(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
7 August 2014 (07.08.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/120949 A1

(51) **International Patent Classification**:
C12Q 1/68 (2006.01) ***C07H 21/04*** (2006.01)
C12N 5/078 (2010.01) ***A61K 35/14*** (2006.01)

(21) **International Application Number**:
PCT/US2014/013896

(22) **International Filing Date**:
30 January 2014 (30.01.2014)

(25) **Filing Language**: English

(26) **Publication Language**: English

(30) **Priority Data**:
61/758,558 30 January 2013 (30.01.2013) US

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(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

WO 2014/120949 A1

(54) **Title**: DONOR KIR3DL1 AND HLA-B SUBTYPES AND LEUKEMIA CONTROL IN HLA-COMPATIBLE ALLOGENIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

(57) **Abstract**: This disclosure generally relates to donor selection for hematopoietic stem cell transplantation. In particular, this disclosure relates to typing *KIR3DL1* and *HLA-B* alleles as basis for donor selection.

Donor *KIR3DL1* And *HLA-B* Subtypes And Leukemia Control In HLA-Compatible Allogeneic Hematopoietic Stem Cell Transplantation

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of priority from U.S. Provisional Application No. 61/758,558, filed January 30, 2013.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] This invention was made with Government Support under Contract No. U01 AI69197, R01 HL088134, and P01 CA23766 awarded by NIH. The Government has certain rights in this invention.

FIELD OF THE DISCLOSURE

[0003] This disclosure generally relates to donor selection for hematopoietic stem cell transplantation. In particular, this disclosure relates to typing *KIR3DL1* and *HLA-B* alleles as basis for donor selection.

BACKGROUND ART

[0004] Natural killer (NK) cells play an important innate immune role in controlling viral infection and malignancy. An increasing understanding of how NK immunogenetics control NK function has led to important associations between NK immunogenetics and disease outcomes. The most widely studied family of genes controlling human NK cell response is the polymorphic killer immunoglobulin-like receptor (*KIR*) gene family, with wide inter-individual variability based on differences in gene content and allelic polymorphism[1]. Recognition of self-specific HLA class I molecules on autologous cells by inhibitory KIR leads to NK cell inhibition. Conversely, non-engagement of the inhibitory KIR by target cells with reduced class I expression permits NK activation through simultaneous stimulatory signaling. Furthermore, interaction between self-specific inhibitory KIR and cognate HLA ligands is also fundamental to NK "education"[2],

in which cells expressing inhibitory KIR for self-HLA are "licensed" and have higher capacity for response compared to "unlicensed" NK cells which lack inhibitory KIR for self-HLA[3,4]. Under inflammatory conditions, however, unlicensed NK cells can be stimulated to exhibit higher effector function[2,5,6].

[0005] In hematopoietic cell transplantation (HCT), NK alloreactivity and leukemic control can occur through the stimulation of unlicensed NK cells lacking inhibitory KIR for self-HLA molecules[7-9].

[0006] The *KIR3DL1/S1* gene locus is one of the oldest and most polymorphic loci of the *KIR* gene family and is the only locus whose alleles encode for both inhibitory (KIR3DL1) and activating (KIR3DS1) receptors[10]. Although the ligand for the activating KIR3DS1 allotype remains unknown[11,12], the ligands for the numerous inhibitory KIR3DL1 allotypes are the polymorphic HLA-B molecules characterized by the Bw4 motif at positions 77-83[13], with dimorphism between isoleucine or threonine at position 80 (Bw4-I^{80} vs Bw4-T^{80}) important for differences in inhibitory response upon interaction with KIR3DL1 allotypes[14,15]. *KIR3DL1* alleles are expressed at different densities, and their inhibitory activities are closely proportional to their abundance on the NK cell surface[14-18]. This variability has been translated into an operative grouping system: high-expression (KIR3DL1**h*), low-expression (KIR3DL1**l*) and null subtypes with no surface expression (KIR3DL1**n*). Among the groups, there are known dichotomies in inhibitory response to the Bw4-I^{80} and Bw4-T^{80} subtypes, with some evidence linking highly inhibitory interactions to higher NK function[14,19]. In patients infected with human immunodeficiency virus (HIV), *KIR3DL1/Bw4* allele combinations predictive of highly inhibitory interactions and especially those involving *B*57* and *B*2705*, are associated with stronger protection from HIV progression compared to weak inhibition combinations[20,21]. The association of strong inhibitory combinations with protection from AIDS progression may reflect the greater efficiency of highly educated NK cells at clearing the infected target cell, whose class I expression is down-regulated by the HIV Nef protein[19,22-24].

[0007] In allogeneic HCT, where differences in *HLA* alleles between the stem cell donor and patient markedly influence transplant outcome, the role of KIR alleles, particularly in the context of differentially inhibiting class I ligand alleles, is unknown.

SUMMARY OF THE DISCLOSURE

[0008] The inventors evaluated the effects of *KIR3DL1/HLA-Bw4* subtype combinations with known differences in strengths of inhibition/interaction on transplant outcomes in leukemia patients receiving an *HLA*-compatible stem cell allograft from an unrelated donor (URD). The data show that donor *KIR3DL1* and donor-recipient *HLA-Bw4* allele combinations predictive of weak NK inhibition in the patient are associated with improved leukemia control and superior survival following HCT compared to combinations predictive of strong inhibitory interactions. These findings illuminate inhibitory capacity as a significant dimension controlling anti-tumor NK activity in allogeneic HLA-matched HCT and highlight the importance of identifying donor KIR subtypes to protect patients from leukemic relapse following allogeneic HCT.

[0009] Accordingly, this disclosure provides methods for improved donor selection for hematopoietic stem cell transplantation for leukemia based on typing *KIR3DL1* and *HLA-B* alleles in candidate donors and selecting donors with desirable *KIR3DL1* alleles in combination with donor-recipient *HLA-B* alleles.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] Fig. 1. Donor *KIR3DL1/HLA-Bw4* subtype pairs associated with weak inhibitory interactions are protective from relapse in AML patients following HCT. Among donors with *KIR3DL1-H* subtypes, those with *Bw4-T*^{80} (weak interaction) are associated with lower relapse compared to those with *Bw4-I*^{80} (strong interaction) in AML patients after HCT. Among donors with *3DL1-L* subtypes, those with *Bw4-I*^{80} (weak interaction) are associated with lower relapse in AML patients after HCT, compared to those with *Bw4-T*^{80} (strong interaction). **(a)** Combined, *3DL1-Bw4* allelic combinations with weak inhibitory interaction are associated with lower relapse compared to combinations with strong inhibitory interaction. *Bw6/Bw6* donors are also associated with lower relapse compared to strong inhibitory combinations. **(b)** Donors with high inhibitory interactions further subdivided into those with *3DL1-H* with *HLA-B*2705* or *B*57* are associated with the highest relapse rate compared to donors with *3DL1-H* + non-*B*2705* or non-*B*57* alleles and compared with other *HLA-B* subtype groups.

[0011] Fig. 2. Strength of KIR3DL1 inhibition is associated with mortality in AML patients following HCT. Weak inhibitory subtype combinations (*3DL1-H* + *Bw4-T*^{80} (not including *B*2705*) and *3DL1-L* + *Bw4-I*^{80}) are associated with lower mortality compared to strong inhibitory combinations (*3DL1-H* + *Bw4-I*^{80}, *3DL1-H* + *B*2705,* and *3DL1-L* + non-*B*2705 Bw4-T*^{80}). *Bw6* homozygous donors are associated with intermediate protection from mortality.

[0012] Figure 3. *KIR3DL1* + *HLA-B* allele combinations define a hierarchy of protection from AML relapse following allogeneic HCT. Donor allotype pairs are arranged by relative hazard ratios for AML relapse after HCT.

DETAILED DESCRIPTION

[0013] For patients undergoing allogeneic HCT, donor preference is given to those matched for up to 12 *HLA* alleles[36,37]. While GvHD and graft rejection have decreased due to tighter *HLA* matching, risks of leukemic relapse and overall mortality remain disappointingly high. It has been shown herein that donor *KIR3DL1* and *HLA-Bw4* allele combinations with low inhibitory interactions were associated with lower AML relapse and higher survival, and, were not associated with a higher risk for GvHD. Conversely, high inhibitory combinations and, in particular, combinations with *B*2705* and *B*57* were associated with progressively higher relapse risk and lower survival. Nearly all (99%) patients exhibited the same HLA-B epitopes (Bw6, Bw4-T^{80}, Bw4-I^{80}) as their donors, indicating that even in *HLA*-matched allogeneic HCT, epistatic interactions between donor KIR and HLA class I at the allotype resolution can have a profound impact on transplant outcome.

