction affects NK cell responses [48]. Remarkably, activating KIRs

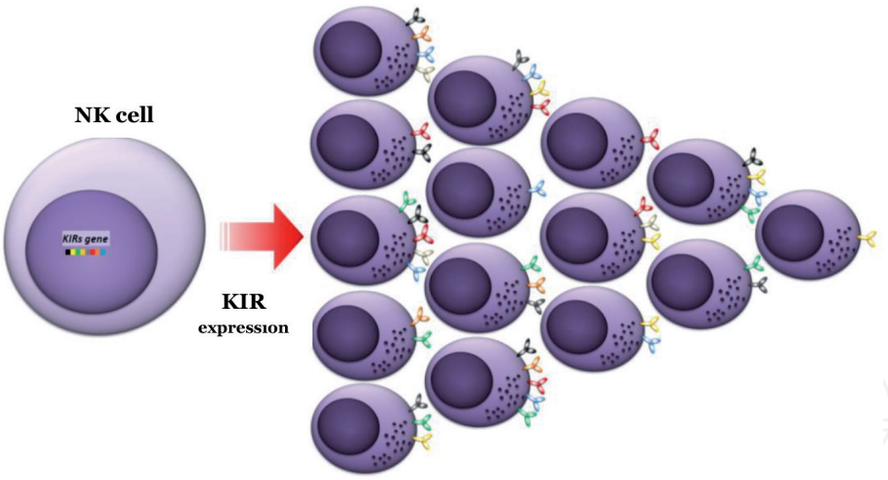

Figure 1.
NK cell repertoires. Individual KIR genotype generates a diverse repertoire of NK cells by stochastic expression. KIR expression on NK cells is influenced by HLA class I and CD94:NKG2A.

KIR	KIR ligand (HLA)	Function
2DL1	HLA-C2 (HLA-C^{Lys80})	Inhibition
2DL2	HLA-C1 (HLA-C^{Ans80})	Inhibition
2DL3	HLA-C1, HLA-C2 (weak)	Inhibition
2DL4	HLA-G?*	Activation/inhibition?
2DL5	Unknown	Inhibition
2DS1	HLA-C2 (HLA-C^{Lys80})	Activation
2DS2	HLA-C1 (HLA-C^{Ans80}), β_2-Microglobulin	Activation
2DS3	Unknown	Activation
2DS4	HLA-A11 and some HLA-C alleles	Activation
2DS5	Unknown	Inhibition
3DL1	HLA-Bw4	Inhibition
3DL2	HLA-A3, -A11	Inhibition
3DL3	Unknown	Inhibition
3DS1	HLA-Bw4?	Activation

**The ligand for KIR2DL4 is still controversy [44, 45].*

Table 2.
KIR and their ligands (HLA) specificity.

(KIR2DS1/2) and inhibitory KIRs (KIR2DL1/2) can bind the same HLA molecules. However, the affinity of inhibitory KIR-binding HLA is higher than activating KIR-binding HLA [49, 50]. It is believed that the lower affinity of activating KIR and HLA interaction would be evolved to avoid self-aggression.

4. Alloreactive NK cells from transplantation to adoptive immunotherapy

Over the past decade, adoptive transfer allogeneic NK cells have been emerged as promising immunotherapy for hematological malignancies [8, 51, 52]. The role of alloreactive NK cells is considered to be beneficial in achieving better outcomes

after haploidentical HSCT (haplo-HSCT). Presently, haplo-HSCT is an alternative option when completely matched related or unrelated donors are not available. Historically, although haplo-HSCT can lead to graft versus host disease (GvHD) which has undesirable effect in post HSCT, this problem has been currently solved by performing of T cell depletion before graft infusion. After chemotherapy in AML patients, T cell prophylaxis has been used together with high stem cell doses, resulting in fast NK cell alloreactivities and slow T cell reconstitution. Moreover, graft versus leukemic cells mediated by NK cell, alloreactivities have been exploited which they can beneficially lead to reduced relapse, and improve survival [53]. Based on the interactions between NK cell receptors and their ligands, it was believed that allogeneic NK cells do not receive inhibition signals from the recipient HLA, leading NK cells to exert powerful anti-leukemia activity [54]. Regarding KIR-ligand mismatches between donor and recipient under haplo HSCT setting, alloreactive NK cells play crucial roles against leukemic cells, recipients' DCs and T cells [55], resulting in reduced leukemic relapses, GvHD and graft rejection, respectively (**Figure 2**). With these reasons, a number of studies have extensively investigated the role of KIR-ligand mismatches in both pre-clinical and clinical setting to evaluate the success in leukemia therapy. Additionally, four situations in predicting NK cell alloreactivities after haplo-HSCT have been proposed based on the deference in definition of KIR mismatches between the donor NK cells and the recipient's HLA [56] (**Figure 3**).

4.1 KIR-ligand (HLA) mismatch or missing-self-model

The KIR-ligand mismatch model, also called ligand incompatibility, has been proposed that an expression of HLA class I molecules (KIR ligand) on donor are

