RESEARCH

HLA-B*44 and the Bw4-80T motif are associated with poor outcome of relapse-preventive immunotherapy in acute myeloid leukemia

Hana Komic[1,2] · Alexander Hallner[1] · Brwa Ali Hussein[1,2] · Chiara Badami[1,2] · Anne Wöhr[1,2] · Kristoffer Hellstrand[1,3] · Elin Bernson[1,4] · Fredrik B. Thorén[1,2]

Abstract

HLA-B alleles are associated with outcomes in various pathologies, including autoimmune diseases and malignancies. The encoded HLA-B proteins are pivotal in antigen presentation to cytotoxic T cells, and some variants containing a Bw4 motif also serve as ligands to the killer immunoglobulin-like receptors (KIR) 3DL1/S1 of NK cells. We investigated the potential impact of HLA-B genotypes on the efficacy of immunotherapy for relapse prevention in acute myeloid leukemia (AML). Seventy-eight non-transplanted AML patients receiving HDC/IL-2 in the post-consolidation phase were genotyped for HLA-B and KIR genes. HLA-B*44 heralded impaired LFS (leukemia-free survival) and overall survival (OS), but the negative association with outcome was not shared across alleles of the HLA-B44 supertype. Notably, HLA-B*44 is one of few HLA-B44 supertype alleles containing a Bw4 motif with a threonine at position 80, which typically results in weak binding to the inhibitory NK receptor, KIR3DL1. Accordingly, a strong interaction between KIR3DL1 and Bw4 was associated with superior LFS and OS ($p = 0.014$ and $p = 0.027$, respectively). KIR3DL1$^+$ NK cells from 80 T-Bw4 donors showed significantly lower degranulation responses and cytokine responses than NK cells from 80I-Bw4 donors, suggesting impaired KIR3DL1-mediated education in 80 T-Bw4 subjects. We propose that presence of a strong KIR3DL1$^+$–Bw4 interaction improves NK cell education and thus is advantageous in AML patients receiving HDC/IL-2 immunotherapy for relapse prevention.

Keywords NK cells · KIR3DL1 · HLA-B*44 · AML · IL-2 · Histamine

Introduction

Acute myeloid leukemia (AML) is characterized by clonal expansion of immature myeloid cells in the bone marrow and peripheral blood [1]. Intensive induction chemotherapy yields high rates of complete remission (CR) but many patients relapse despite repeated courses of consolidation chemotherapy in the post-CR phase. Eligible patients may receive allogeneic stem cell transplants and older patients may benefit from post-consolidation chemotherapy for remission maintenance [2] but there remains a need for additional relapse-preventive therapies [3]. A phase III trial of 320 AML patients in remission showed that treatment with histamine dihydrochloride and interleukin-2 (HDC/IL-2) significantly improved leukemia-free survival (LFS) versus standard of care in patients below 60 years of age [4]. In this regimen, IL-2 activates natural killer (NK) and T cells, while HDC targets H$_2$ receptors expressed by myeloid

Hana Komic and Alexander Hallner have contributed eqally to this work.

✉ Fredrik B. Thorén
  fredrik.thoren@gu.se

[1] TIMM Laboratory at Sahlgrenska Center for Cancer Research, University of Gothenburg, Gothenburg, Sweden

[2] Department of Medical Biochemistry and Cell Biology, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

[3] Department of Infectious Diseases, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

[4] Department of Obstetrics and Gynecology, Institute of Clinical Sciences, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

cells to inhibit the formation of immunosuppressive oxygen radicals [5–7].

Human leukocyte antigen (HLA) class I genes are highly polymorphic, and the encoded proteins have a key role in antigen presentation. Peptides derived from intracellular proteins bind to the groove of the HLA molecule and are presented to cytotoxic CD8^{+} T cells. Different HLA alleles that share capacity to present similar peptides are commonly grouped into nine HLA supertypes [8]. A particular neoantigen from malignant cells can preferentially be presented by HLA molecules of a specific supertype, leading to detection by specific T cells and the elimination of malignant cells. For example, HLA-B44 supertype molecules feature an electropositive binding pocket and preferentially display peptides with negatively charged amino acid anchors. Mutations causing glycine-to-glutamic acid substitutions are common in melanoma, and it was proposed that this contributes to the favorable outcome of HLA-B44 supertype patients in immune checkpoint blockade (ICB)-treated melanoma [9]. However, the beneficial impact of the B44 supertype was not observed in lung cancer [10], B cell lymphoma (DLBCL) [11] or uveal melanoma [12]. Conversely, the HLA-B62 supertype and the HLA-A03 allele have been associated with inferior outcome of ICB therapy [9, 13].

Like T cells, NK cells express a battery of receptors that recognize HLA molecules, such as the killer immunoglobulin-like receptors (KIRs). The *KIR* locus harbors a family of highly homologous and polymorphic genes, and the encoded receptors recognize specific HLA molecules. Thereby, NK cells will in general spare healthy cells with intact expression of HLA class I molecules, while cells with absent or reduced HLA class I expression will fail to generate sufficient inhibitory signals in NK cells and are killed in a process known as missing self-recognition. All HLA-C alleles serve as ligands to KIR2D receptors, while subsets of HLA-A and HLA-B alleles contain a Bw4 motif that ligates KIR3DL1. A dimorphism at HLA-B Bw4 position 80, with isoleucine (Bw4-80I) or threonine (Bw4-80 T) variants, has been reported to determine the affinity for KIR3DL1 [14]. Interestingly, such Bw4 variants were reported to be of clinical relevance in viral infections and malignancies [15–17]. Bw4 motifs are also present in a few HLA-A alleles, but it is still unclear to what extent these alleles contribute to KIR3DL1-mediated education and inhibition [18–20].