[0014] The findings disclosed herein confirm a key role of interaction between *3DL1* and *HLA-B* allotypes in determining HCT outcomes, providing valuable insight to inhibitory controls of NK effects in an *HLA*-matched setting. The findings support *KIR3DL1* allele typing to select stem cell donors with favorable *KIR-HLA* allelic combinations to minimize inhibition, maximize leukemic toxicity, lower relapse rates and increase survival. Considering the high frequencies of both *HLA-Bw4* and *KIR3DL1* in the population (70% and 95%, respectively)[46], selection of a donor with advantageous

KIR3DL1 alleles is highly relevant and feasible in clinical practice, where genetic selection criteria currently focus solely on *HLA* allele-matching.

[0015] In one aspect, this disclosure provides a method of selecting a hemotopoietic cell donor for allogeneic hemotopoietic cell transplantation (HCT) to an acute myelogenous leukemia (AML) patient.

[0016] In some embodiments, the patient requiring HCT expresses *HLA-Bw4*. Typically, for a patient requiring HCT, more than one donor is identified. Thus, for a given patient, potential donors, equivalent based on HLA-matching, would be screened in accordance with the method disclosed herein in order to select the best donor based on donor and recipient *KIR3DL1* and *HLA-Bw4* allele combinations.

[0017] In accordance with the present method, a genomic DNA-containing sample is obtained from a candidate donor matched for *HLA-Bw4*. The sample can be a tissue or blood sample, including, but not limited to, blood, fractions of blood, peripheral blood cells, skin or tissue biopsies, buccal swab samples, and umbilical cord blood. In some embodiments, the sample is processed to permit allele typing, e.g., a cell-containing fraction is obtained from the sample, and genomic DNA is isolated. In other embodiments, a sample is used directly in allele typing.

[0018] The genomic DNA from the sample, processed or otherwise, is then analyzed to determine whether the *KIR3DL1* gene is present (96% of individuals), and which allele(s), *KIR3DL1-h, KIR3DL1-l, KIR3DL1-n,* or *KIR-3DS1,* is present; i.e., allele typing of the *KIR3DL1* gene. Allele typing of the *KIR3DL1* gene is achieved by using various approaches described in the art, including, but not limited to, hybridization based on sequence-specific oligonucleotides, sequencing, PCR-SSP ("sequence-specific primer"), and combinations thereof.

[0019] A "*KIR3DL1-h*", as used herein, refers to an allele which expresses the KIR3DL1 receptor at high densities on the cell surface of NK cells detectable by cell surface staining, or an allele which is yet to be characterized for surface staining but shares substantial sequence similarity to an allele which expresses the KIR3DL1 receptor at high densities on the cell surface of NK cells detectable by cell surface staining. By

"substantial sequence similarity", it is meant that the relevant sequences share at least about 90%, 95%, 98%, 99% or higher identity at the nucleotide level, or at least about 90%, 95%, 98%, 99% or higher similarity or identity at the amino acid level.

[0020] Cell surface staining can be performed using an antibody directed to KIR3DL1 receptor. Examples of suitable antibodies include Z27 or DX9, both widely available, for example, from BD Biosciences (San Jose, California) or ThermoFisher Scientific (Waltham, MA).

[0021] *KIR3DL1-h* alleles are those alleles which have been characterized by cell surface staining, including but not limited to *KIR3DL1**001, *002, *008, *015, *020, *033, and *052. Alleles yet to be characterized for surface staining but which share substantial sequence similarity to a *KIR3DL1-h* allele characterized by high density cell surface staining include but are not limited to *009, *016, *043, *067, *026, *052, *034, *035, *022, *017, *066, *029, *038, *025, *054, *018, *051, *023, *028, *062, *030, *024N, *031, *059, *060, *061, *064, *065, *074, *075, *076, *077, and *057.

[0022] A "*KIR3DL1-l*", as used herein, refers to an allele which expresses the KIR3DL1 receptor at low densities on the cell surface of NK cells detectable by cell surface staining (e.g., using Z27 or DX9), or an allele which is yet to be characterized for surface staining but shares substantial sequence similarity to an allele which expresses the KIR3DL1 receptor at low densities on the cell surface of NK cells detectable by cell surface staining.

[0023] *KIR3DL1-l* alleles which have been characterized by cell surface staining include but are not limited to *KIR3DL1**005, *007, and *053. Alleles yet to be characterized for surface staining but which share substantial sequence similarity to a *KIR3DL1-l* allele characterized by low density cell surface staining include but not limited to *032, *033, *068, *044, and *041.

[0024] A "*KIR3DL1-n*", as used herein, refers to an allele which expresses KIR3DL1 molecules retained intracellularly and not detectable by cell surface staining (e.g., using Z27 or DX9). *KIR3DL1-n* alleles include but are not limited to *004, *019, and *056. Alleles yet to be characterized for surface staining but which share substantial sequence

similarity to a *KIR3DL1-n* allele characterized by low density cell surface staining include but not limited to *021, *036, *037, *039, *056, *072, *063, and *040.

[0025] A *KIR3DS1* allele expresses KIR3DS1 molecules, detectable by surface staining with Z27 but not DX9. *KIR3DS1* alleles include but are not limited to *KIR3DS1**013, *047, *010, *011, *012, *014, *045, *046, *048, *049N, *050, *055, and *058.

[0026] Once the *KIR3DL1* allele typing information is obtained, the donor can be assigned to one of the following subtype groups based on its allele combination: *KIR3DL1-H (KIR3DL1*h/*h,* or *KIR3DL1*h/KIR3DS1), KIR3DL1-L (KIR3DL1*l/*l, KIR3DL1*l/*h,* or *KIR3DL1*l/KIR3DS1),* or *KIR3DL1-N (KIR3DL1*n/*n, KIR3DL1*n/*h, KIR3DL1*n/*l,* or *KIR3DL1*n/KIR3DS1).*

[0027] One can then determine the inhibition potential of the donor *KIR3DL1* subtype and the *HLA-Bw4* allele combination, wherein the combinations of *KIR3DL1-L* and *HLA-Bw4-I80,* and *KIR3DL1-H* and *HLA-Bw4-T80* represent low inhibitory combinations, the combinations of *KIR3DL1-L* and *HLA-Bw4-T80,* and *KIR3DL1-H* and *HLA-Bw4-I80* represent high inhibitory combinations, and where *HLA-B*57* and *B*2705* are highly inhibitory *Bw4* alleles in specific combinations with *KIR3DL1. HLA-B*2705* is a *Bw4-T80* allele but appears to have a highly inhibitory relationship with *3DL1-H.* For purposes of this determination, donors are considered *Bw4-I*^{80}, *Bw4-T*^{80}, or *Bw4*-negative (*Bw6/Bw6*). All donors positive for *Bw4-I*^{80}, homozygous or heterozygous, are considered in the *Bw4-I*^{80} group, independent of the presence/absence of *HLA-Bw4-T*^{80}.

[0028] A donor can be selected on the basis that the donor and recipient genotypes provide a low inhibitory combination of donor *KIR3DL1* and donor/recipient *HLA-Bw4* alleles. Such donor is associated with a reduced risk of AML relapse and increased rate of survival in the AML recipient.

[0029] The present description is further illustrated by the following examples, which should not be construed as limiting in any way. The contents of all cited references (including literature references, issued patents, and published patent applications as cited throughout this application) are hereby expressly incorporated by reference.

EXAMPLE.

[0030] In experiments described in this Example, we tested the hypothesis that differences in KIR3DL1-mediated NK inhibition by HLA-Bw4 subtypes can influence donor NK activity against leukemia in hematopoietic stem cell transplantation (HCT). Among 299 patients with acute myelogenous leukemia (AML) who received HLA-compatible HCT, allelic combinations of donor *KIR3DL1* and *HLA-Bw4* predictive of low inhibitory interactions were strongly and consistently associated with lower relapse and improved survival. These data are the first to demonstrate the importance of allotypic KIR-HLA interactions in leukemic control and indicate that NK cells with lower capacity for inhibition have the most potent anti-leukemic effects. Considering the high frequencies of *HLA-Bw4* and *KIR3DL1* among patients and donors, consideration of *KIR3DL1* allele typing for stem cell donor selection is feasible and may provide better disease control for AML patients undergoing allogeneic HCT.

[0031] RESULTS

[0032] ***Impact of donor KIR3DL1 or HLA–Bw4 allele groups on allogeneic HCT***

[0033] We evaluated donor *KIR3DL1* and *HLA–Bw4* alleles for 299 AML patients who received an allogeneic HCT from an *HLA*-compatible donor (**Table 1**). Patients and donors were matched for 10 *HLA* alleles in 78.9%, and matched for *HLA-B* alleles in 94.6%. All but 3 donor-patient pairs were matched for *HLA-B* subtype (**Table 1**).