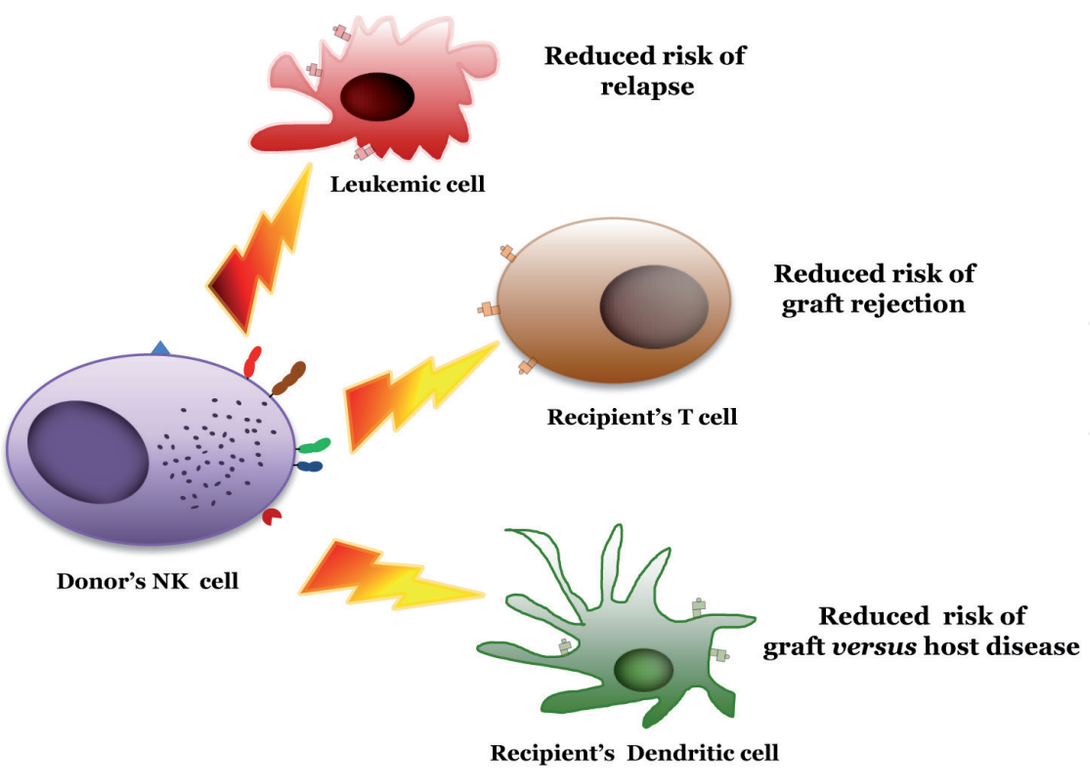

Figure 2.
The role of NK cell alloreactivities in acute leukemia. Beneficial effects of NK cell alloreactivities on the outcomes of acute leukemia under haplo-HSCT setting, adoptive transfer NK cells mediated activity against residual leukemic cells, recipient's T cells and dendritic cells, resulting in reduced the risk of relapse, graft rejection and GvHD, respectively.

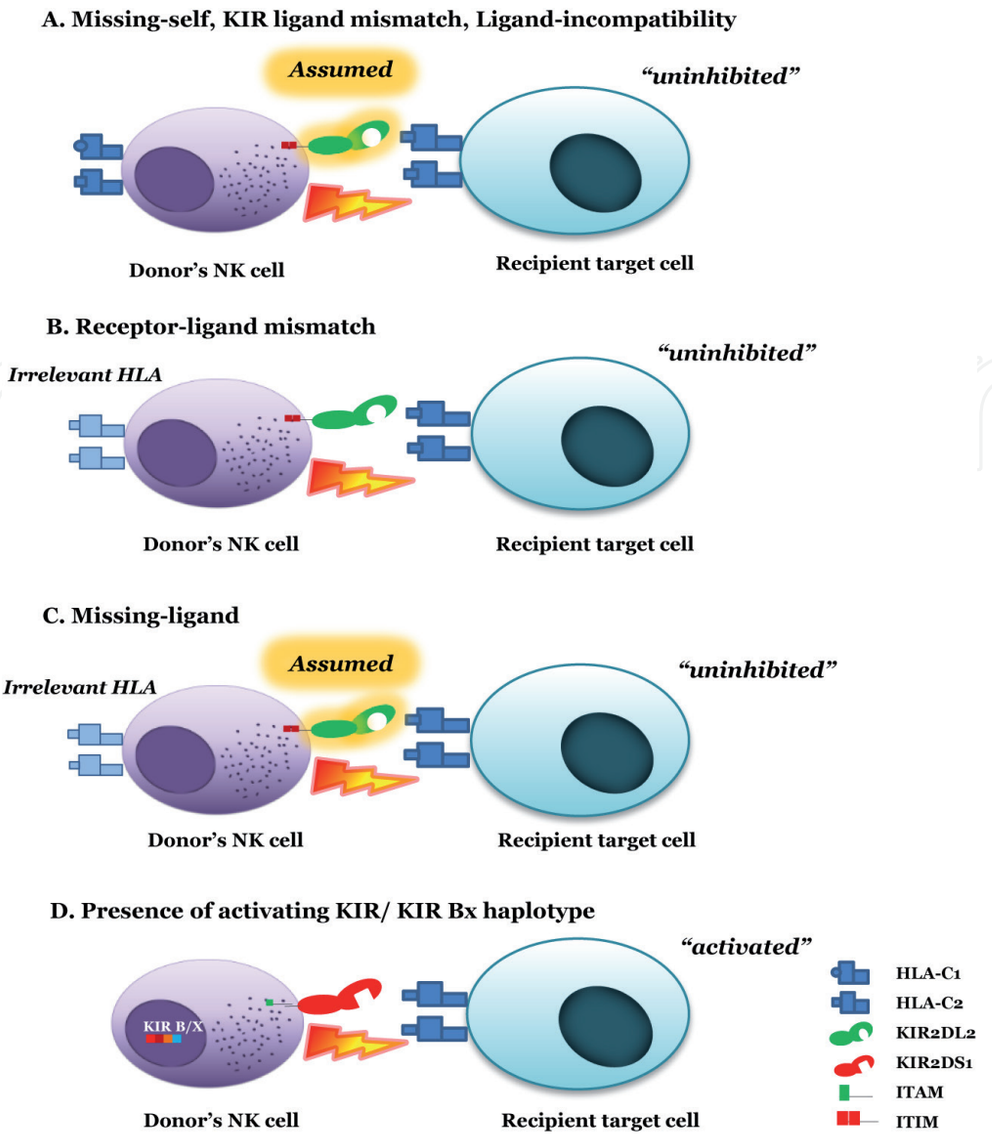

Figure 3.
Situations of NK cell alloreactivity in post-HSCT. (A) The KIR-ligand (HLA) mismatch or missing-self-model; NK cell alloreactivity is mediated by the lack of HLA ligand expression on recipient for inhibitory KIR on donor's NK cell which the presence of KIR expression on donor is assumed. High resolution HLA genotyping is performed in donor and recipient. (B) The receptor-ligand model; NK cell alloreactivity is mediated by the lack of HLA ligand expression on recipient for inhibitory KIR which the presence of KIR expression on donor NK cell is verified by genotyping and flow cytometry. The donor HLA is irrelevant to recipient. (C) The missing-ligand model; NK cell alloreactivity is mediated by the lack of at least one of HLA ligand (HLA-C1, -C2 or -Bw4) expression on recipient for inhibitory KIR and the donor HLA is irrelevant to recipient. (D) The presence of activating KIR model; NK cell alloreactivity is mediated by interaction of activating KIR on donor NK cell and HLA ligand on recipient target cell which KIR B haplotype contains more activating KIR than A/A haplotype. KIR genotyping was investigated in donor and recipient and some studies detected activating KIR on donor cells.