This study aimed at determining the potential impact of HLA-B genotypes on outcome of HDC/IL-2-based immunotherapy in AML. We observed that HLA-B*44, but not other members of the HLA-B44 supertype, was associated with distinctly poor LFS. Notably, the HLA-B*44 alleles encode a Bw4-80 T motif with low affinity for KIR3DL1. Individuals with this Bw4 variant harbored KIR3DL1^{+} NK cells with inferior degranulation and cytokine release responses

to leukemic cells, which may contribute to the poor survival after AML immunotherapy.

Materials and methods

Patients

An open-label single-arm phase IV study (Re:Mission, NCT01347996) enrolled 84 (age 18–79) patients in first CR with de novo or secondary AML. Patients received ten 21-day courses of HDC (0.5 mg by subcutaneous injection twice daily) and IL-2 (16 400 IU/kg by subcutaneous injection twice daily) over a period of 18 months or until relapse/death. A primary objective of the study was to monitor quantitative and qualitative pharmacodynamics effects with focus on the phenotype and function of NK, T and myeloid cells. All patients gave written informed consent prior to enrollment and the trial was approved by the Ethical committees in all participating regions. This study was conducted according to the Declaration of Helsinki principles. Results from the trial have been previously published [21–24]. Detailed patient characteristics can be found in previously published papers [21, 22].

Sampling of peripheral blood

Peripheral blood was collected before and after the first and third treatment cycles. Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation using Lymphoprep (Stemcell Technologies), cryopreserved at local sites and shipped on dry ice to the central laboratory at University of Gothenburg for analysis by flow cytometry. Samples with $< 25\%$ viability were excluded from any analyzes.

Leukopacks from healthy blood donors were obtained from Sahlgrenska University Hospital, and PBMCs were isolated by density gradient centrifugation using Lymphoprep (Stemcell Technologies) and cryopreserved in liquid nitrogen. Isolation of NK cells was performed by negative selection using human NK cell isolation kit (Miltenyi Biotec) in accordance with manufacturer's instructions.

DNA extraction and KIR/HLA genotyping

DNA was extracted from whole blood using a Roche MagNAPure 96 or Qiagen® DNeasy Blood & Tissue kit according to the manufacturer's instructions. KIR3DL1 typing was performed using PCR primers described by Boudreau et al. to detect the major alleles *004 (null),

*001, *002 (high), *013 (3DS1) and *005, *007 (low). [25]. Furthermore, flow cytometry was used to determine the KIR3DL1 expression level by staining cells with anti-CD3-APC-H7 (SK7), anti-CD14-APC-H7 (MφP9), anti-CD16-BV786 (3G8), anti-CD56-BV711 (NCAM1), anti-CD57-BV605 (NK-1), anti-CD19-APC-H7 (SJ25C1; all from BD Biosciences), anti-NKG2C-Alexa Fluor 488 (134,591), anti-KIR2DL1 Alexa Fluor 700 (143211, both R&D Systems), anti-NKG2A-PE (Z199), anti-KIR2DL1/S1-Pe-Cy7 (EB6B), anti-KIR2DL2/L3/S2-Pe-Cy5.5 (GL 183; all from Beckman Coulter), anti-KIR3DL1-APC (DX9; BioLegend). Stained samples were analyzed on a five laser BD LSR Fortessa SORP instrument. Data was analyzed using FACSDiva (v.8.0.1 or later) and FlowJo (v.10.8.1 or later) software (BD Biosciences). The median frequency of KIR3DL1$^+$ cells among CD16$^+$CD56$^+$ NK cells was 11% (range 7.71–56.5%). The HLA-B and -C allele genotype was determined using LABType SSO Class I Locus Typing Tests from One Lambda as described elsewhere [26]. Complementary KIR ligand typing for HLA-A Bw4 was performed using the Olerup SSP KIR HLA Ligand kit.

HLA-B and HLA-Bw4 staining

To determine the expression levels of HLA-B and HLA-Bw4 in 80I/T versus 80 T groups of donors, cryopreserved PBMCs were stained with anti-CD3-FITC (HIT3a), anti-CD14-PE-Cy7 (M5E2), anti-CD56-BV711 (NCAM 16.2), anti-HLA-B-PE (YTH 76.3rMAb, all BD Biosciences), live-dead marker (near IR-APC-Cy7, Life Technologies) and anti-CD3-PerCP-Cy5.5 (SK7), anti-CD14-PE-Cy7 (M5E2), anti-CD56-BV711 (NCAM 16.2), anti-HLA-Bw4-FITC (REA274, Miltenyi Biotec), live-dead marker (near IR-APC-Cy7, Life Technologies). Analyzes were performed on a five laser BD LSR Fortessa SORP instrument and FlowJo software (v.10.8.1 or later) (BD Biosciences).

