Integrating killer cell immunoglobulin-like receptor high-resolution genotyping for predicting transplant outcomes in allogeneic hematopoietic stem cell transplantation

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Supplementary material

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Supplementary methods

1. Study cohort

The post-transplant immunosuppression protocol for GvHD prophylaxis consisted of either a calcineurin inhibitor with methotrexate or a calcineurin inhibitor with mycophenolate mofetil. Anti-thymocyte globulin (ATG) was used in 10/10 matched unrelated transplants and in 9/10 matched unrelated transplants until 2015. Post-transplant cyclophosphamide (PTCy) was used in 9/10 matched unrelated transplants from 2016 onward.

2. High-resolution *KIR* genotyping

Genomic DNA was directly extracted from whole blood samples and purified using the QIAGEN Blood and Tissue Kit according to the manufacturer's instructions. DNA purity and concentration were assessed by Qubit fluorometer. All samples were stored at 4°C until use. For library preparation, 500 ng of genomic DNA was first fragmented by digestive enzymes (New England Biolabs, Boston, MA, USA), followed by barcode ligation with unique adaptors (IDT, Coralville, Iowa, USA). After post-ligation cleanup, dual size selection was performed with AMPure magnetic beads (Beckman Coulter, Brea, California, USA) to acquire fragment sizes of 800 to 1200 bp length. In a second step, a pool of oligonucleotide probes specific for the KIR and HLA genomic regions was used for the targeted capture (1). Final enriched libraries were normalized to a concentration of 12 pmol/l. Paired-end sequencing was performed using a NovaSeq instrument with a sequencing length of 2 x 250 bp (Illumina, San Diego, CA, USA).

3. High-resolution *HLA* genotyping

High-resolution *HLA* genotyping was performed on all recipients and donors using reverse PCR-sequence-specific oligonucleotide microbead arrays, high-throughput sequencing (One Lambda, Canoga Park, CA, USA) or PCR-sequence-specific primers (Genovision, Milan Analytika AG, Switzerland).

4. *KIR* haplotype assignment

The centromeric portion is defined as *KIR* genes present in between the framework genes *KIR3DL3* and *KIR3DP1*, while the telomeric part encompasses genes from *KIR2DL4* to *KIR3DL2*. The presence of one or more of the following KIRs – *2DL2*, *2DL5*, *3DS1*, *2DS1*, *2DS2*, *2DS3* and *2DS5* – defines Bx haplotypes, whereas the presence of *2DL1*, *2DL3*, *3DL1* and *2DS4* marks A haplotypes.

5. KIR allotype assignment

KIR allotype refers to a distinct amino acid sequence and KIR alleles were grouped according to published known KIR allotypes: KIR3DL1 expression levels were classified into high, low or null as previously described (2, 3). KIR2DS4 alleles were classified into the full-length version or the truncated variant (4). KIR2DL1 alleles were segregated into strongly and weakly inhibiting based on the presence of an arginine or a cysteine group at position 245, respectively (5). Functionally stronger KIR2DL2 and KIR2DL3 alleles were defined as alleles having a glutamic acid at position 35, whereas weak alleles were defined by the presence of a glutamine at position 35 (6) (Supplemental Tables S3 and S4).

6. KIR and HLA interactions

HLA-A, -B and -C alleles were categorized according to their relevant epitopes following known classifications using the IPD database (7). KIR-HLA pairs were then summed up for each individual, with homozygous KIR or HLA alleles counted twice: HLA-C (C1 epitope) and HLA-B*46/B*73 with KIR2DL2 and KIR2DL3 (8), HLA-C (C2 epitope) with KIR2DL1 (8), HLA-C (C2 epitope) with KIR2DS1, HLA-C2 (**02:02*, **04:01, *05:01*), HLA-C1 (**01:02*, **14:02, *16:01*) and HLA-A*11 with *KIR2DS4*001* (8-10), HLA-C1 (**16*, **01:02*) and HLA-A**11:01* with KIR2DS2 (10-12), HLA-A (Bw4 epitope) and HLA-B (Bw4 epitope) with KIR3DL1 (13, 14). HLA-A25 and HLA-A23 molecules were not considered ligands for KIR3DL1 as they do not educate KIR3DL1$^+$ NK cells (15, 16). *KIR2DS5*003*, **004*, **005*, **006*, **007*, **008* with HLA-C2 (17). The interaction between KIR3DS1 and HLA-B was assumed based on its 97% sequence homology in the extracellular domain with the KIR3DL1 receptor, despite the lack of *in vitro* demonstration (18).