incompatible with recipient's HLA class I molecules [57, 58]. This model has been assumed that donor NK cells have inhibitory KIR that is missing its ligand on recipient. For example, NK cells from a HLA-C1/C2 donor will be alloreactive against a HLA-C2/C2 recipient, where it is assumed that KIR2DL2 is expressed on donor NK cells (**Figure 3A**).

4.2 Receptor-ligand mismatch model

The receptor-ligand mismatch model states that donor NK cells represent inhibitory KIR in mismatching with HLA class I on the recipient target cells,

leading to NK cell alloreactivities in graft versus host direction [59]. This model is therefore required for KIR genotyping in donor as well as HLA typing in recipient (**Figure 3B**).

4.3 Missing-ligand model

Notably, the HLA is only genotyped in recipients and missing HLA-C1, C2 or Bw4 for inhibitory KIR on donor can lead to NK cell alloreactivities [58]. For example, recipient represents HLA-C2/C2, therefore, it is assumed (not investigated) that donor NK cells expressing KIR2DL2 to be alloreactive due to missing HLA-C1 ligand (**Figure 3C**).

4.4 Presence of activating KIR model

Here, in this model, activating KIR on donor cells is measured to predict NK cell alloreactivities because the interactions between donor activating KIRs and their ligands on recipient can lead to NK cells achieving activation signals [60] (**Figure 3D**).

5. The role of NK cell alloreactivities in post HSCT

Several studies have revealed an influence of alloreactive NK cells in acute leukemia patients, where alloreactive NK cells deliver promising better outcomes in term of anti-leukemia activity. Predominantly, KIR-mediated NK alloreactivity has been demonstrated to be the most clinically significant relevance to AML, while its role in ALL remains unclear.

5.1 Acute myeloblastic leukemia (AML)

As acute myeloblastic leukemia was more susceptible to NK cell cytotoxicity than solid tumors [61], the role of adoptive transfer NK cells against leukemia was investigated in AML patients [62]. Anti-leukemic effect of allogeneic NK cells has been extensively studied under haplo-HSCT with the mismatches of KIR and cognate ligands between donor and recipient. Interestingly, Ruggeri and coworkers initially reported allogeneic NK cells mediated cytotoxicity against recipients' leukemic cells [53]. Later, the impact of NK cells alloreactivity in preventing AML relapse, GVHD and rejection was confirmed in clinical setting and mouse model [63]. Taken together, this condition has been explored under T cell depleted to avoid graft versus host effect and high doses of infused stem cell transplantation [54, 63, 64]. Remarkably, this approach was investigated in 21 AML children who received haplo-HSCT, showing donor-derived alloreactive NK cells killed leukemic cells in KIR-ligand mismatches, even late after transplantation [7]. Altogether, the doses of infused NK cell and immunosuppression were evaluated in children with AML to consider safety and effectiveness of alloreactive NK cells therapy [65]. Moreover, successfully transferred NK cells immunotherapy were reported in elderly AML who were not candidates for HSCT, demonstrating this approach was feasible and safe in elderly patients [8, 66].

Haplo-HSCT with KIR-ligand mismatches has been a promising strategy in AML for adoptive transfer of NK cells for immunotherapy [67, 68]. The incompatibility of three main inhibitory *KIR* loci (*2DL1*, *2DL2/3* and *3DL1*) with their ligands (*HLA-C2*, *HLA-C1* and *HLA-Bw4*) has been extensively investigated, and

found to be relevant for better clinical outcomes [69, 70]. To explain the potential benefits of alloreactive NK cells against leukemia based on an absence of inhibitory signal delivered by KIR on donor's NK cells, it has been shown that KIR2DL1$^+$ NK cells lyse leukemia in HLA-C1/C1 recipients, whereas KIR2DL2/3$^+$ NK cells partially lyse leukemia in HLA-C2/C2 recipients due to low-affinity binding, and that KIR3DL1 + NK cells lyse HLA-Bw4$^-$ leukemia of recipients, where HLA-Bw4$^-$ would be HLA-A or HLA-B as determined by the serotypic specificity. However, for the activating KIR model, KIR2DS1$^+$NK cells have demonstrated a potent reactivity against HLA-C2 expressing allogeneic target cells *in vitro* [60, 71], particularly on T cells and dendritic cells. As mentioned, although NK cell-based immunotherapy has been a promising approach in adult and childhood [8, 65, 66], there are additional issues that need to be considered in achieving a successful therapy. Firstly, the minimum or optimal number of infused NK cells is really required to achieve therapeutic effect that remains inconclusive due to lack of standardized technical procedure for qualifying alloreactive NK cells [67, 68]. Additionally, it became clear that infused NK cells favor IL-2 and IL-15 for expansion and survival [51, 72], however, administration of IL-15 after infusion has been recommended because IL-2 can promote host regulatory T cells to inhibit allogeneic NK cells.