[0034] There were no statistically significant associations of donor *HLA-Bw4* subtype, segregated into *Bw4-I*^{80} and *Bw4-T*^{80} subtypes, on the outcomes of overall mortality (OM), non-relapse mortality (NRM), relapse, and acute graft-versus-host disease (GvHD) among all patients and among AML versus MDS/CML patients (data not shown). Patients homozygous for *HLA-Bw6* alleles (*Bw6/Bw6*) and therefore lacking the Bw4 epitope, represented 41% of patients. When AML patients were segregated according to absence or presence of *Bw4 (Bw6/Bw6* vs *Bw4/Bw4* or *Bw4/Bw6*), there was no statistically significant reduction in relapse (HR 0.83, p=0.45).

[0035] Among donors, 95.5% possessed the *KIR3DL1* gene. Allele typing successfully assigned alleles to all *KIR3DL1*-positive donors and identified three new alleles:

*KIR3DL1*00403* (GenBank accession number GU063854); *KIR3DL1*072* (GenBank accession number GU063855) and *KIR3DL1*073* (GenBank accession number GU063857). Frequencies of *KIR3DL1*h*, *KIR3DL1*l* and *KIR3DL1*n* alleles were 64.6%, 29.3% and 27.9%, respectively (**Table 1**). Of the 17 different *KIR3DL1* alleles identified, 4 had frequencies higher than 20% (*001, *002, *004 and *005), consistent with published data[30], and 10 had frequencies less than 5%. Donors were segregated by *KIR3DL1* alleles into functionally relevant groups similar to published criteria[20]: donors with only high-expressing *KIR3DL1* alleles comprised group *3DL1-H* (KIR3DL1**h*/**h*, *KIR3DL1*h/KIR3DS1*); donors with at least one low-expressing *KIR3DL1* allele comprised group *3DL1-L* (*KIR3DL1*l/*l*, *KIR3DL1*l/*h*, *KIR3DL1*l/KIR3DS1*); and donors with at least one null allele comprised group *3DL1-N* (*KIR3DL1*n/*n*, *KIR3DL1*n/*h*, *KIR3DL1*n/*l*, *KIR3DL1*n/KIR3DS1*). Among donors, frequencies of *3DL1-H, 3DL1-L,* and *3DL1-N* were 43%, 27%, and 29%, respectively (**Table 1**). Four donors could not be assigned to a group due to lack of information regarding expression of their *KIR3DL1* alleles and were therefore not included in the analysis. No group (*3DL1-H*, *3DL1-L* or *3DL1-N*) was significantly associated with differences in HCT outcome from the others, regardless of disease category (data not shown).

[0036] ***Weak inhibitory donor 3DL1-H and Bw4-T^{80} combinations are associated with lower AML relapse following HCT***

[0037] We then considered *KIR3DL1* groups in combination with cognate *HLA-Bw4* ligand subtypes on HCT outcomes, segregating combinations according to strong or weak inhibition combinations and comparing to *Bw6/Bw6* individuals. All results are adjusted for disease status. Among AML patients with a *3DL1-H* donor, those whose donor exhibited the weak inhibitory combination *3DL1-H + Bw4-T^{80}* experienced lower relapse when compared to those with the highly inhibitory *3DL1-H + Bw4-I^{80}* combination (HR 0.47; P=..099). Patients with *Bw6/Bw6* donors also had lower relapse compared to patients transplanted from donors with strong inhibition subtype combinations (HR 0.7; P= .33).

[0038] ***Inhibitory donor 3DL1-H and Bw4-B*2705 or Bw4-B*57 combinations are associated with highest AML relapse following HCT***

[0039] *HLA-B*2705* and *B*57* alleles are known to have particularly high protective effect against HIV [20,31], likely related to higher NK functional capacity via interaction with KIR3DL1[19,23]. To determine if these alleles specifically enhance risk for relapse, we divided *3DL1-H* donors with *B*57 or B*2705* or non-*B*57 Bw4-I*^{80} alleles. The protection from relapse seen in the weakly inhibitory group (*3DL1-H + Bw4-T*^{80}) relative to the highly inhibitory group was even more striking when the highly inhibitory group was restricted to those with *B*2705* or *B*57* (HR 0.3; P=.02). These data support a model where licensed but weakly inhibited NK cells are most effective at controlling leukemia. In contrast, NK cells that are more inhibitable, despite being highly licensed, are less effective at tumor clearance.

[0040] Weak inhibitory donor KIR3DL1-L and Bw4 allele combinations are associated with lower AML relapse

[0041] In contrast to the *3DL1-H* group, we found that among *3DL1-L* donors, those with the weak inhibitory *3DL1-L + HLA-Bw4-I*^{80} combination were associated with lower AML relapse in the patient when compared to donors with the highly inhibitory *3DL1-L + Bw4-T*^{80} combinations (HR 0.47; P=.2). Patients transplanted from a *3DL1-L, Bw6/Bw6* donor also had a lower rate of relapse compared to patients transplanted from *KIR3DL1-L + Bw4-T*^{80} donors (HR 0.6; p=.3).

[0042] ***Strength of NK inhibition is associated with AML relapse and mortality after HCT***

[0043] Consistent findings among the donor *3DL1-H* and *3DL1-L* subtype groups, where strong and weak inhibitory interactions with Bw4 subtypes led to higher and lower relapse respectively, permitted combination of the two groups. Among the combined groups, weak inhibitory subtype combinations (*KIR3DL1-L + Bw4-I*^{80} and *KIR3DL1-H + Bw4-T*^{80}), were strongly associated with lower relapse in AML patients compared to the strong inhibitory combinations (*3DL1-L + Bw4-T*^{80}, and *3DL1-H + Bw4-I*^{80}) (HR 0.46; P=.02; **Fig. 1a, Table 2**), and even more striking when compared to the strongly inhibitory combinations of *3DL1-H + B*57 or + B*2705* (HR 0.3; P=.006; **Fig. 1b, Table 2**). Correspondingly, there was lower mortality among patients transplanted from donors with weak or non-inhibitory combinations compared to patients transplanted from donors with

strong inhibitory combinations (HR 0.65; P=.05; **Fig. 2**). Moreover, distribution of KIR haplotype-A homozygosity, an unfavorable KIR genotype, was equivalent between donors with high inhibitory combinations (21%) and low inhibitory combinations (22%).

[0044] From these analyses, a hierarchy of protection from AML relapse is identified, ranging from the highly protective weakly inhibitory *KIR3DL1/Bw4* allotype combinations to the intermediate protective effect of *Bw6/Bw6* to the strongly inhibitory *KIR3DL1/Bw4* subtype combinations associated with susceptibility and, in the case of the *Bw4-I80 B*2705* and *B*57* alleles, "super-susceptibility," to relapse (**Fig. 3**). In contrast to relapse and survival, there was no statistically significant association between subtype combinations and graft-versus-host disease (data not shown).

[0045] ***HLA-A alleles bearing the Bw4-I^{80} epitope do not influence AML relapse***

[0046] Some HLA-A allotypes exhibit the Bw4-I^{80} epitope, binding weakly and variably to KIR3DL1[14,32-34]. When donors with *HLA-A* alleles containing the Bw4-I^{80} epitope were included with *HLA-B* alleles in the analysis, there was no added effect on relapse (data not shown), suggesting that interaction between KIR3DL1 and the Bw4 epitope presented by HLA-A subtypes may not play as significant a role in NK education and leukemic surveillance following HCT as the HLA-B Bw4 subtypes.

[0047] ***Donor KIR3DL1-N and HLA-Bw4 combinations are not associated with HCT outcomes***

[0048] Studies in HIV-infected individuals identified an association between combinations of *3DL1-N* and *Bw4* subtypes with improved HIV control, although the biological mechanism is unclear[20]. AML patients transplanted from donors with *3DL1-N* in aggregate experienced a higher relapse rate compared to the most favorable low-affinity combination *3DL1-H* + *Bw4-T^{80}* (HR 2.2, **Fig. 3**). There were no statistically significant differences in outcome in this cohort for AML patients transplanted from donors with specific combinations of *3DL1-N* with *Bw4-I^{80}*, *Bw4-T^{80}* or *Bw6/Bw6* (data not shown).