Functional assays

NK cells were cultured overnight with 100 IU/ml of IL-2 (Proleukin, Novartis Pharmaceuticals) or non-stimulated (resting) in 0.1 million cells/well before addition of 0.1 million K562 cells/well. Anti-CD107a-BUV395 (H4A3, BD Biosciences) was added to measure the degranulation. After 4 h-incubation, cells were stained with anti-CD56-BV711 (NCAM 16.2, BD Biosciences), anti-NKG2A-PE (Z199), anti-KIR2DL1/S1-PE-Cy7 (EB6B), anti-KIR2DL2/L3/S2-PE-Cy5.5 (GL183; all Beckman Coulter) and anti-KIR3DL1-APC (DX9, Biolegend). Gating strategy is shown in Supplementary Fig. 1.

In intracellular cytokine assays, 50 000 NK cells/well were cultured and stimulated overnight, using same conditions as in degranulation assays. A five-hour co-culture with 50 000 K562 cells/well at 37 °C was performed, adding Brefeldin A (GolgiPlug, BD Biosciences) after one hour. The cells were stained with the same antibody panel as mentioned above, followed by fixation and permeabilization with BD Cytofix/Cytoperm (BD Biosciences). At the end, cells were stained with anti-IFNγ-BUV395 (B27) and anti-TNFα-AF488 (Mab 11; both BD Biosciences).

Stained samples were analyzed using a five laser BD LSR Fortessa SORP instrument. Data was analyzed using FACSDiva (v.8.0.1 or later) and FlowJo (v.10.8.1) software (BD Biosciences).

Statistical analyses

All statistical analyses were performed in Prism v. 9 or later (GraphPad Software). The logrank test (Mantel-Cox) was used to compare survival between patient groups. Incidence of relapse was calculated using Chi square test. The impact of HLA-B and HLA-A alleles on LFS was analyzed by univariate and multivariate Cox regression analyses using IBM SPSS Statistics (v. 29). Mann–Whitney test was used to compare responses in degranulation and intracellular cytokine staining assays between groups.

Results

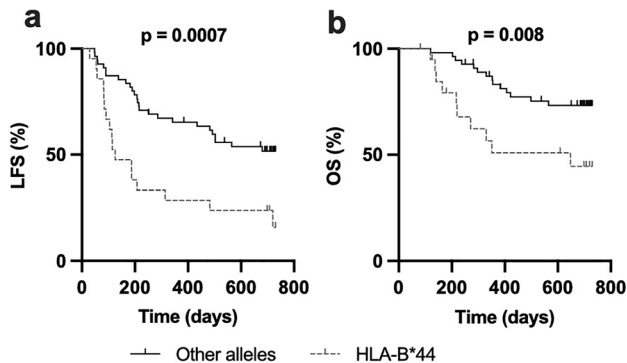### HLA-B*44 associates with inferior leukemia-free survival

Seventy-six patients from the Re:Mission trial were genotyped for HLA-B alleles. Out of 24 detected alleles, the most common was B*44 (detected in 28% of patients, allele frequency = 0.15), followed by B*35 (21%, 0.11), B*7 (20%, 0.11) and B*8 (20%, 0.11). The other alleles detected in > 10% of patients were B*51 and B*40 (Table 1).

To determine whether HLA-B allotypes had an impact on clinical outcome, we performed univariate and multivariate Cox regression analyses of leukemia-free survival (LFS) for all alleles with an allele frequency of > 10% in the cohort. As shown in Table 1, patients with at least one HLA-B*44 allele showed significantly inferior LFS. Accordingly, Kaplan–Meier survival analyses showed that patients harboring an HLA-B*44 (n = 21) allele had inferior LFS and overall survival (OS) when compared to patients with other alleles (n = 55) (Fig. 1a, b; p = 0.0007 and p = 0.008, respectively).

Some HLA-B alleles display a similar capacity to bind and present peptides, and such alleles are thus commonly grouped into supertypes [27]. Survival analyses comparing the B44 supertype alleles (HLA-B*18, *37, *40, *41, *44, *47, *49 and *50) (n = 41) with all other B supertypes

Table 1 HLA-B allele frequency and impact on LFS

Allele	Detected in% patients	Allele frequency	Univariate Cox regression analysis of LFS	Multivariate Cox regression analysis of LFS
B*07	20	0.112	0.055	0.517
B*08	20	0.105	0.379	0.971
B*35	21	0.112	0.993	0.245
B*40	14	0.072	0.562	0.102
B*44	28	0.151	<0.001	0.005
B*51	17	0.086	0.524	0.836

Fig. 1 The HLA*B44 allele is associated with significantly inferior LFS and OS. **a** Leukemia-free survival and **b** Overall survival for patients with at least one HLA*B44 allele (n=21) compared with patients lacking HLA-B*44 alleles (n=55). (Log rank test for trend)
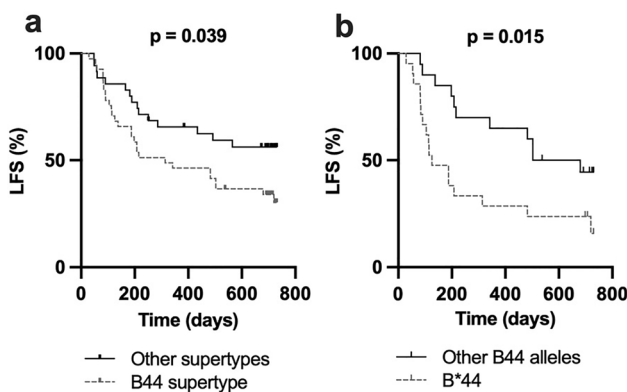
Fig. 2 Inferior survival in the HLA-B44 supertype is driven by the HLA-B*44 allele **a** Leukemia-free survival of patients with alleles from the HLA-B44 supertype versus patients with alleles from any other HLA-B supertype (B07, B27, B58 and B62), **b** Leukemia-free survival among patients having at least one B*44 allele versus patients carrying any other non-HLA-B*44 allele of the B44 supertype. (Log rank test for trend)

(n=35) showed significantly superior LFS for patients who did not carry any B44 supertype alleles ($p < 0.05$; Fig. 2a). However, the effect of the B44 supertype on clinical outcome was clearly driven by HLA-B*44 as LFS was significantly higher in B*44 allele carriers (n=21) than in non-B*44 allele carriers (n=20) within the HLA-B44 supertype (Fig. 2b).