Given the crucial role of alloreactive NK cells in graft versus leukemia (GVL) effect in haplo-HSCT, the predictive algorithm for donor selection is being developed in AML treatment. Several research groups have explored feasibility of NK cell-based immunotherapy, including *in vivo* and *in vitro* studies as well as clinical trials. Predominantly, mismatches of donors' inhibitory KIR expressing NK cells (2DL1, KIR2DL2/3 and KIR3DL1) with HLA class I of the patients have been well-documented that were relevant to therapeutic effect of NK immunotherapy in AML [7, 66, 73]. For activating KIRs, only KIR2DS1 has been reported to associate with NK cell alloreactivities against target cells expressing HLA-C2 [60, 74]. Moreover, *KIR* haplotypes and clinical outcomes have been observed, showing donors with group B *KIR* haplotype have improved relapse—free survival for AML patients under unrelated HSCT [75]. With these reasons, KIR-mediated NK cell functions in haplo-HSCT should be taken in to account for donor selections, since the potential benefit of NK alloreactivity has improved survival and clinical outcomes in AML patients. Moreover, phase I and II clinical trials have been being studied to evaluate the feasibility and safety [76, 77] for further applications. Therefore, haplo-HSCT with T cell depletion, KIR-mediated NK cell alloreactivities should be considered for donor selections using an algorithm in which KIR-ligand mismatches could be predicted. This is available as an online calculator (https://www.ebi.ac.uk/ipd/kir/ligand.html).

5.2 Acute lymphoblastic leukemia (ALL)

Since the role of alloreactive NK cells in AML were reported, the influence of KIR on the outcome of ALL patients in haplo-HSCT setting has been investigated. Like AML, the approach based upon KIR-ligand mismatches and the presence of donor's KIR2DS1$^+$ NK cells with HLA-C2 expressing target cells mediated NK cell alloreactivities against leukemic blasts has been tested [7]. In addition, ALL children transplanted from a *KIR* haplotype B donor showed significantly reduced risk of relapse, particularly in donors with high B content score [78]. However, the beneficial effect of NK cell alloreactivity has not been obviously noticeable in ALL patients in particular mechanisms of NK cells against ALL tumor cells [63, 79, 80]. The clinical studies of beneficial effect of NK cell alloreactivities in acute leukemia are summarized in **Table 3**.

Study	Disease	Model	Beneficial effect	Ref.
Ruggeri et al. (1999)	AML, ALL, CML	KIR-ligand mismatch	Antileukemic effect	[53]
Ruggeri et al. (2002)	AML, ALL	KIR-ligand mismatch	Reduced relapse, Reduces graft rejection, protected GvHD	[63]
Miller et al. (2007)	AML, MDS, CML	Missing-ligand	Reduced relapse	[81]
Pende et al. (2008)	AML, ALL	KIR-ligand mismatch	Antileukemic effect	[7]
Rubnitz et al. (2009)	AML	Receptor-ligand mismatch	No GvHD	[65]
Willemze et al. (2009)	AML, ALL	KIR-ligand mismatch	Reduced relapse	[82]
Cooley et al. (2009)	AML	KIR haplotype, KIR-ligand mismatch	Improved survival rate	[75]
Cooley et al. (2010)	AML, ALL	KIR haplotype	Reduced relapse	[83]
Venstrom et al. (2010)	AML, MDS, CML, ALL	KIR haplotype	Decreased acute GvHD	[84]
Curti et al. (2011)	AML	KIR-ligand mismatch	Antileukemic effect	[8]
Venstrom et al. (2012)	AML	Missing-ligand, receptor-ligand mismatch, presence of activating *KIR*	KIR2DS1 associated with lower relapse, KIR3DS1 associated with lower mortality	[74]
Cooley et al. (2014)	AML	Missing-ligand, KIR haplotype	Reduced relapse	[85]
Curti et al. (2016)	AML	KIR-ligand mismatch	Reduced relapse	[66]

AML: acute myeloid leukemia; ALL, acute lymphoid leukemia; CML: chronic myeloid leukemia; MDS: myelodysplastic syndromes; GvHD: graft versus host disease.

Table 3.
Clinical studies of beneficial effects of NK cell alloreactivities in acute leukemia.

6. Conclusion

This chapter sheds light on adoptive transfer NK cell immunotherapy in haplo-HSCT setting after immunosuppressive chemotherapy. KIR-ligand mismatches, activating KIR with cognate ligand as well as *KIR* B haplotype, could contribute to the potential benefit of allogeneic NK cells against acute leukemia in improving relapse-free and survival in AML patients. Additionally, the donor selection algorithm based on KIR-ligand mismatches is provided and available. Conflicts resulted from studies could be explained by the different definitions of KIR ligand mismatches, failure of infused NK cells to expand and persist, extensive genetic polymorphisms as well as the stochastic surface expressions of specific KIRs on individual NK cells. However, some limitations still need to be elucidated in further studies to overcome obstacles and achieve successful NK cell immunotherapy and clinical impact. For example, the ligands for KIR2DL5 and KIR2DS3 are still

unknown. In addition, the molecular mechanisms of KIR and NK cells need to be well-established. Eventually, this chapter provides a promising approach of NK cell-based immunotherapy that has revolutionized hematologic malignancy treatment, particularly AML, however, the feasibility has been challenged by complexity and understanding of NK cell biology as well as KIRs.

Acknowledgements

SC was supported by Thailand Research Fund and the Commission on Higher Education under the Grant for New Researcher (No. MRG6180172).

Conflict of interest

The authors declare no conflict of interest.

Author details

Suwit Chaisri[1*] and Chanvit Leelayuwat[2]

1 Chulabhorn International College of Medicine (CICM), Thammasat University, Pathum Thani, Thailand

2 The Centre for Research and Development of Medical Diagnostic Laboratories (CMDL), Department of Clinical Immunology and Transfusion Sciences, Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen, Thailand

*Address all correspondence to: chaisrisuw@gmail.com

References

[1] Rezvani K, Rouce RH. The application of natural killer cell immunotherapy for the treatment of cancer. Frontiers in Immunology. 2015;**6**:578. DOI: 10.3389/fimmu.2015.00578

[2] Paul S, Lal G. The molecular mechanism of natural killer cells function and its importance in cancer immunotherapy. Frontiers in Immunology. 2017;**8**:1124. DOI: 10.3389/fimmu.2017.01124

[3] Zhang J, Zheng H, Diao Y. Natural killer cells and current applications of chimeric antigen receptor-modified NK-92 cells in tumor immunotherapy. International Journal of Molecular Sciences. 2019;**20**(2):317. DOI: 10.3390/ijms20020317