[0049] MATERIALS AND METHODS

[0050] ***Patients, Donors and Transplant characteristics***

[0051] We evaluated 299 patients who received an allogeneic hematopoietic stem allograft from an unrelated donor (URD) following myeloablative conditioning as treatment for a myeloid malignancy (AML 70.2%, CML 6.3%, MDS 23.5%). All transplants were facilitated by the National Marrow Donor Program (NMDP) between 1995 and 2002. Clinical data, *HLA* allele typing, *KIR3DL1* allele typing, and donor cell lines or donor genomic DNA for *KIR* genotyping were provided by the Center for International Blood and Marrow Transplant Research (CIBMTR). The CIBMTR is a research affiliation of the International Bone Marrow Transplant Registry, Autologous Blood and Marrow Transplant Registry, and the National Marrow Donor Program (NMDP), comprising a group of more than 450 centers worldwide that contribute hematopoietic stem cell transplant data to the Statistical Center at the Medical College of Wisconsin in Milwaukee and the NMDP Coordinating Center in Minneapolis, MN. Patients were followed longitudinally, with yearly follow-up. Studies conducted by the CIBMTR were performed in compliance with the Privacy Rule (HIPAA) as a Public Health Authority, and in compliance with federal regulations pertaining to the protection of human research participants as determined by the Institutional Review Boards of the NMDP and the Medical College of Wisconsin. 236 patients (78.9%) received an allograft from an unrelated donor matched at 10 of 10 possible *HLA* alleles, and 63 (21.1%) received and URD allograft matched at 9 of 10 alleles. 283 donor-recipient pairs (94.6%) were matched for both *HLA-B* alleles, with the remaining 16 pairs mismatched for one *HLA-B* allele. Only 3 patients (1.0%) were HLA-B epitope/KIR3DL1 ligand mismatched with their donors. A total of 257 (85.9%) allografts were T-cell replete, and 268 (95.7%) were of marrow origin. All patients received an ablative conditioning regimen. Cyclosporine and methotrexate were used as GVHD prophylaxis in 252 (84.3%) patients; 5 patients received tacrolimus-based GVHD prophylaxis. The 42 (14.0%) patients who received a T-cell depleted transplant did not receive additional GVHD prophylaxis.

[0052] *HLA and KIR3DL1 allele typing*

[0053] High-resolution *HLA* genotyping (HLA-A, B, C, DRB1 and DQB1) of patients and donors was obtained from CIBMTR. Typing for donor *KIR* gene presence/absence was performed using previously described methods[47] or by Invitrogen KIR genotyping kit as per manufacturer's instructions. *KIR3DL1* allele level typing was executed using PCR-

based methods and sequencing, as previously described[48-51]. All *KIR3DL1* alleles were verified by sequencing. All samples were typed for *KIR3DL1* alleles independently in two different laboratories, with any discrepancies resolved by a third independent laboratory.

[0054] ***HLA and KIR3DL1 subtype grouping***

[0055] *KIR3DL1* alleles were divided into high-, low- and null-expressing groups based on published data[14,16-18]. Specifically, the high-expressing alleles (*KIR3DL1-h*) present in this patient cohort included KIR3DL1*001, *002, *008, *020, *015, *033 and *052; the low-expressing alleles (*KIR3DL1-l*) were *005, *007, and *053. The *KIR3DL1* allele *004 was considered as a non-expressed allele (*KIR3DL1-n*). To investigate the different effects of high- versus low-expressing subtype combinations, donors were segregated similar to published grouping criteria[20]: donors with only *KIR3DL1-h* alleles (*KIR3DL1*h/*h*; *KIR3DL1*h/3DS1*; grouped as *KIR3DL1-H*) were segregated from those with at least one *KIR3DL1-l* allele (*KIR3DL1*l/*l*, *KIR3DL1*l/*h*, *KIR3DL1*l/3DS1*; grouped as *KIR3DL1-L*). Considering the different biological role of the intracellularly sequestered *KIR3DL1**004 null allele, donors with at least one *004 allele (*3DL1*n/*n*, *3DL1*n/*h*, *3DL1*n/*l*, *3DL1*n/3DS1*; grouped as *3DL1-N*) were also evaluated separately. Donors were considered *Bw4-I*^{80}, *Bw4-T*^{80}, or *Bw4*-negative (*Bw6/Bw6*). All donors positive for *Bw4-I*^{80}, homozygous or heterozygous, were considered in the *Bw4-I*^{80} group, independent of the presence/absence of *HLA-Bw4-T*^{80}.

[0056] ***Statistical analysis***

[0057] Subtype combinations of *KIR3DL1* and *HLA-Bw4* were compared using Cox regression for the time-to-event outcomes relapse and overall mortality. Variables included age, disease severity, degree of *HLA*-matching, type of disease (AML versus MDS/CML, where appropriate), type of transplant (T-cell deplete versus T-cell replete), and presence of *KIR3DS1* (the group assignments did not remove 3DS1). Due to relatively limited numbers of events for some comparisons (particularly for relapse among KIR3DL1 subgroups), the ability to adjust for non-*KIR/HLA* variables was limited; in some cases, at most one additional variable was included in the regression model, with each variable included sequentially. Estimates of the probability of overall survival were obtained using the method of Kaplan and Meier, and estimates of the probability of relapse

were summarized using cumulative incidence estimates, where death without relapse was regarded as a competing risk for relapse.

Table 1. Transplant characteristics

	AML
Number of patients	299
Median age, y (range)	39.3 (1.8-68.9)
Disease Status at transplant*	
Early	89 (29.8)
Intermediate	84 (28.1)
Advanced	126 (42.1)
Other MDS	
***HLA* match**	
10/10	236 (78.9)
9/10	63 (21.1)
8/10	0
***HLA-B* match**	
2/2	283 (94.7)
1/2	16 (5.3)
HLA-B subtype match$^{\Omega}$	296 (99.0)
HLA-B subtype mismatch	3 (1.0)
Patient *Bw4-I*^{80} →Donor *Bw4-T*^{80}	1
Patient *Bw4-T*^{80} →Donor *Bw4-I*^{80}	1
Patient *Bw6/Bw6*→Donor *Bw4-I*^{80}	1
Transplant Type	
Ablative	299 (100)
T-deplete	42 (14.0)
T-replete	257 (85.9)
Graft type	
Bone marrow	268 (89.6)
Peripheral blood stem cells	31 (10.4)
GVHD prophylaxis	
Cyclosporine A + Methotrexate	252 (84.3)
Non-Cyclosporine A	5 (1.7)
T-cell depletion	42 (14.0)
Donor *KIR3DL1* allotype	284 (95.0)
Donor *KIR3DL1-h* positive	205 (68.6)
Donor *KIR3DL1-l* positive	80 (26.8)
Donor *KIR3DL1-n* positive	83 (27.8)
Donor *KIR3DS1*-positive	115 (38.5)
Donor *KIR3DL1* subtype groups	
Donor *KIR3DL1-H*	130 (45.8)

Donor *KIR3DL1-L*	69 (24.3)
Donor *KIR3DL1-N*	82 (28.9)
Not assessable#	3 (1.0)
Donor *HLA-B* subtype groups	
Bw6 homozygous	123 (41.1)
Bw4-I^{80}	86 (28.8)
Bw4-T^{80}	90 (30.1)

Values are n (%) unless otherwise noted.

* Disease status was defined as follows. AML: "early" for first complete remission, "intermediate" for second complete remission or higher complete remission, and "advanced" for primary induction failure, first relapse, second or higher relapse.

Ω HLA-B subtype match or mismatch is defined as presence or absence of a different HLA-B subtype in the patient and donor, where HLA-B subtypes are defined as HLA-Bw6, HLA-Bw4-I^{80}, or HLA-Bw4-T^{80}.

KIR3DL1 alleles not classifiable as high, low or null as described in "METHODS", based on available literature.

AML, acute myeloid leukemia; CML, chronic myeloid leukemia; MDS, myelodysplastic syndrome; GVHD, graft versus host disease.