7. Statistical endpoints and analysis

The following covariates were tested by forward selection in univariable analysis and by backward selection to eliminate non-significant variables in multiple regression models: recipient and donor age, disease type, HLA matching, Karnofsky performance status, EBMT risk score, disease status, conditioning regimen, graft source, *in vitro* T-cell depletion, total body irradiation, comorbid conditions, donor/recipient cytomegalovirus matching, gender matching and transplant center.

Descriptive results are presented as medians and interquartile ranges (IQRs) for continuous variables and counts and percentages for categorical variables.

Table S1. *KIR* alleles characteristics of activating KIRs in the cohort (*n* = 1247).

KIR2DS1	*k*	%	% (Amorim *et al.*) *	*KIR2DS4*	*k*	%	% (Amorim *et al.*)
**null*	1951	78.23	77.63	**null*	551	22.1	22.65
**001*	1	0.04	-	**00101*	479	19.2	27.82
**00201*	462	18.52	17.42	**00104*	2	0.008	1.92
**00502*	21	0.84	0.02347	**00301*	576	23.1	14.62
**006*	31	1.24	0.28	**00401*	71	2.85	3.57
**008*	1	0.04	-	**00601*	426	17.08	12.04
**011*	2	0.08	-	**010*	359	14.4	14.34
**unresolved*	25	1.0	4.58	**016*	1	0.004	-
KIR2DS2	*k*	%	% (Amorim *et al.*)	**022*	2	0.008	-
**null*	1738	69.68	70.63	**unresolved*	27	1.08	2.89
**00101*	701	28.11	24.11	*KIR3DS1*	*k*	%	% (Amorim *et al.*)
**00102*	2	0.08	-	**null*	1950	77.97	-
**00104*	1	0.04	-	**01301*	525	21	18.87
**00106*	2	0.08	-	**014*	2	0.008	-
**00107*	1	0.04	-	**049N*	19	0.76	-
**002*	34	1.36	0.89	**1107*	1	0.004	-
**02001*	6	0.24	-	**unresolved*	4	0.16	-
**005*	1	0.04	-				
**007*	1	0.04	-				
**008*	1	0.04	0.0235				
**unresolved*	6	0.24	4.30				

*Comparative allele frequencies from the publication by Amorim *et al* .are shown (19).

Table S2. *KIR* alleles characteristics of inhibitory KIRs in the cohort (*n* = 1247).

KIR2DL1	*k*	%	% (Amorim *et al.*) *	*KIR2DL2/L3*	*k*	*%*	% (Amorim *et al.*)
**00101*	116	4.63	5.35	*3*00101*	957	38.7	38.36
**00201*	583	23.25	24.29	*3*00110*	14	0.56	1.10
**00302*	914	36.46	38.12	*3*00201*	591	23.6	25.21
**00303*	1	0.004	-	*3*003*	12	0.48	0.68
**00401*	281	11.21	11.48	*3*00501*	116	4.65	4.30
**00402*	12	0.48	0.26	*3*006*	1	0.04	0.07
**007*	34	1.36	1.17	*3*009*	3	0.12	0.07
**008*	4	0.16	0.40	*3*015*	2	0.08	0.12
**010*	1	0.04	-	*3*030*	1	0.04	0.0469
**01201*	1	0.04	-	**unresolved*	45	1.8	2.42
**01202*	2	0.08	-				
**014*	1	0.04	0.02347				
**020*	4	0.16	0.33				
**029*	1	0.04	-				
**3201*	8	0.32	0.40				
**03701*	9	0.36	-				
**040*	1	0.04	-				
**04301*	5	0.2	-				
**044*	4	0.16	-				
**049*	1	0.04	-				
**05401*	1	0.04	-				
**057*	5	0.2	-				
**063*	1	0.04	-				
**null*	425	16.95	16.76				
**unresolved*	92	3.67	1.03				
KIR2DL2/L3	*k*	%	% (Amorim *et al.*)				
*2*00101*	402	16.1	14.81				
*2*00301*	322	12.9	11.55				
*2*00303*	2	0.08	-				
*2*012*	3	0.12	0.05				
*2*unresolved*	23	0.92	-				