[4] Rezvani K, Rouce R, Liu E, Shpall E. Engineering natural killer cells for cancer immunotherapy. Molecular Therapy. 2017;**25**(8):1769-1781. DOI: 10.1016/j.ymthe.2017.06.012

[5] Mehta RS, Randolph B, Daher M, Rezvani K. NK cell therapy for hematologic malignancies. International Journal of Hematology. 2018;**107**(3):262-270. DOI: 10.1007/s12185-018-2407-5

[6] Handgretinger R, Lang P, Andre MC. Exploitation of natural killer cells for the treatment of acute leukemia. Blood. 2016;**127**(26):3341-3349. DOI: 10.1182/blood-2015-12-629055

[7] Pende D, Marcenaro S, Falco M, Martini S, Bernardo ME, Montagna D, et al. Anti-leukemia activity of alloreactive NK cells in KIR ligand-mismatched haploidentical HSCT for pediatric patients: Evaluation of the functional role of activating KIR and redefinition of inhibitory KIR specificity. Blood. 2009;**113**(13):3119-3129. DOI: 10.1182/blood-2008-06-164103

[8] Curti A, Ruggeri L, D'Addio A, Bontadini A, Dan E, Motta MR, et al. Successful transfer of alloreactive haploidentical KIR ligand-mismatched natural killer cells after infusion in elderly high risk acute myeloid leukemia patients. Blood. 2011;**118**(12):3273-3279. DOI: 10.1182/blood-2011-01-329508

[9] Cerwenka A, Lanier LL. Natural killer cell memory in infection, inflammation and cancer. Nature Reviews. Immunology. 2016;**16**(2):112-123. DOI: 10.1038/nri.2015.9

[10] Hammer Q, Ruckert T, Romagnani C. Natural killer cell specificity for viral infections. Nature Immunology. 2018;**19**(8):800-808. DOI: 10.1038/s41590-018-0163-6

[11] Vivier E, Tomasello E, Baratin M, Walzer T, Ugolini S. Functions of natural killer cells. Nature Immunology. 2008;**9**(5):503-510. DOI: 10.1038/ni1582

[12] Vivier E, Nunes JA, Vely F. Natural killer cell signaling pathways. Science. 2004;**306**(5701):1517-1519. DOI: 10.1126/science.1103478

[13] Long EO, Kim HS, Liu D, Peterson ME, Rajagopalan S. Controlling natural killer cell responses: Integration of signals for activation and inhibition. Annual Review of Immunology. 2013;**31**:227-258. DOI: 10.1146/annurev-immunol-020711-075005

[14] Kim S, Poursine-Laurent J, Truscott SM, Lybarger L, Song YJ, Yang L, et al. Licensing of natural killer cells by host major histocompatibility complex class I molecules. Nature. 2005;**436**(7051):709-713. DOI: 10.1038/nature03847

[15] Topham NJ, Hewitt EW. Natural killer cell cytotoxicity: How do they pull the trigger? Immunology. 2009;**128**(1):7-15. DOI: 10.1111/j.1365-2567.2009.03123.x

[16] Mercer F, Kozhaya L, Unutmaz D. Expression and function of TNF and IL-1 receptors on human regulatory T cells. PLoS One. 2010;**5**(1):e8639. DOI: 10.1371/journal.pone.0008639

[17] Wajant H. The Fas signaling pathway: More than a paradigm. Science. 2002;**296**(5573):1635-1636. DOI: 10.1126/science.1071553

[18] Chiossone L, Dumas PY, Vienne M, Vivier E. Natural killer cells and other innate lymphoid cells in cancer. Nature Reviews. Immunology. 2018;**18**(11): 671-688. DOI: 10.1038/s41577-018-0061-z

[19] Chaisri S, Traherne JA, Jayaraman J, Romphruk A, Trowsdale J, Leelayuwat C. Novel KIR genotypes and gene copy number variations in northeastern Thais. Immunology. 2018;**153**(3): 380-386. DOI: 10.1111/imm.12847

[20] Yawata M, Yawata N, Draghi M, Little AM, Partheniou F, Parham P. Roles for HLA and KIR polymorphisms in natural killer cell repertoire selection and modulation of effector function. The Journal of Experimental Medicine. 2006;**203**(3):633-645. DOI: 10.1084/jem.20051884

[21] Kulkarni S, Martin MP, Carrington M. The Yin and Yang of HLA and KIR in human disease. Seminars in Immunology. 2008;**20**(6):343-352. DOI: 10.1016/j.smim.2008.06.003

[22] Bakker AB, Phillips JH, Figdor CG, Lanier LL. Killer cell inhibitory receptors for MHC class I molecules regulate lysis of melanoma cells mediated by NK cells, gamma delta T cells, and antigen-specific CTL. Journal of Immunology. 1998;**160**(11):5239-5245. Epub 1998/05/30

[23] Mingari MC, Ponte M, Vitale C, Bellomo R, Moretta L. Expression of HLA class I-specific inhibitory receptors in human cytolytic T lymphocytes: A regulated mechanism that controls T-cell activation and function. Human Immunology. 2000;**61**(1):44-50. Epub 2000/02/05. DOI: S0198-8859(99)00158-5 [pii]

[24] Trowsdale J, Barten R, Haude A, Stewart CA, Beck S, Wilson MJ. The genomic context of natural killer receptor extended gene families. Immunological Reviews. 2001;**181**: 20-38. Epub 2001/08/22

[25] Wende H, Colonna M, Ziegler A, Volz A. Organization of the leukocyte receptor cluster (LRC) on human chromosome 19q13.4. Mammalian Genome. 1999;**10**(2):154-160. Epub 1999/01/29

[26] Hsu KC, Chida S, Geraghty DE, Dupont B. The killer cell immunoglobulin-like receptor (KIR) genomic region: Gene-order, haplotypes and allelic polymorphism. Immunological Reviews. 2002;**190**: 40-52. Epub 2002/12/21

[27] Lanier LL, Corliss BC, Wu J, Leong C, Phillips JH. Immunoreceptor DAP12 bearing a tyrosine-based activation motif is involved in activating NK cells. Nature. 1998;**391**(6668):703-707. Epub 1998/03/07. DOI: 10.1038/35642

[28] Rajagopalan S, Moyle MW, Joosten I, Long EO. DNA-PKcs controls an endosomal signaling pathway for a proinflammatory response by natural killer cells. Science Signaling. 2010;**3**(110):ra14. Epub 2010/02/25. DOI: 10.1126/scisignal.2000467

[29] Faure M, Long EO. KIR2DL4 (CD158d), an NK cell-activating receptor with inhibitory potential. Journal of Immunology. 2002;**168**(12):6208-6214. Epub 2002/06/11

[30] Robinson J, Halliwell JA, McWilliam H, Lopez R, Marsh SG.