Table 2. Subtype combinations of *KIR3DL1* and *HLA-B* and relapse after HCT for AML, adjusted results

KIR3DL1 + *HLA-B** subtype	Hazard Ratio	95% CI	P-value
Extra-high: *3DL1-H + B*57* or *3DL1-H + B*2705*	1	---	---
High: *3DL1-H + Bw4-I*^{80} (non-*B57*) or *3DL1-L +Bw4-T*^{80} (non-*B*2705*)	0.54	0.24 – 1.21	.13
No Bw4: *3DL1-H + Bw6/Bw6* or *3DL1-L +Bw6/Bw6*	0.4		.014
Low: *3DL1-H + Bw4-T*^{80} or *3DL1-L + Bw4-I*^{80}	0.3	0.19 – 0.83 0.13-0.7	.006

* "Extra-high", "high", and low" refer to strength of inhibition

REFERENCES

1. Parham, P. MHC class I molecules and KIRs in human history, health and survival. *Nat Rev Immunol* **5**, 201-214 (2005).
2. Kim, S. et al. Licensing of natural killer cells by host major histocompatibility complex class I molecules. *Nature* **436**, 709-13 (2005).
3. Anfossi, N. et al. Human NK cell education by inhibitory receptors for MHC class I. *Immunity* **25**, 331-42 (2006).
4. Yu, J. et al. Hierarchy of the human natural killer cell response is determined by class and quantity of inhibitory receptors for self-HLA-B and HLA-C ligands. *J Immunol* **179**, 5977-89 (2007).
5. Fernandez, N. et al. A subset of natural killer cells achieves self-tolerance without expressing inhibitory receptors specific for self-MHC molecules. *Blood* **105**, 4416-4423 (2005).
6. Yokoyama, W. & Kim, S. Licensing of natural killer cells by self-major histocompatibility complex class I. *Immunol Rev* **214**, 143-154 (2006).
7. Hsu, K. et al. Improved outcome in allogeneic hematopoietic stem cell transplantation in acute myelogenous leukemia (AML) predicted by donor KIR genotype and recipient HLA genotype in T-cell depleted HLA-identical sibling transplants. *Blood* **105**, 4878-4884 (2005).
8. Yu, J. et al. Breaking tolerance to self, circulating natural killer cells expressing inhibitory KIR for non-self HLA exhibit effector function after T cell-depleted allogeneic hematopoietic cell transplantation. *Blood* **113**, 3875-84 (2009).
9. Miller, J. et al. Missing KIR-ligands is associated with less relapse and increased graft versus host disease (GVHD) following unrelated donor allogeneic HCT. *Blood* **109**, 5058-5061 (2007).
10. Parham, P., Norman, P.J., Abi-Rached, L. & Guethlein, L. Variable NK cell receptors exemplified by human KIR3DL1/S1. *J Immunol* **187**, 11-19 (2011).
11. Gillespie, G. et al. Lack of KIR3DS1 binding to MHC class I Bw4 tetramers in complex with CD8+ T cell epitopes. *AIDS Res Hum Retroviruses* **23**, 451-455 (2007).
12. O'Connor, G. et al. Functional polymorphism of the KIR3DL1/S1 receptor on human NK cells. *J Immunol* **178**, 235-241 (2007).
13. Vivian, J.P. et al. Killer cell immunoglobulin-like receptor 3DL1-mediated recognition of human leukocyte antigen B. *Nature* **479**, 401-5 (2011).
14. Yawata, M. et al. Roles for HLA and KIR polymorphisms in natural killer cell repertoire selection and modulation of effector function. *J Exp Med* **203**, 633-645 (2006).
15. Carr, W., Pando, M. & Parham, P. *KIR3DL1* polymorphisms that affect NK cell inhibition by HLA-Bw4 ligand. *J Immunol* **175**, 5222-5229 (2005).
16. Thomas, R. et al. Novel KIR3DL1 alleles and their expression levels on NK cells: convergent evolution of KIR3DL1 phenotype variation? *J Immunol* **180**, 6743-6750 (2008).
17. Pando, M.J., Gardiner, C.M., Gleimer, M., McQueen, K.L. & P.Parham. The protein made from a common allele of KIR3DL1 (3DL1*004) is poorly expressed at cell surfaces due to substitution at positions 86 in Ig domain 0 and 182 in Ig domain 1. *J Immunol* **171**, 6640-6649 (2003).

18. Gardiner, C.M. et al. Different NK cell surface phenotypes defined by the DX9 antibody are due to *KIR3DL1* gene polymorphism. *J Immunol* **166**, 2992-3001 (2001).
19. Boulet, S. et al. HIV protective KIR3DL1 and HLA-B genotypes influence NK cell function following stimulation with HLA-devoid cells. *J Immunol* **184**, 2057-2064 (2010).
20. Martin, M. et al. Innate partnership of HLA-B and KIR3DL1 subtypes against HIV-1. *Nat Genet* **39**, 733–740 (2007).
21. Martin, M.P. et al. Epistatic interaction between KIR3DS1 and HLA-B delays the progression to AIDS. *Nature Genetics* **31**, 429-434 (2002).
22. Alter, G. et al. Differential natural killer cell-mediated inhibition of HIV-1 replication based on distinct KIR/HLA subtypes. *J Exp Med* **204**, 3027-3036 (2007).
23. Kamya, P. et al. Receptor-ligand requirements for increased NK cell polyfunctional potential in slow progressors infected with HIV-1 coexpressing KIR3DL1*h/*y and HLA-B*57. *J Virol* **85**, 5949-5960 (2011).
24. Schwartz, O., Marechal, V., Le Gall, S., Lemonnier, F. & Heard, J. Endocytosis of major histocompatibility complex class I molecules is induced by the HIV-1 Nef protein. *Nat Med* **2**, 338-342 (1996).
25. Wetzler, M. et al. HLA class I antigen cell surface expression is preserved on acute myeloid leukemia blasts at diagnosis and at relapse. *Leukemia* **15**, 128-133 (2001).
26. Ruggeri, L. et al. Donor natural killer cell allorecognition of missing self in haploidentical hematopoietic transplantation for acute myeloid leukemia: challenging its predictive value. *Blood* **110**, 433-440 (2007).
27. Venstrom, J. et al. HLA-C-dependent prevention of leukemia relapse by donor activating KIR2DS1. *New England Journal of Medicine* **367**, 805-816 (2012).
28. Cooley, S. et al. Donors with group B haplotypes improve relapse-free survival after unrelated hemaotpoietic transplantation for acute myeloid leukemia. *Blood* **113**, 726-732 (2009).
29. Hsu, K. et al. KIR ligands and prediction of relapse after unrelated donor hematopoietic cell transplantation for hematologic malignancy. *Biol Blood Mar Transpl* **12**, 828-836 (2006).
30. Hollenbach, J.A. et al. Report from the killer immunoglobulin-like receptor (KIR) anthropology component of the 15th International Histocompatibility Workshop: worldwide variation in the KIR loci and further evidence for the co-evolution of KIR and HLA. *Tissue Antigens* **76**, 9-17 (2010).
31. Carrington, M. & O'Brien, S. The influence of HLA genotype on AIDS. *Annu Rev Med* **54**, 535-551 (2003).
32. Stern, M., L Ruggeri, L., Capanni, M., Mancusi, A. & Velardi, A. Human leukocyte antigens A23, A24 and A32 but not A25 are ligands for KIR3DL1. *Blood* **112**, 708-710 (2008).
33. Foley, B. et al. The reactivity of Bw4+ HLA-B and HLA-A alleles with KIR3DL1: implications for patient and donor suitability for haploidentical stem cell transplantations. *Blood* **112**, 435-443 (2008).
34. Gumperz, J., Litwin, V., Phillips, J., Lanier, L. & Parham, P. The Bw4 public epitope of HLA-B molecules confers reactivity with NK cell clones that express NKB1, a putative HLA receptor. *J Exp Med* **181**, 1133-1144 (1995).

35. Ruggeri, L. et al. Role of natural killer cell alloreactivity in HLA-mismatched hematopoietic stem cell transplantation. *Blood* **94**, 333-339 (1999).
36. Lee, S.J. et al. High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation. *Blood* **110**, 4576-4583 (2007).
37. Petersdorf, E.W. et al. Major-histocompatibility-complex class I alleles and antigens in hematopoietic-cell transplantation. *N Engl J Med* **345**, 1794-800 (2001).
38. Ruggeri, L. et al. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. *Science* **295**, 2097-100 (2002).
39. Giebel, S. et al. Survival advantage with KIR ligand incompatibility in hematopoietic stem cell transplantation from unrelated donors. *Blood* **102**, 814-9 (2003).
40. Cooley, S. et al. Donor selection for natural killer cell receptor genes leads to superior survival after unrelated transplantation for acute myelogenous leukemia. *Blood* **116**, 2411-2419 (2010).
41. Hsu, K.C., Chida, S., Geraghty, D. & Dupont, B. The killer cell immunoglobulin-like receptor (KIR) genomic region: gene-order, haplotypes and allelic polymorphism. *Immunol Rev* **190**, 40-52 (2002).
42. Venstrom, J.M. et al. KIR and HLA genotypes are associated with disease progression and survival following autologous hematopoietic stem cell transplantation for high-risk neuroblastoma. *Clin Cancer Res* **15**, 7330-4 (2009).
43. Tarek, N. et al. Unlicensed natural killer cells target neuroblastoma following anti-GD2 antibody treatment. *J Clin Invest* **122**, 3260-3270 (2012).
44. Thananchai, H. et al. Cutting edge: allele-specific and peptide-dependent interactions between KIR3DL1 and HLA-A and HLA-B. *J Immunol* **178**, 33-37 (2007).
45. Vivian, F. et al. Killer cell immunoglobulin-like receptor 3DL1-mediated recognition of human leukocyte antigen B. *Nature* **479**, 401-406 (2011).
46. Single, R. et al. Global diversity and evidence for coevolution of KIR and HLA. *Nat Genet* **39**, 1114-9 (2007).
47. Hsu, K.C. et al. Killer Ig-like receptor haplotype analysis by gene content: evidence for genomic diversity with a minimum of six basic framework haplotypes, each with multiple subsets. *J Immunol* **169**, 5118-29 (2002).
48. Belle, I. et al. Investigation of killer cell immunoglobulin-like receptor gene diversity in KIR3DL1 and KIR3DS1 in a transplant population. *Tissue Antigens* **71**, 434-439 (2008).
49. Lebedeva, T., Ohashi, M., Zannelli, G., Cullen, R. & Yu, N. Comprehensive approach to high-resolution KIR typing. *Hum Immunol* **68**, 789-796 (2007).
50. Levinson, R. et al. Combination of KIR and HLA gene variants augments the risk of developing birdshot chorioretinopathy in HLA-A*29-positive individuals. *Genes Immun* **9**, 249-258 (2008).
51. Jiang, B., Hou, L., Chen, M., Ng, J. & Hurley, C. The profile of KIR3DL1 and KIR3DS1 alleles in an African American population resembles that found in African populations. *Tissue Antigens* **76**, 64-66 (2010).