Patients with Bw4-80 T have inferior clinical outcome

As stated above, HLA supertypes are groups of HLA alleles that share peptide binding features that allow them to present similar antigens to T cells. The finding that not all alleles within the B44 supertype share the negative association with LFS made us hypothesize that this discrepancy might be related to differential capacity of the encoded HLA-B molecules to interact with NK cell receptors. Although recent studies have demonstrated that some NK cell receptors may display peptide specificity [28], HLA interactions with NK cell receptors are typically less dependent on the presented peptide. Furthermore, NK cells are reportedly important for clinical outcome of HDC/IL-2 immunotherapy in AML [21, 22, 26, 29, 30]. HLA-B molecules contain either a Bw6 motif that does not interact with NK cell receptors, or one of two different Bw4 motifs, Bw4-80I and Bw4-80 T, which are recognized by the NK cell receptor KIR3DL1 [31]. Interestingly, data from the Immune polymorphism database showed that there are large differences within the B44 supertype regarding presence of Bw4 or Bw6 motifs [18]. HLA-B*37, HLA-B*44, and HLA-B*47 alleles harbor the Bw4-80 T motif, the HLA-B*49 allele contains a Bw4-80I motif, while the remaining major HLA-B44 supertype alleles detected encode the Bw6 motif. Thus, we next tested if relapse incidence was associated with Bw4 and Bw6 motifs. We speculated that having only one 80I allele is sufficient to enable education, as HLA-B and HLA-B Bw4 expression is much higher in 80I/T donors compared to 80 T donors (Supplementary Fig. 2). As shown in Fig. 3a patients with at least one Bw4-80 T allele and no 80I allele had higher relapse rates (Chi-square test, $p = 0.003$). Furthermore, these patients had a significantly inferior LFS ($p < 0.0001$) compared to 80I and Bw6 groups (Fig. 3b). Similar results were obtained if 80I/T patients were excluded
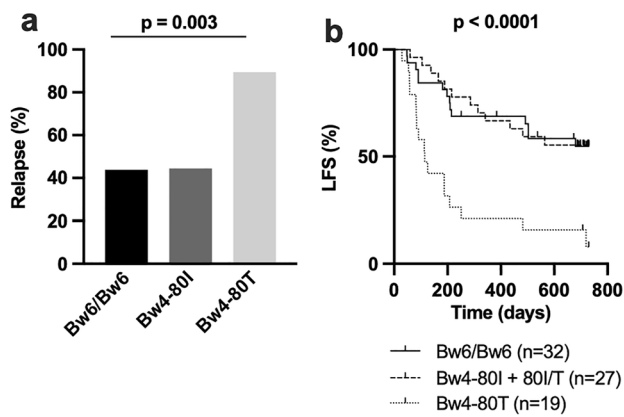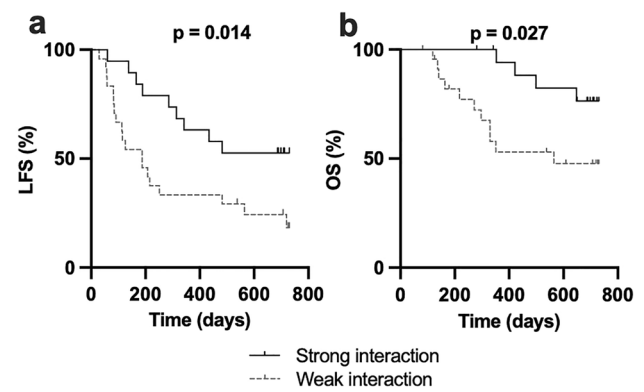
Fig. 3 The Bw4 binding motif impacts survival outcome. **a** Relapse rates and **b** Kaplan–Meier analysis of patients with only a Bw4-80 T motif (Bw4-80 T/Bw4-80 T or Bw4-80 T/Bw6, n=19), at least one Bw4-80I motif (Bw4-80I/Bw4-80I, Bw4-80I/Bw4-80 T or Bw4-80I/Bw6, n=27) or Bw6/Bw6 (n=32). (Log rank test for trend)

Fig. 4 Weak 3DL1–Bw4 interaction is associated with inferior survival. **a** Leukemia -free survival and **b** Overall survival of patients with a strong interacting 3DL1–Bw4 pair (at least one KIR3DL1-high allele without low alleles together with a Bw4-80I allele) or weak interacting pair (KIR3DL1-low together with either of the Bw4 motifs) (Log rank test for trend)

from the analysis ($p < 0.0001$), or alternatively included to 80 T group ($p = 0.001$) (Supplementary Fig. 3a, b).