IPD—The Immuno Polymorphism Database. Nucleic Acids Research. 2013;**41**(Database issue):D1234-D1240. Epub 2012/11/28. DOI: 10.1093/nar/gks1140

[31] Gonzalez-Galarza FF, Takeshita LY, Santos EJ, Kempson F, Maia MH, da Silva AL, et al. Allele frequency net 2015 update: New features for HLA epitopes, KIR and disease and HLA adverse drug reaction associations. Nucleic Acids Research. 2015;**43**(Database issue):D784-D788. Epub 2014/11/22. DOI: 10.1093/nar/gku1166

[32] Pyo CW, Guethlein LA, Vu Q, Wang R, Abi-Rached L, Norman PJ, et al. Different patterns of evolution in the centromeric and telomeric regions of group A and B haplotypes of the human killer cell Ig-like receptor locus. PLoS One. 2010;**5**(12):e15115. Epub 2011/01/06. DOI: 10.1371/journal.pone.0015115

[33] Shilling HG, Young N, Guethlein LA, Cheng NW, Gardiner CM, Tyan D, et al. Genetic control of human NK cell repertoire. Journal of Immunology. 2002;**169**(1):239-247

[34] Williams AP, Bateman AR, Khakoo SI. Hanging in the balance. KIR and their role in disease. Molecular Interventions. 2005;**5**(4):226-240. Epub 2005/08/27. DOI: 10.1124/mi.5.4.6

[35] Bashirova AA, Martin MP, McVicar DW, Carrington M. The killer immunoglobulin-like receptor gene cluster: Tuning the genome for defense. Annual Review of Genomics and Human Genetics. 2006;**7**:277-300. Epub 2006/07/11. DOI: 10.1146/annurev.genom.7.080505.115726

[36] Takeshita LY, Gonzalez-Galarza FF, dos Santos EJ, Maia MH, Rahman MM, Zain SM, et al. A database for curating the associations between killer cell immunoglobulin-like receptors and diseases in worldwide populations. Database: The Journal of Biological Databases and Curation. 2013;**2013**:bat021. Epub 2013/04/16. DOI: 10.1093/database/bat021

[37] Uhrberg M, Valiante NM, Shum BP, Shilling HG, Lienert-Weidenbach K, Corliss B, et al. Human diversity in killer cell inhibitory receptor genes. Immunity. 1997;**7**(6):753-763. Epub 1998/01/16. DOI: S1074-7613(00)80394-5 [pii]

[38] Toneva M, Lepage V, Lafay G, Dulphy N, Busson M, Lester S, et al. Genomic diversity of natural killer cell receptor genes in three populations. Tissue Antigens. 2001;**57**(4):358-362. Epub 2001/07/14. DOI: tan570411 [pii]

[39] Norman PJ, Stephens HA, Verity DH, Chandanayingyong D, Vaughan RW. Distribution of natural killer cell immunoglobulin-like receptor sequences in three ethnic groups. Immunogenetics. 2001;**52**(3-4):195-205. Epub 2001/02/28

[40] Rajalingam R, Du Z, Meenagh A, Luo L, Kavitha VJ, Pavithra-Arulvani R, et al. Distinct diversity of KIR genes in three southern Indian populations: Comparison with world populations revealed a link between KIR gene content and pre-historic human migrations. Immunogenetics. 2008;**60**(5):207-217. Epub 2008/03/29. DOI: 10.1007/s00251-008-0286-2

[41] Wu GQ, Zhao YM, Lai XY, Yang KL, Zhu FM, Zhang W, et al. Distribution of killer-cell immunoglobulin-like receptor genes in Eastern mainland Chinese Han and Taiwanese Han populations. Tissue Antigens. 2009;**74**(6):499-507. Epub 2009/09/19. DOI: 10.1111/j.1399-0039.2009.01366.x

[42] Rajalingam R, Krausa P, Shilling HG, Stein JB, Balamurugan A, McGinnis MD, et al. Distinctive KIR and HLA diversity in a panel of north Indian Hindus. Immunogenetics. 2002;**53**(12):

1009-1019. Epub 2002/03/21. DOI: 10.1007/s00251-001-0425-5

[43] Chaisri S, Kitcharoen K, Romphruk AV, Romphruk A, Witt CS, Leelayuwat C. Polymorphisms of killer immunoglobulin-like receptors (KIRs) and HLA ligands in northeastern Thais. Immunogenetics. 2013;**65**(9):645-653. Epub 2013/07/03. DOI: 10.1007/s00251-013-0716-7

[44] Moradi S, Berry R, Pymm P, Hitchen C, Beckham SA, Wilce MC, et al. The structure of the atypical killer cell immunoglobulin-like receptor, KIR2DL4. The Journal of Biological Chemistry. 2015;**290**(16):10460-10471. DOI: 10.1074/jbc.M114.612291

[45] Le Page ME, Goodridge JP, John E, Christiansen FT, Witt CS. Response to comment on "killer Ig-like receptor 2DL4 does not mediate NK cell IFN-gamma responses to soluble HLA-G preparations". Journal of Immunology. 2014;**192**(9):4003-4004. DOI: 10.4049/jimmunol.1400492