WHAT IS CLAIMED IS:

1. A method of selecting a hematopoietic cell donor for allogeneic hematopoietic cell transplantation (HCT) to an acute myelogenous leukemia (AML) patient recipient expressing *HLA-Bw4*, comprising:

i. obtaining a sample from a candidate donor expressing a matching *HLA-Bw4* allele and exhibiting *KIR3DL1* ;

ii. allele typing for the *KIR3DL1* gene in the donor sample to determine whether a *KIR3DL1-h, KIR3DL1-l, KIR3DL1-n,* or *KIR-3DS1* allele is present;

iii. subtyping the donor as *KIR3DL1-H, KIR3DL1-L* or *KIR3DL1-N* based on the allele typing results;

iv. determining the inhibition potential of the donor *KIR3DL1* subtype and the *HLA-Bw4 allele* combination, wherein the combinations of *KIR3DL1-L* and *HLA-Bw4-I80*, and *KIR3DL1-H* and *HLA-Bw4-T80* represent low inhibitory combinations, and the combinations of *KIR3DL1-L* and *HLA-Bw4-T80*, and *KIR3DL1-H* and *HLA-Bw4-I80* represent high inhibitory combinations; and

v. selecting the candidate donor on the basis that the donor and recipient genotypes provide a low inhibitory combination of donor *KIR3DL1* and donor/recipient *HLA-Bw4* alleles.

2. The method claim 1, wherein said sample from the candidate donor is a sample containing genomic nucleic acids selected from the group consisting of blood, fractions of blood, peripheral blood cells, skin or tissue biopsies, buccal swab samples, and umbilical cord blood.

3. The method of claim 2, wherein genomic nucleic acids are isolated from the sample for *KIR3DL1* allele typing.

4. The method of claim 1, wherein allele typing of the *KIR3DL1* gene is achieved by an approach selected from the group consisting of hybridization using sequence-specific oligonucleotides, sequencing, PCR-SSP, and combinations thereof.

5. The method of claim 1, wherein a *KIR3DL1-h* allele is an allele which expresses the KIR3DL1 receptor at high densities on the cell surface of NK cells detectable by cell surface staining, or an allele which shares substantial sequence similarity to an allele which expresses the KIR3DL1 receptor at high densities on the cell surface of NK cells detectable by cell surface staining.

6. The method of claim 5, wherein cell surface staining is done using an antibody selected from Z27 or DX9.

7. The method of claim 5, wherein a *KIR3DL1-h* allele is selected from the group consisting of *KIR3DL1**001, *002, *008, *009, *015, *016, *020, *043, *067, *026, *052, *034, *035, *022, *017, *066, *029, *038, *025, *054, *018, *051, *068, *023, *028, *062, *030, *024N, *031, *042, *057, *059, *060, *061, *064, *065, 074, *075, *076, and *077.

8. The method of claim 1, wherein a *KIR3DL1-l* allele expresses KIR3DL1 at low densities on the cell surface of NK cells, detectable by surface staining.

9. The method of claim 8, wherein cell surface staining is done using an antibody Z27 or DX9.

10. The method of claim 8, wherein a *KIR3DL1-l* allele is selected from the group consisting of *KIR3DL1**005, *007, *053, *044, *033, *032, *068 and *041.

11. The method of claim 1, wherein a *KIR3DL1-n* allele expresses KIR3DL1 molecules which are retained intracellularly and are not detectable by cell surface staining.

12. The method of claim 11, wherein cell surface staining is done using antibody Z27 or DX9.

13. The method of claim 11, wherein a *KIR3DL1-n* allele is selected from the group consisting of *004, *019, *021, *036, *037, *039, *056, *072, *063, and *040.

14. The method of claim 1, wherein a *KIR3DS1* allele expresses KIR3DS1 molecules, detectable by surface staining with Z27 but not DX9.

15. The method of claim 14, wherein a *KIR3DS1* allele is selected from the group consisting of *KIR3DS1**013, *047, *010, *011, *012, *014, *045, *046, *048, *049N, *050, *055, and *058.

16. The method of claim 1, wherein the donor is assigned to a *KIR3DL1-H* subtype based on an allele combination of *KIR3DL1*h/*h,* or *KIR3DL1*h/KIR3DS1.*

17. The method of claim 1, wherein the donor is assigned to a *KIR3DL1-L* subtype based on an allele combination of *KIR3DL1*l/*l, KIR3DL1*l/*h,* or *KIR3DL1*l/KIR3DS1.*

18. The method of claim 1, wherein the donor is assigned to a *KIR3DL1-N* subtype based on an allele combination of *KIR3DL1*n/*n, KIR3DL1*n/*h, KIR3DL1*n/*l,* or *KIR3DL1*n/KIR3DS1.*

19. The method of claim 1, wherein the donor selected for the AML patient on the basis of providing a weakly inhibitory combination of donor *KIR3DL1* alleles and donor-recipient *HLA-Bw4* alleles provides a reduced risk of AML relapse in the patient.