A few HLA-A alleles contain a Bw4 motif but it is still unclear to what extent these alleles contribute to KIR3DL1-mediated education and inhibition. In our patient cohort, HLA-A Bw4 did not show significant effect on LFS in 80I and 80 T groups ($p = 0.35$ and $p = 0.71$, respectively) (Supplementary Fig. 4). Additionally, to address to what extent HLA-A Bw4 presence affects the impact of HLA-B Bw4 alleles on the LFS, we performed univariate and multivariate Cox regression analyses, using HLA-A Bw4 presence/absence in combination with B*44 allele and HLA-B Bw4 motif as covariates, respectively. The findings regarding B*44 and B-80I versus 80 T remained highly significant (Supplementary Tables 1 and 2).

Strong interacting 3DL1/HLA-B Bw4 pair favors leukemia-free survival

Different combinations of KIR3DL1 and Bw4-containing HLA-B alleles result in 3DL1/HLA-B Bw4 interactions of varying affinity. KIR3DL1 alleles are divided into subgroups based on surface expression and function, and the subtypes KIR3DL1-high or KIR3DL1-low bind HLA-B Bw4-80I and 80 T with different affinity. We thus genotyped all HLA-B Bw4+ patients for KIR3DL1 alleles and grouped them into either high or low allele, using classification proposed by Boudreau et al. [32]. Strong KIR3DL1/HLA-B Bw4 interaction was defined by presence of at least one KIR3DL1-high allele without low alleles along with an HLA-B Bw4-80I, while low KIR3DL1 receptor in combination with either of the two HLA-B Bw4 motifs was classified as weak interaction. Two patients with *013/*004 (3DS1/null) allele combinations

were excluded from the interaction figure. As shown in Fig. 4a, b, patients with a strong interacting pair (n=19) showed significantly improved LFS and OS compared to patients with a weak interacting pair (n=24). Similar results were obtained when KIR3DL1-high patients were defined by factual expression as determined by flow cytometry (Supplementary Fig. 5a, b).

KIR3DL1 education

To address the possible mechanisms underlying the inferior outcome for Bw4-80 T patients, we first determined the education status and ability to mount a missing-self response in KIR3DL1-single positive (sp) NK cells. In agreement with previous reports [33] there were significant differences between KIR3DL1+ NK cells from 80 T to 80I individuals in degranulation assays towards HLA-negative, NK cell-sensitive K562 cells. In the presence or absence of IL-2 pre-stimulation, the KIR3DL1+ NK cells from Bw4-80I donors degranulated significantly more than KIR3DL1+ NK cells from Bw4-80 T donors. Importantly, no such difference was seen when comparing NK cells educated via KIR2DL receptors in the same patients, suggesting that the observed difference was due to enhanced KIR3DL1 education in 80I individuals (Fig. 5a, b). The vast majority of NK cells from AML patients in the remission cohort were NKG2A+ cells. Accordingly, we tested and confirmed that the enhanced degranulation response was also seen in the corresponding NKG2A+KIR3DL1sp NK