[46] Jamil KM, Khakoo SI. KIR/HLA interactions and pathogen immunity. Journal of Biomedicine & Biotechnology. 2011;**2011**:298348. Epub 2011/06/02. DOI: 10.1155/2011/298348

[47] Thiruchelvam-Kyle L, Hoelsbrekken SE, Saether PC, Bjornsen EG, Pende D, Fossum S, et al. The activating human NK cell receptor KIR2DS2 recognizes a beta2-microglobulin-independent ligand on cancer cells. Journal of Immunology. 2017;**198**(7):2556-2567. DOI: 10.4049/jimmunol.1600930

[48] Purdy AK, Campbell KS. Natural killer cells and cancer: Regulation by the killer cell Ig-like receptors (KIR). Cancer Biology & Therapy. 2009;**8**(23):2211-2220. Epub 2009/11/20

[49] Biassoni R, Pessino A, Malaspina A, Cantoni C, Bottino C, Sivori S, et al. Role of amino acid position 70 in the binding affinity of p50.1 and p58.1 receptors for HLA-Cw4 molecules. European Journal of Immunology. 1997;**27**(12):3095-3099. Epub 1998/02/17. DOI: 10.1002/eji.1830271203

[50] Vales-Gomez M, Erskine RA, Deacon MP, Strominger JL, Reyburn HT. The role of zinc in the binding of killer cell Ig-like receptors to class I MHC proteins. Proceedings of the National Academy of Sciences of the United States of America. 2001;**98**(4):1734-1739. Epub 2001/02/15. DOI: 10.1073/pnas.041618298

[51] Miller JS, Soignier Y, Panoskaltsis-Mortari A, McNearney SA, Yun GH, Fautsch SK, et al. Successful adoptive transfer and in vivo expansion of human haploidentical NK cells in patients with cancer. Blood. 2005;**105**(8):3051-3057. DOI: 10.1182/blood-2004-07-2974

[52] Rodriguez NA, Meier PP, Groer MW, Zeller JM, Engstrom JL, Fogg L. A pilot study to determine the safety and feasibility of oropharyngeal administration of own mother's colostrum to extremely low-birth-weight infants. Advances in Neonatal Care. 2010;**10**(4):206-212. DOI: 10.1097/ANC.0b013e3181e94133

[53] Ruggeri L, Capanni M, Casucci M, Volpi I, Tosti A, Perruccio K, et al. Role of natural killer cell alloreactivity in HLA-mismatched hematopoietic stem cell transplantation. Blood. 1999;**94**(1):333-339

[54] Aversa F, Tabilio A, Terenzi A, Velardi A, Falzetti F, Giannoni C, et al. Successful engraftment of T-cell-depleted haploidentical "three-loci" incompatible transplants in leukemia patients by addition of recombinant human granulocyte colony-stimulating factor-mobilized peripheral blood progenitor cells to bone marrow inoculum. Blood. 1994;**84**(11):3948-3955

[55] Locatelli F, Pende D, Mingari MC, Bertaina A, Falco M, Moretta A, et al. Cellular and molecular basis of haploidentical hematopoietic stem cell transplantation in the successful treatment of high-risk leukemias: Role of alloreactive NK cells. Frontiers in Immunology. 2013;**4**:15. DOI: 10.3389/fimmu.2013.00015

[56] Heidenreich S, Kroger N. Reduction of relapse after unrelated donor stem cell transplantation by KIR-based graft selection. Frontiers in Immunology. 2017;**8**:41. DOI: 10.3389/fimmu.2017.00041

[57] Ljunggren HG, Karre K. In search of the 'missing self': MHC molecules and NK cell recognition. Immunology Today. 1990;**11**(7):237-244

[58] Symons HJ, Fuchs EJ. Hematopoietic SCT from partially HLA-mismatched (HLA-haploidentical) related donors. Bone Marrow Transplantation. 2008;**42**(6):365-377. DOI: 10.1038/bmt.2008.215

[59] Ruggeri L, Mancusi A, Capanni M, Urbani E, Carotti A, Aloisi T, et al. Donor natural killer cell allorecognition of missing self in haploidentical hematopoietic transplantation for acute myeloid leukemia: Challenging its predictive value. Blood. 2007;**110**(1):433-440. DOI: 10.1182/blood-2006-07-038687

[60] Chewning JH, Gudme CN, Hsu KC, Selvakumar A, Dupont B. KIR2DS1-positive NK cells mediate alloresponse against the C2 HLA-KIR ligand group in vitro. Journal of Immunology. 2007;**179**(2):854-868

[61] Guillerey C, Huntington ND, Smyth MJ. Targeting natural killer cells in cancer immunotherapy. Nature Immunology. 2016;**17**(9):1025-1036. DOI: 10.1038/ni.3518

[62] Ruggeri L, Parisi S, Urbani E, Curti A. Alloreactive natural killer cells for the treatment of acute myeloid leukemia: From stem cell transplantation to adoptive immunotherapy. Frontiers in Immunology. 2015;**6**:479. DOI: 10.3389/fimmu.2015.00479

[63] Ruggeri L, Capanni M, Urbani E, Perruccio K, Shlomchik WD, Tosti A, et al. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science. 2002;**295**(5562):2097-2100. DOI: 10.1126/science.1068440

[64] Koehl U, Sorensen J, Esser R, Zimmermann S, Gruttner HP, Tonn T, et al. IL-2 activated NK cell immunotherapy of three children after haploidentical stem cell transplantation. Blood Cells, Molecules & Diseases. 2004;**33**(3):261-266. DOI: 10.1016/j.bcmd.2004.08.013

[65] Rubnitz JE, Inaba H, Ribeiro RC, Pounds S, Rooney B, Bell T, et al. NKAML: A pilot study to determine the safety and feasibility of haploidentical natural killer cell transplantation in childhood acute myeloid leukemia. Journal of Clinical Oncology. 2010;**28**(6):955-959. DOI: 10.1200/JCO.2009.24.4590

[66] Curti A, Ruggeri L, Parisi S, Bontadini A, Dan E, Motta MR, et al. Larger size of donor alloreactive NK cell repertoire correlates with better response to NK cell immunotherapy in elderly acute myeloid leukemia patients. Clinical Cancer Research. 2016;**22**(8):1914-1921. DOI: 10.1158/1078-0432.CCR-15-1604