Figure 1a

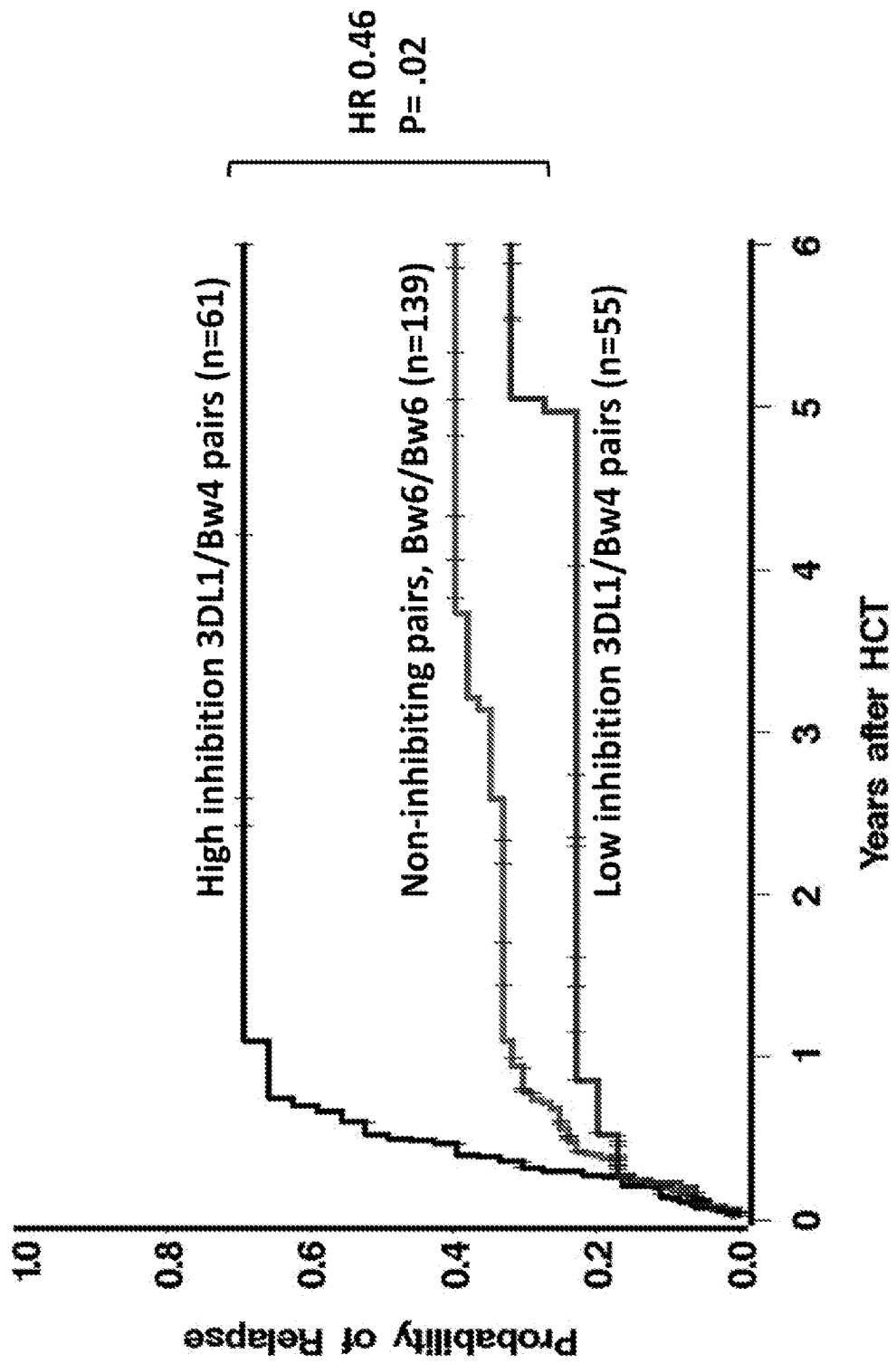

HR 0.46
P= .02
High inhibition 3DL1/Bw4 pairs (n=61)
Non-inhibiting pairs, Bw6/Bw6 (n=139)
Low inhibition 3DL1/Bw4 pairs (n=55)
Probability of Relapse
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
6
Years after HCT

Figure 1b

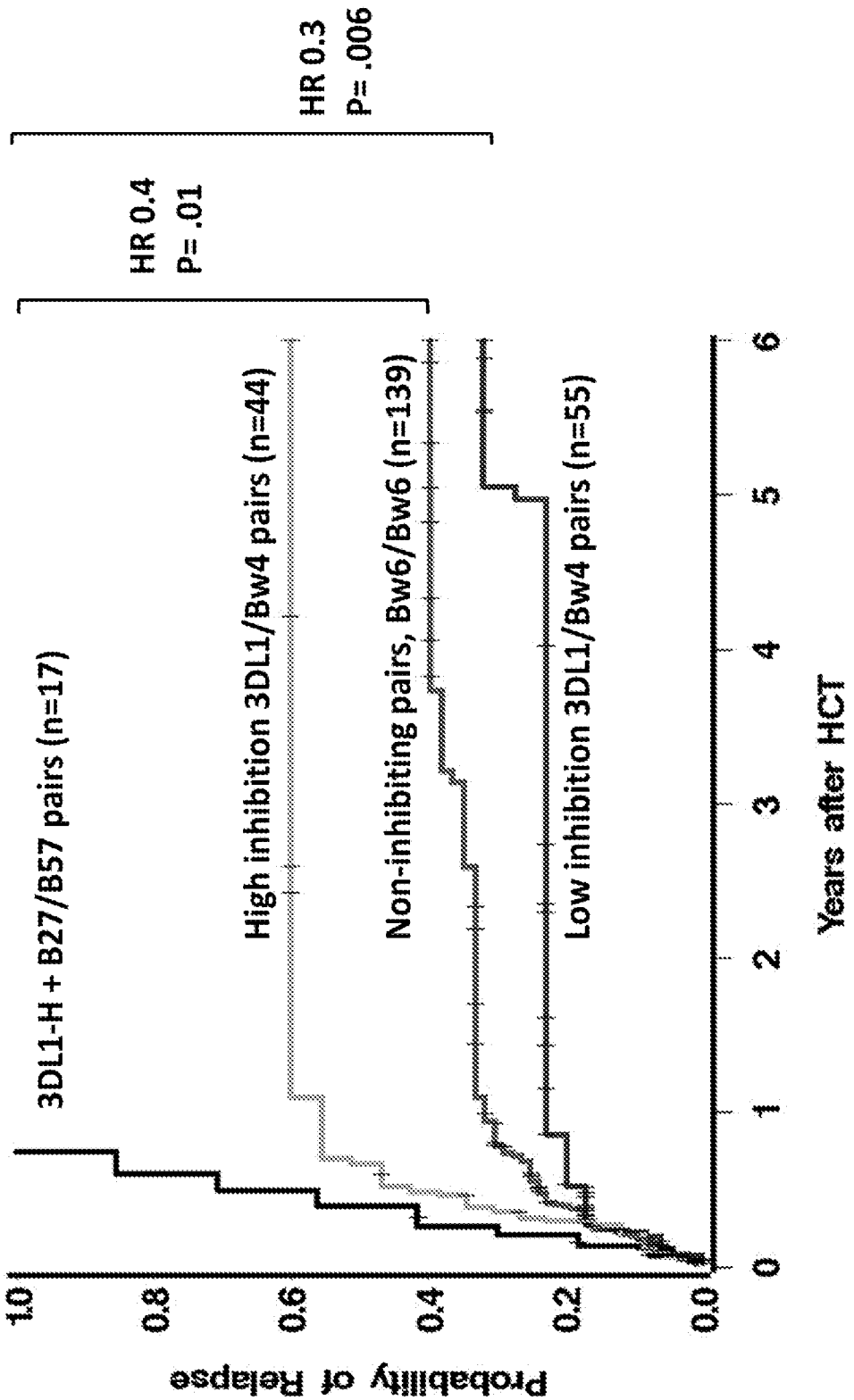

HR 0.3
P=.006
HR 0.4
P=.01
3DL1-H + B27/B57 pairs (n=17)
High inhibition 3DL1/Bw4 pairs (n=44)
Non-inhibiting pairs, Bw6/Bw6 (n=139)
Low inhibition 3DL1/Bw4 pairs (n=55)
Probability of Relapse
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
6
Years after HCT