Degranulation
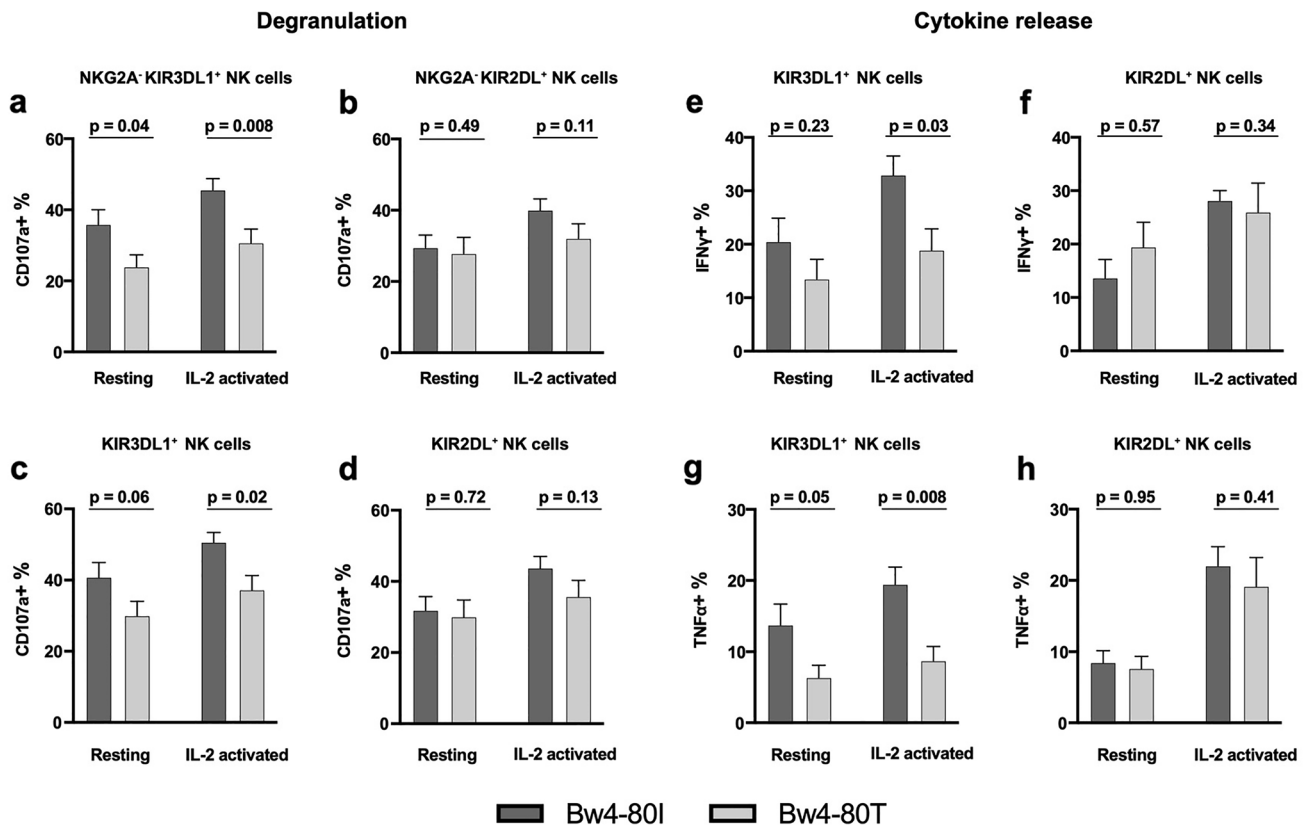
Cytokine release

Fig. 5 Degranulation assay in resting and IL-2 activated NK cells (a–d). **a** NKG2A-KIR3DL1sp cells, **b** NKG2A−KIR2D+ cells, **c** KIR3DL1+ cells, **d** KIR2D+ cells, (n=19). **Cytokine release e–h. e** IFN-g production in KIR3DL1+ cells, **f** KIR2D+ cells, **g** TNF-a in 3DL1+ cells, **h** KIR2D.+ cells, (n=13). Error bars represent SEM and Mann–Whitney test was used for statistical analyses. The mean degranulation response without K562 cells in absence of IL-2 was 1.6% (80I) and 1.0% (80 T), and 1.7% (80I) and 0.7% (80 T) in presence of IL-2. Corresponding results in the absence of K562 cells in intracellular cytokine release assays were consistently <1% (data not shown)

cells but not in NKG2A+KIR2DL+KIR3DL1− cells (Fig. 5c, d). A similar pattern of increased NK cell responses to K562 cells within the Bw4-80I group was observed for IFNγ and TNFα production in KIR3DL1 cells but not in KIR2DL cells (Fig. 5f–h, Supplementary Fig. 6).

Discussion

HLA-B is a highly polymorphic molecule implicated in immune cell recognition of malignant cells, and HLA-B genotypes have been linked to disease severity and treatment outcome in various pathologies. In this study, we set out to determine to what extent HLA-B genotypes are associated with treatment outcome in AML patients receiving immunotherapy for relapse prevention. The most striking finding in our study was that patients carrying B*44 alleles showed significantly inferior LFS and OS after HDC/IL-2 treatment. In contrast to these findings, ICB-treated melanoma patients with HLA-B44 supertype alleles reportedly

show prolonged survival [9]. In that study it was proposed that the B44 supertype encompasses an electropositive binding pocket to preferentially display peptides with negatively charged amino acid anchors, which are common among melanoma neoantigens [9].

In our study the association with outcome appeared to be specific for the B*44 alleles as patients carrying HLA-B*44 alleles showed inferior outcome as compared to patients carrying non-HLA-B*44 alleles. Supertypes comprise HLA alleles that share peptide preferences, but the ability to serve as a KIR3DL1 ligand may vary within one HLA-B supertype. Within the B44 supertype, HLA B*37, B*44 and B*47 contain the Bw4-80 T epitope, which is a weak ligand to KIR3DL1 while other alleles encode either Bw6 or Bw4-80I, with the latter typically showing high affinity to KIR3DL1 [14, 18, 34, 35]. Intuitively, a weak inhibitory interaction between NK cells and leukemic targets should be advantageous and favor NK cell cytotoxicity. However, a weak interaction also means that the homeostatic inhibitory signaling to circulating NK

cells via KIR3DL1 is low. These signals set the functional threshold for NK cells in a process known as education [36]. The more inhibitory input the NK cell receives at steady-state, the more vigorously the cell can respond to a target with down-regulated MHC class I expression. Accordingly, our data indicated that individuals carrying HLA-B Bw4-80 T harbored less functional single-positive KIR3DL1$^+$ NK cells than donors with Bw4-80I, which is in agreement with previous reports [33]. Notably, it was reported that AML blasts display lower expression of HLA-Bw4 antigens than cells from healthy volunteers [37]. It is thus conceivable that NK cells can exert missing-self cytotoxicity against AML blasts, and that the response may be stronger if these anti-leukemic cells are highly educated via KIR3DL1 interactions with the high-affinity Bw4-80I ligands.

In line with this reasoning, studies of HIV infection show that NK cells from individuals with strong educating KIR3DL1–Bw4 interactions mount more efficient cytotoxic responses to HIV-infected cells [19] and that such individuals are less likely to progress to AIDS [17]. Furthermore, in uveal melanoma, which is a rare malignancy in which NK cells are believed to be key mediators in preventing metastasis formation, HLA-B*44 allele carriers were reported to have a significantly inferior survival [12]. The interaction between KIR3DL1 and Bw4 variants has also been the focus of multiple studies of allogeneic stem cell transplantation in AML. Some studies have, in contrast to the studies above, suggested reduced relapse risk and a survival benefit in patients receiving grafts resulting in low KIR3DL1 inhibition [32, 38] but a large retrospective study of over 2000 transplanted patients did not confirm these results [39]. More studies are thus warranted to clarify the role of KIR3DL1–Bw4 interactions in transplanted and non-transplanted AML.