[67] Parisi S, Lecciso M, Ocadlikova D, Salvestrini V, Ciciarello M, Forte D, et al. The more, the better: "Do the right thing" for natural killer immunotherapy in acute myeloid leukemia. Frontiers in Immunology. 2017;**8**:1330. DOI: 10.3389/fimmu.2017.01330

[68] Muntasell A, Lopez-Botet M. Natural killer cell-based immunotherapy

in acute myeloid leukemia: Lessons for the future. Clinical Cancer Research. 2016;**22**(8):1831-1833. DOI: 10.1158/1078-0432.CCR-15-3168

[69] Giebel S, Locatelli F, Lamparelli T, Velardi A, Davies S, Frumento G, et al. Survival advantage with KIR ligand incompatibility in hematopoietic stem cell transplantation from unrelated donors. Blood. 2003;**102**(3):814-819. DOI: 10.1182/blood-2003-01-0091

[70] Hsu KC, Gooley T, Malkki M, Pinto-Agnello C, Dupont B, Bignon JD, et al. KIR ligands and prediction of relapse after unrelated donor hematopoietic cell transplantation for hematologic malignancy. Biology of Blood and Marrow Transplantation. 2006;**12**(8):828-836. DOI: 10.1016/j.bbmt.2006.04.008

[71] Sivori S, Carlomagno S, Falco M, Romeo E, Moretta L, Moretta A. Natural killer cells expressing the KIR2DS1-activating receptor efficiently kill T-cell blasts and dendritic cells: Implications in haploidentical HSCT. Blood. 2011;**117**(16):4284-4292. DOI: 10.1182/blood-2010-10-316125

[72] Bachanova V, Cooley S, Defor TE, Verneris MR, Zhang B, McKenna DH, et al. Clearance of acute myeloid leukemia by haploidentical natural killer cells is improved using IL-2 diphtheria toxin fusion protein. Blood. 2014;**123**(25):3855-3863. DOI: 10.1182/blood-2013-10-532531

[73] Boudreau JE, Giglio F, Gooley TA, Stevenson PA, Le Luduec JB, Shaffer BC, et al. KIR3DL1/HL A-B subtypes govern acute myelogenous leukemia relapse after hematopoietic cell transplantation. Journal of Clinical Oncology. 2017;**35**(20):2268-2278. DOI: 10.1200/JCO.2016.70.7059

[74] Venstrom JM, Pittari G, Gooley TA, Chewning JH, Spellman S, Haagenson M, et al. HLA-C-dependent prevention of leukemia relapse by donor activating KIR2DS1. The New England Journal of Medicine. 2012;**367**(9):805-816. DOI: 10.1056/NEJMoa1200503

[75] Cooley S, Trachtenberg E, Bergemann TL, Saeteurn 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):726-732. DOI: 10.1182/blood-2008-07-171926

[76] Shaffer BC, Le Luduec JB, Forlenza C, Jakubowski AA, Perales MA, Young JW, et al. Phase II study of haploidentical natural killer cell infusion for treatment of relapsed or persistent myeloid malignancies following allogeneic hematopoietic cell transplantation. Biology of Blood and Marrow Transplantation. 2016;**22**(4):705-709. DOI: 10.1016/j.bbmt.2015.12.028

[77] Lee DA, Denman CJ, Rondon G, Woodworth G, Chen J, Fisher T, et al. Haploidentical natural killer cells infused before allogeneic stem cell transplantation for myeloid malignancies: A phase I trial. Biology of Blood and Marrow Transplantation. 2016;**22**(7):1290-1298. DOI: 10.1016/j.bbmt.2016.04.009

[78] Oevermann L, Michaelis SU, 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):2744-2747. DOI: 10.1182/blood-2014-03-565069

[79] Brentjens RJ. Cellular therapies in acute lymphoblastic leukemia. Current Opinion in Molecular Therapeutics. 2009;**11**(4):375-382

[80] Romanski A, Bug G, Becker S, Kampfmann M, Seifried E, Hoelzer D, et al. Mechanisms of resistance to natural killer cell-mediated

cytotoxicity in acute lymphoblastic leukemia. Experimental Hematology. 2005;**33**(3):344-352. DOI: 10.1016/j.exphem.2004.11.006

[81] Miller JS, Cooley S, Parham P, Farag SS, Verneris MR, McQueen KL, et al. Missing KIR ligands are associated with less relapse and increased graft-versus-host disease (GVHD) following unrelated donor allogeneic HCT. Blood. 2007;**109**(11):5058-5061. DOI: 10.1182/blood-2007-01-065383

[82] Willemze R, Rodrigues CA, Labopin M, Sanz G, Michel G, Socie G, et al. KIR-ligand incompatibility in the graft-versus-host direction improves outcomes after umbilical cord blood transplantation for acute leukemia. Leukemia. 2009;**23**(3):492-500. DOI: 10.1038/leu.2008.365

[83] Cooley S, Weisdorf DJ, Guethlein LA, Klein JP, Wang T, Le CT, et al. Donor selection for natural killer cell receptor genes leads to superior survival after unrelated transplantation for acute myelogenous leukemia. Blood. 2010;**116**(14):2411-2419. DOI: 10.1182/blood-2010-05-283051

[84] Venstrom JM, Gooley TA, Spellman S, Pring J, Malkki M, Dupont B, et al. Donor activating KIR3DS1 is associated with decreased acute GVHD in unrelated allogeneic hematopoietic stem cell transplantation. Blood. 2010;**115**(15):3162-3165. DOI: 10.1182/blood-2009-08-236943

[85] Cooley S, Weisdorf DJ, Guethlein LA, Klein JP, Wang T, Marsh SG, et al. Donor killer cell Ig-like receptor B haplotypes, recipient HLA-C1, and HLA-C mismatch enhance the clinical benefit of unrelated transplantation for acute myelogenous leukemia. Journal of Immunology. 2014;**192**(10):4592-4600. DOI: 10.4049/jimmunol.1302517