KIR3DL1	*k*	%	% (Amorim *et al.*) *
**00101*	385	15.4	16.38
**00103*	1	0.04	-
**00201*	286	11.43	9.46
**00401*	360	14.4	13.85
**00402*	30	1.2	1.57
**00501*	344	13.75	12.18
**00701*	64	2.56	2.68
**00801*	149	5.96	4.81
**00901*	18	0.72	0.99
**01501*	5	0.2	0.19
**01502*	149	5.96	6.24
**01702*	1	0.04	0.05
**019*	17	0.68	0.82
**02001*	59	2.36	-
**021*	1	0.04	0.05
**02901*	3	0.12	0.05
**03101*	2	0.08	-
**033*	3	0.12	0.05
**039*	1	0.04	0.16
**043*	2	0.08	0.02
**052*	1	0.04	0.05
**053*	14	0.56	-
**072*	1	0.04	0.02
**089*	1	0.04	-
**110*	1	0.04	-
** null*	43	1.72	-
** unresolved*	50	2	-

*Comparative allele frequencies from the publication by Amorim *et al* .are shown (19).

Table S3. Frequency of KIR2DS4, KIR2DL1, KIR2DL2/L3 and KIR3DL1 allotypes in the cohort according to their functional aspects (*n* = 1247).

KIR2DS4	*n*	%
Expressed/expressed	60	4.81
Expressed	361	28.9
Not expressed	808	64.8
Unresolved	18	1.44
KIR2DL1	*n*	%
Strong/strong or strong	854	68.48
Strong/weak	221	17.7
Weak/weak or weak	82	6.57
Unresolved	51	4.1
KIR2DL2/L3	*n*	%
E^{35} homozygous	157	12.6
E^{35} / Q^{35} heterozygous	545	43.7
Q^{35} homozygous	519	41.6
Unresolved	26	2.1
KIR3DL1	*n*	%
High/high or high	644	51.64
High/low	167	13.4
Low/low or low	215	17.24
Not expressed	130	10.4
Unresolved	30	2.41

E^{35}; glutamic acid at position 35, Q^{35}; glutamine at the position 35

Table S4. Stratification of *KIR2DS4*, *KIR2DL1*, *KIR2DL2/L3* and *KIR3DL1* alleles in the cohort according to their functional aspects (*n* = 1247).

KIR2DL1	Alleles	Reference
Strong	**00101, *00201, *00301, *00302, *00303, *00501, *008, *01201, *01202, *014, *010, *020, *040, *044, *049, *057, *063, *4301*	(5)
Weak	**00401, *00402, *00701,*007,*05401,*029*	
Not expressed	**03201*	
KIR2DL2/L3		
2DL2 E^{35}	**00101, *00301, *00303,*012*	(6)
2DL3 E^{35}	**00501, *015*	
2DL3 Q^{35}	**00101, *01101, *00201,*003,*00501,*006,*00701,*009,*015,*030*	
KIR3DL1		
High	**00101, *00103, *00201, *00801, *00901,*01501,*01502,*01702,*02001,*02901,*043,*052,*089*	(2, 3)
Low	**00501, *00701, *033, *03101, *053*	
Not expressed	**00401, *00402 *039, *019, *072*	
KIR2DS4		
Expressed	**00101, *00104*	(4)
Not expressed	**00301, *00401, *00601,*010, *016, *022*	

E^{35}; glutamic acid at position 35, Q^{35}; glutamine at the position 35

Table S5. Multivariable Cox regression analysis of the KIR variables tested on six different transplant outcomes (*n* = 1247).