In summary, we show that expression of host HLA-B*44 is associated with inferior survival in AML patients receiving immunotherapy for relapse prevention. We hypothesize that this HLA-B variant, which is a weak ligand to the NK cell receptor, KIR3DL1, results in hypofunctional KIR3DL1$^+$ NK cells that fail to mount strong anti-leukemic responses.

Declarations

References

1. Dohner H, Weisdorf DJ, Bloomfield CD (2015) Acute myeloid leukemia. N Engl J Med 373:1136–1152. https://doi.org/10.1056/NEJMra1406184

2. Wei AH, Dohner H, Pocock C et al (2020) Oral azacitidine maintenance therapy for acute myeloid leukemia in first remission. N Engl J Med 383:2526–2537. https://doi.org/10.1056/NEJMoa2004444

3. Isidori A, Cerchione C, Daver N et al (2021) Immunotherapy in acute myeloid leukemia: where we stand. Front Oncol 11:656218. https://doi.org/10.3389/fonc.2021.656218

4. Brune M, Castaigne S, Catalano J et al (2006) Improved leukemia-free survival after postconsolidation immunotherapy with histamine dihydrochloride and interleukin-2 in acute myeloid leukemia: results of a randomized phase 3 trial. Blood 108:88–96. https://doi.org/10.1182/blood-2005-10-4073

5. Hansson M, Hermodsson S, Brune M, Mellqvist UH, Naredi P, Betten A, Gehlsen KR, Hellstrand K (1999) Histamine protects T cells and natural killer cells against oxidative stress. J Interferon Cytokine Res 19:1135–1144. https://doi.org/10.1089/107999093 13073

6. Hellstrand K, Asea A, Hermodsson S (1990) Role of histamine in natural killer cell-mediated resistance against tumor cells. J Immunol 145:4365–4370

7. Martner A, Wiktorin HG, Lenox B et al (2015) Histamine promotes the development of monocyte-derived dendritic cells and reduces tumor growth by targeting the myeloid NADPH oxidase. J Immunol 194:5014–5021. https://doi.org/10.4049/jimmunol.1402991

8. Sidney J, Peters B, Frahm N, Brander C, Sette A (2008) HLA class I supertypes: a revised and updated classification. BMC Immunol 9:1. https://doi.org/10.1186/1471-2172-9-1

9. Chowell D, Morris LGT, Grigg CM et al (2018) Patient HLA class I genotype influences cancer response to checkpoint blockade immunotherapy. Science 359:582–587. https://doi.org/10.1126/science.aao4572

10. Cummings AL, Gukasyan J, Lu HY et al (2020) Mutational landscape influences immunotherapy outcomes among patients

with non-small-cell lung cancer with human leukocyte antigen supertype B44. Nat Cancer 1:1167–1175. https://doi.org/10.1038/s43018-020-00140-1

11. Alcoceba M, Sebastian E, Marin L et al (2013) HLA specificities are related to development and prognosis of diffuse large B-cell lymphoma. Blood 122:1448–1454. https://doi.org/10.1182/blood-2013-02-483420

12. Maat W, Haasnoot GW, Claas FH, Schalij-Delfos NE, Schreuder GM, Jager MJ (2006) HLA Class I and II genotype in uveal melanoma: relation to occurrence and prognosis. Invest Ophthalmol Vis Sci 47:3–6. https://doi.org/10.1167/iovs.05-1122

13. Naranbhai V, Viard M, Dean M et al (2022) HLA-A*03 and response to immune checkpoint blockade in cancer: an epidemiological biomarker study. Lancet Oncol 23:172–184. https://doi.org/10.1016/S1470-2045(21)00582-9

14. Vivian JP, Duncan RC, Berry R et al (2011) Killer cell immunoglobulin-like receptor 3DL1-mediated recognition of human leukocyte antigen B. Nature 479:401–405. https://doi.org/10.1038/nature10517

15. Luque I, Solana R, Galiani MD, Gonzalez R, Garcia F, Lopez de Castro JA, Pena J (1996) Threonine 80 on HLA-B27 confers protection against lysis by a group of natural killer clones. Eur J Immunol 26:1974–1977. https://doi.org/10.1002/eji.1830260845

16. Martin MP, Gao X, Lee JH et al (2002) Epistatic interaction between KIR3DS1 and HLA-B delays the progression to AIDS. Nat Genet 31:429–434. https://doi.org/10.1038/ng934

17. Martin MP, Qi Y, Gao X et al (2007) Innate partnership of HLA-B and KIR3DL1 subtypes against HIV-1. Nat Genet 39:733–740. https://doi.org/10.1038/ng2035

18. EMBL-EBI Immuno polymorphism database. https://www.ebi.ac.uk/ipd/

19. Boudreau JE, Mulrooney TJ, Le Luduec JB, Barker E, Hsu KC (2016) KIR3DL1 and HLA-B density and binding calibrate NK education and response to HIV. J Immunol 196:3398–3410. https://doi.org/10.4049/jimmunol.1502469

20. van der Ploeg K, Le Luduec JB, Stevenson PA, Park S, Gooley TA, Petersdorf EW, Shaffer BC, Hsu KC (2020) HLA-A alleles influencing NK cell function impact AML relapse following allogeneic hematopoietic cell transplantation. Blood Adv 4:4955–4964. https://doi.org/10.1182/bloodadvances.2020002086

21. Martner A, Rydstrom A, Riise RE, Aurelius J, Anderson H, Brune M, Foa R, Hellstrand K, Thoren FB (2016) Role of natural killer cell subsets and natural cytotoxicity receptors for the outcome of immunotherapy in acute myeloid leukemia. Oncoimmunology 5:e1041701. https://doi.org/10.1080/2162402X.2015.1041701