Figure 2

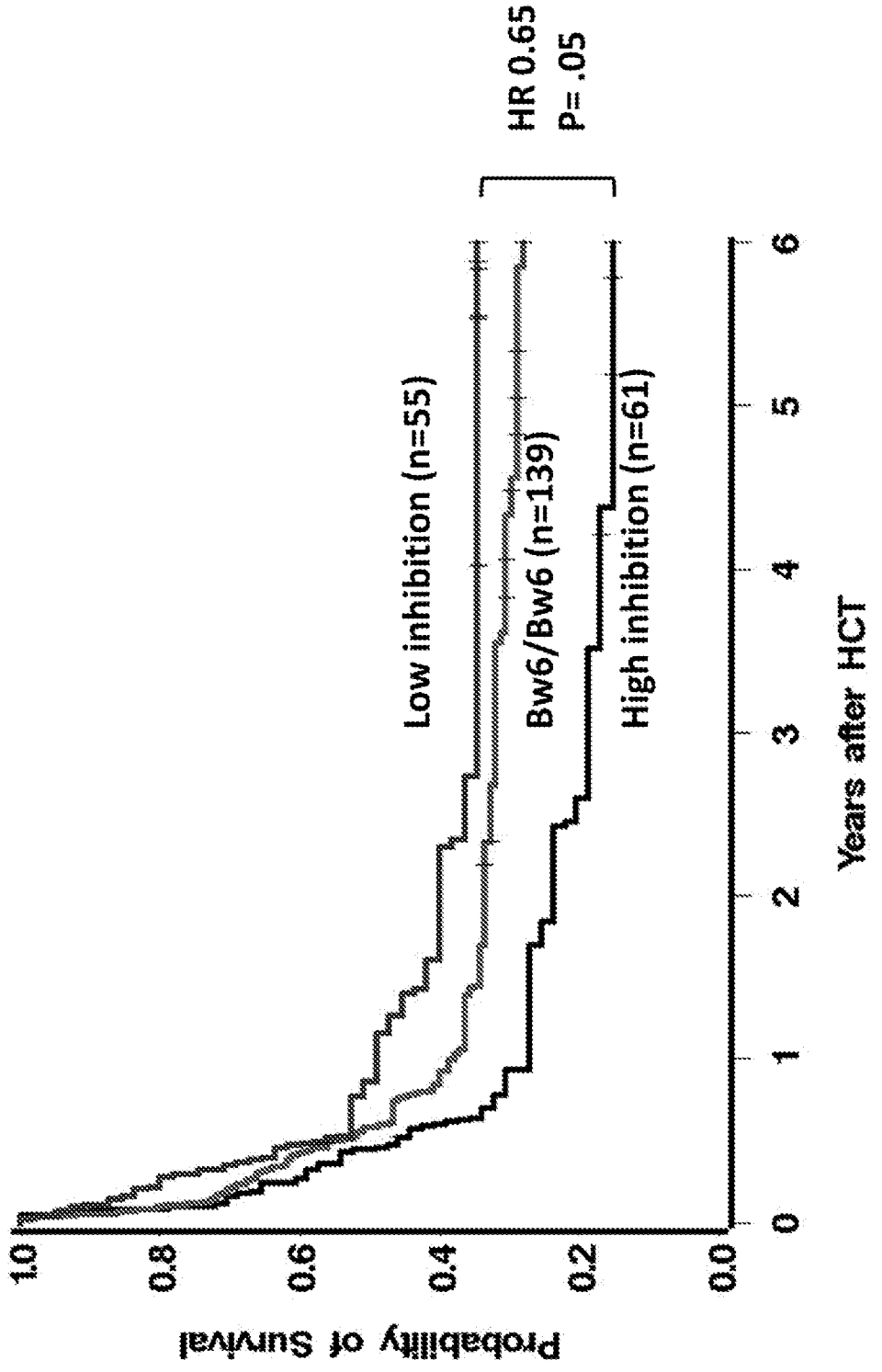
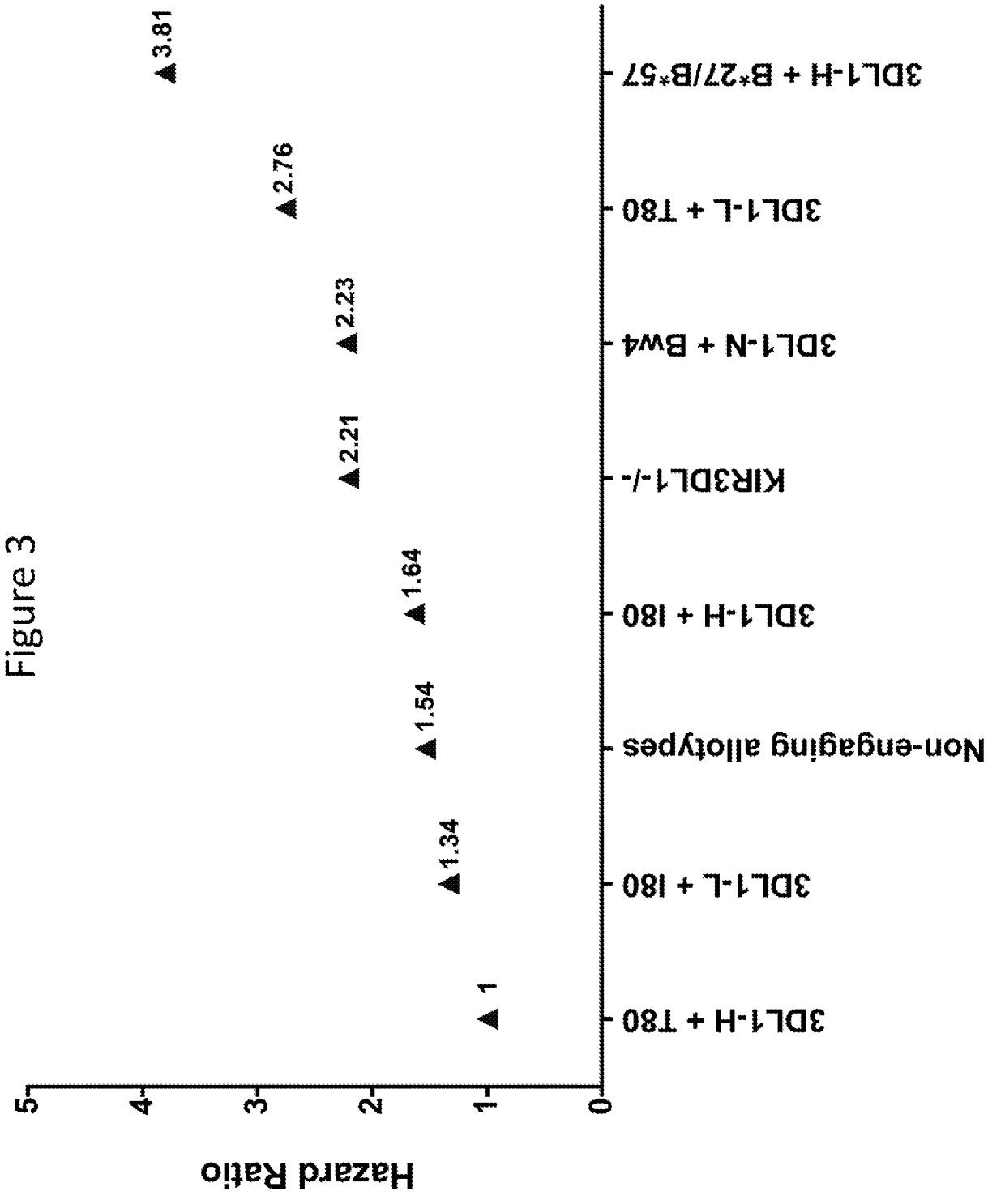

HR 0.65
P= .05
Low inhibition (n=55)
Bw6/Bw6 (n=139)
High inhibition (n=61)
Probability of Survival
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
6
Years after HCT

Figure 3


Hazard Ratio
0
1
2
3
4
5
1
1.34
1.54
1.64
2.21
2.23
2.76
3.81
3DL1-H + T80
3DL1-L + I80
Non-engaging allotypes
3DL1-H + I80
KIR3DL1-/-
3DL1-N + Bw4
3DL1-L + T80
3DL1-H + B*27/B*57

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/13896

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68; C12N 5/078; C07H 21/04; A61K 35/14 (2014.01)
USPC - 435/372, 325, 6.1, 529
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): C07H 21/04; C12Q 1/68; C12N 5/071, 5/078; A61K 35/14; A61F 2/02 (2014.01)
USPC: 435/372, 366, 363, 325, 6.1, 4; 424/529; 536/24.33; 623/1.41, 23.72, 23.63

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); Google; Google Scholar; PubMed; KEYWORDS: hematopoietic cell transplant; acute myelogenous leukemia (AML); HLA-Bw4 (T80 or I80); KIR3DL1*; KIR3DS1*; KIR3DL1*h/*h; Z27; DX9

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	GIGLIO, F et al. Donor KIR3DL1 And HLA-B Allotypes Control Leukemia Relapse After Allogeneic Hematopoietic Stem Cell Transplantation [abstract]. In: 54th ASH Annual Meeting and Exposition; 8-11 December 2012. Atlanta, GA: ASH; 2012; abstract nr 349; paragraphs 1-4.	1, 4, 11, 19 ----- 2, 3, 5-10, 12-18
Y	US 2011/0129830 A1 (LADNER, M et al.) June 2, 2011; paragraphs [0035], [0041], [0078]	2, 3
Y	MORVAN, M et al. Phenotypic And Functional Analyses Of KIR3DL1+ And KIR3DS1+ NK Cell Subsets Demonstrate Differential Regulation By Bw4 Molecules And Induced KIR3DS1 Expression On Stimulated NK Cells. J. Immunol. June 1, 2009. Vol. 182, No. 11: pages 6727-6735; page 6727, column 2, paragraph 3; page 6728; column 2, paragraphs 1-2; figures 1A-C, 2A, 2C, 3A, 4A.	5-10, 12, 13
Y	TRUNDLEY, A et al. Allelic Expression Patterns Of KIR3DS1 And 3DL1 Using The Z27 And DX9 Antibodies. Eur. J. Immunol. March 2007. Vol. 37, No. 3: pages 780-787; abstract; page 781, column 1, paragraph 4; page 781, column2, paragraphs 3-4; page 782, column 2, paragraphs 2-3; figure 3.	14, 15
Y	MARTIN, MP et al. Innate Partnership Of HLA-B And KIR3DL1 Subtypes Against HIV-1. Nat. Gent. June 2007. Vol. 39, No. 6: pages 733-740; page 733, column 2, paragraph 1; page 735, column 1, paragraphs 2-3; page 735, column 2, paragraph 1; page 736, column 1, paragraph 1.	16-18

☐ Further documents are listed in the continuation of Box C. ☐

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Date of the actual completion of the international search	Date of mailing of the international search report
07 April 2014 (07.04.2014)	29 APR 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)