		OS		**TRM**	
		Adjusted for Karnofsky score, disease state, disease type, presence of comorbidities		*Adjusted for Karnofsky score, disease type, transplant center, source of transplant*	
Variable	*n*	HR (95%-CI)	*p*	HR (95%-CI)	*p*
KIR genotype					
AA	406	1		1	
Bx	841	0.71 (0.59 – 0.87)	**< 0.001**	0.73 (0.56 – 0.96)	**0.026**
Cen AA	607	1		1	
Cen Bx	640	0.76 (0.63 – 0.92)	**0.004**	0.76 (0.58 – 1.0)	0.05
Tel AA	742	1		1	
Tel Bx	505	0.96 (0.79 – 1.16)	0.69	0.93 (0.71 – 1.23)	0.63
Centromeric genotype					
AA	587	1		1	
cA01/cB01	212	0.82 (0.63 - 1.07)	0.16	0.79 (0.54 – 1.17)	0.24
cA01/cB02	276	0.72 (0.56 – 0.92)	**0.009**	0.82 (0.58 – 1.16)	0.26
cB01/x	61	0.8 (0.51 – 1.26)	0.34	0.47 (0.2 – 1.06)	0.07
cB02/cB02	40	0.78 (0.44 – 1.38)	0.39	1.1 (0.53 – 2.27)	0.8
B content score					
Neutral	860	1		1	
Better	267	1.01 (0.8 – 1.27)	0.93	1.12 (0.81 – 1.54)	0.49
Best	120	0.83 (0.59 – 1.17)	0.29	0.7 (0.44 – 1.24)	0.25
KIR2DS1 – HLA-C2					
Absence	947	1		1	
Presence	300	0.95 (0.76 – 1.18)	0.65	1.03 (0.76 – 1.41)	0.85
KIR2DS1 – HLA-C2					
Absence	947	1		1	
2DS1 – C1/x	233	0.93 (0.73 – 1.2)	0.59	0.98 (0.69 – 1.39)	0.9
2DS1 – C2/C2	67	1.00 (0.67 – 1.49)	1.0	1.21 (0.7 – 2.1)	0.49
KIR2DS2*00101 – HLA-C*16,C*01:02, A*11:01					

Absence	1035	1		1	
Presence	212	1.15 (0.9 – 1.46)	0.25	0.97 (0.68 – 1.39)	0.89
KIR2DS4*00101 – HLA-C1/C2/A11					
Absence	1000	1		1	
Presence	229	1.26 (1 – 1.59)	**0.047**	1.65 (1.2 – 2.27)	**0.002**
KIR3DS1					
Absence	779	1		1	
Presence	468	0.96 (0.79 – 1.17)	0.72	1.0 (0.76 – 1.31)	0.98
KIR3DS1 – Bw4 (HLA-B)					
Absence	946	1		1	
Presence	301	0.88 (0.71 – 1.1)	0.27	1.02 (0.75 – 1.4)	0.89
KIR2DL1 – HLA-C2					
Strong	671	1		1	
Weak	52	0.74 (0.43 – 1.28)	0.28	0.38 (0.14 – 1.03)	0.06
Missing ligand	429	1.06 (0.87 – 1.3)	0.56	0.83 (0.62 – 1.11)	0.2
KIR2DL2/L3 – HLA-C1					
≥ 1 KIR2DL2 – C1	549	1		1	
KIR2DL3 – C1	512	1.38 (1.13 – 1.69)	**0.002**	1.29 (0.96 – 1.73)	0.09
Missing ligand	186	1.0 (0.75 – 1.34)	1.0	1.2 (0.81 – 1.78)	0.36
KIR2DL2/L3 – HLA-C1					
Weak/weak	437	1		1	
Weak/strong	253	0.83 (0.65 – 1.07)	0.16	0.89 (0.62 – 1.29)	0.55
Strong/strong	342	0.72 (0.56 – 0.91)	**0.007**	0.68 (0.48 – 0.97)	**0.033**
Missing ligand	186	0.73 (0.55 – 0.99)	**0.041**	0.92 (0.62 – 0.97)	0.67
KIR2DL2/L3 – HLA-C1					
No strong 2DL3	949	1		1	
Strong 2DL3	88	0.95 (0.67 – 1.35)	0.77	0.97 (0.57 – 1.66)	0.92
Missing ligand	186	0.85 (0.65 – 1.12)	0.26	1.06 (0.73 – 1.52)	0.77
KIR3DL1 – Bw4 (HLA-B)					

Strong inhibiting	375	1		1	
Weak inhibiting	307	1.23 (0.95 – 1.59)	0.11	1.06 (0.75 – 1.51)	0.73
Non-inhibiting	410	1.05 (0.82 – 1.35)	0.69	0.83 (0.59 – 1.17)	0.3
KIR3DL1 – Bw4 (HLA-A and -B)					
Strong inhibiting	375	1		1	
Weak inhibiting	307	1.23 (0.96 – 1.59)	0.11	1.06 (0.74 – 1.51)	0.75
Non-inhibiting	434	1.06 (0.83 – 1.35)	0.65	0.83 (0.59 – 1.16)	0.27
HLA-A*24 and A*32	100	1.27 (0.88 – 1.83)	0.21	1.1 (0.67 – 1.8)	0.7
KIR3DL1 and KIR2DS1					
Strong + 2DS1	85	1		1	
Weak + 2DS1	75	1.0 (0.59 – 1.69)	1.0	1.74 (0.85 – 3.57)	0.13
None + 2DS1	112	0.86 (0.51 – 1.43)	0.56	1.08 (0.52 – 2.26)	0.84
Strong without 2DS1	290	0.92 (0.6 – 1.42)	0.72	1.35 (0.74 – 2.48)	0.33
Weak without 2DS1	232	1.22 (0.6 – 1.42)	0.37	1.21 (0.64 – 2.29)	0.56
None without 2DS1	298	1.04 (0.68 – 1.58)	0.87	1.05 (0.56 – 1.95)	0.88