22. Martner A, Rydstrom A, Riise RE, Aurelius J, Brune M, Foa R, Hellstrand K, Thoren FB (2015) NK cell expression of natural cytotoxicity receptors may determine relapse risk in older AML patients undergoing immunotherapy for remission maintenance. Oncotarget 6:42569–42574. https://doi.org/10.18632/oncotarget.5559

23. Rydstrom A, Hallner A, Aurelius J, Sander FE, Bernson E, Kiffin R, Thoren FB, Hellstrand K, Martner A (2017) Dynamics of myeloid cell populations during relapse-preventive immunotherapy in acute myeloid leukemia. J Leukoc Biol 102:467–474. https://doi.org/10.1189/jlb.5VMA1116-455R

24. Sander FE, Rydstrom A, Bernson E et al (2016) Dynamics of cytotoxic T cell subsets during immunotherapy predicts outcome in acute myeloid leukemia. Oncotarget 7:7586–7596. https://doi.org/10.18632/oncotarget.7210

25. Boudreau JE, Le Luduec JB, Hsu KC (2014) Development of a novel multiplex PCR assay to detect functional subtypes of KIR3DL1 alleles. PLoS ONE 9:e99543. https://doi.org/10.1371/journal.pone.0099543

26. Hallner A, Bernson E, Hussein BA, Ewald Sander F, Brune M, Aurelius J, Martner A, Hellstrand K, Thoren FB (2019) The HLA-B -21 dimorphism impacts on NK cell education and clinical outcome of immunotherapy in acute myeloid leukemia. Blood 133:1479–1488. https://doi.org/10.1182/blood-2018-09-874990

27. Wang M, Claesson MH (2014) Classification of human leukocyte antigen (HLA) supertypes. Methods Mol Biol 1184:309–317. https://doi.org/10.1007/978-1-4939-1115-8_17

28. Sim MJW, Brennan P, Wahl KL, Lu J, Rajagopalan S, Sun PD, Long EO (2023) Innate receptors with high specificity for HLA class I–peptide complexes. bioRxiv. 2023.02.06.527249. https://doi.org/10.1101/2023.02.06.527249

29. Bernson E, Hallner A, Sander FE et al (2018) Cytomegalovirus serostatus affects autoreactive NK cells and outcomes of IL2-based immunotherapy in acute myeloid leukemia. Cancer Immunol Res 6:1110–1119. https://doi.org/10.1158/2326-6066.CIR-17-0711

30. Bernson E, Hallner A, Sander FE et al (2017) Impact of killer-immunoglobulin-like receptor and human leukocyte antigen genotypes on the efficacy of immunotherapy in acute myeloid leukemia. Leukemia 31:2552–2559. https://doi.org/10.1038/leu.2017.151

31. Gumperz JE, Litwin V, Phillips JH, Lanier LL, Parham P (1995) The Bw4 public epitope of HLA-B molecules confers reactivity with natural killer cell clones that express NKB1, a putative HLA receptor. J Exp Med 181:1133–1144. https://doi.org/10.1084/jem.181.3.1133

32. Boudreau JE, Giglio F, Gooley TA et al (2017) KIR3DL1/HLA-B subtypes govern acute myelogenous leukemia relapse after hematopoietic cell transplantation. J Clin Oncol 35:2268–2278. https://doi.org/10.1200/JCO.2016.70.7059

33. Yawata M, Yawata N, Draghi M, Partheniou F, Little AM, Parham P (2008) MHC class I-specific inhibitory receptors and their ligands structure diverse human NK-cell repertoires toward a balance of missing self-response. Blood 112:2369–2380. https://doi.org/10.1182/blood-2008-03-143727

34. Carr WH, Pando MJ, Parham P (2005) KIR3DL1 polymorphisms that affect NK cell inhibition by HLA-Bw4 ligand. J Immunol 175:5222–5229. https://doi.org/10.4049/jimmunol.175.8.5222

35. Cella M, Longo A, Ferrara GB, Strominger JL, Colonna M (1994) NK3-specific natural killer cells are selectively inhibited by Bw4-positive HLA alleles with isoleucine 80. J Exp Med 180:1235–1242. https://doi.org/10.1084/jem.180.4.1235

36. Anfossi N, Andre P, Guia S et al (2006) Human NK cell education by inhibitory receptors for MHC class I. Immunity 25:331–342. https://doi.org/10.1016/j.immuni.2006.06.013

37. Verheyden S, Ferrone S, Mulder A, Claas FH, Schots R, De Moerloose B, Benoit Y, Demanet C (2009) Role of the inhibitory KIR ligand HLA-Bw4 and HLA-C expression levels in the recognition of leukemic cells by natural killer cells. Cancer Immunol Immunother 58:855–865. https://doi.org/10.1007/s00262-008-0601-7

38. Shaffer BC, Le Luduec JB, Park S et al (2021) Prospective KIR genotype evaluation of hematopoietic cell donors is feasible with potential to benefit patients with AML. Blood Adv 5:2003–2011. https://doi.org/10.1182/bloodadvances.2020002701

39. Schetelig J, Baldauf H, Heidenreich F et al (2020) External validation of models for KIR2DS1/KIR3DL1-informed selection of hematopoietic cell donors fails. Blood 135:1386–1395. https://doi.org/10.1182/blood.2019002887

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.