		Relapse/progression		**PFS**	
		Adjusted for age of recipient, disease type, disease state, risk score, source of transplant, transplant center		*Adjusted for Karnofsky score, disease state, disease type, presence of comorbidities, transplant center*	
Variable	*n*	HR (95%-CI)	*p*	HR (95%-CI)	*p*
KIR genotype					
AA	406	1		1	
Bx	841	1.04 (0.84 – 1.27)	0.73	0.82 (0.69 – 0.98)	**0.032**
Cen AA	607	1		1	
Cen Bx	640	0.98 (0.81 – 1.19)	0.83	0.88 (0.74 – 1.04)	0.13
Tel AA	742	1		1	
Tel Bx	505	1.02 (0.84 – 1.24)	0.83	0.96 (0.8 – 1.14)	0.63
Centromeric genotype					
AA	587	1		1	
cA01/cB01	212	1.23 (0.95 – 1.6)	0.12	1.01 (0.8 – 1.29)	0.87
cA01/cB02	276	0.88 (0.69 – 1.13)	0.32	0.83 (0.67 – 1.03)	0.09
cB01/x	61	0.95 (0.61 – 1.49)	0.83	0.72 (0.47 – 1.1)	0.13
cB02/cB02	40	1.0 (0.56 – 1.81)	0.99	1.02 (0.61 – 1.7)	0.95
B content score					
Neutral	860	1		1	
Better	267	0.99 (0.78 – 1.26)	0.95	1.09 (0.89 – 1.34)	0.41
Best	120	0.87 (0.62 – 1.23)	0.43	0.78 (0.57 – 1.06)	0.11
KIR2DS1 – HLA-C2					
Absence	947	1		1	
Presence	300	0.99 (0.79 – 1.23)	0.91	0.95 (0.78 – 1.16)	0.6
KIR2DS1 – HLA-C2					
Absence	947	1		1	
2DS1 – C1/x	233	0.97 (0.75 – 1.24)	0.78	0.94 (0.76 – 1.18)	0.62
2DS1 – C2/C2	67	1.06 (0.7 – 1.62)	0.77	0.9 6(0.67 – 1.39)	0.83

KIR2DS2*00101 – HLA-C*16,C*01:02,A*11					
Absence	1035	1		1	
Presence	212	1.17 (0.92 – 1.49)	0.19	1.15 (0.92 – 1.43)	0.22
KIR2DS4*00101 – C1/C2/A11					
Absence	1000	1		1	
Presence	229	1.25 (0.98 – 1.59)	0.07	1.39 (1.12 – 1.71)	**0.002**
KIR3DS1					
Absence	779	1		1	
Presence	468	1.04 (0.86 – 1.27)	0.67	0.99 (0.83 – 1.18)	0.88
KIR3DS1 – Bw4 (HLA-B)					
Absence	946	1		1	
Presence	301	1.0 (0.8 – 1.25)	0.97	0.97 (0.8 – 1.19)	0.8
KIR2DL1 – HLA-C2					
Strong	671	1		1	
Weak	52	0.78 (0.46 – 1.32)	0.36	0.67 (0.41 – 1.09)	0.11
Missing ligand	429	1.11 (0.91 – 1.37)	0.3	1.04 (0.86 – 1.25)	0.68
KIR2DL2/L3 – HLA-C1					
≥ 1 KIR2DL2 – C1	549	1		1	
KIR2DL3 – C1	512	1.08 (0.88 – 1.33)	0.46	1.19 (0.99 – 1.44)	0.06
Missing ligand	186	0.97 (0.72 – 1.3)	0.84	0.95 (0.73 – 1.23)	0.64
KIR2DL2/L3 – HLA-C1					
Weak/weak	437	1		1	
Weak/strong	253	0.97 (0.75 – 1.26)	0.81	0.91 (0.72 – 1.14)	0.41
Strong/strong	342	0.91 (0.71 – 1.16)	0.43	0.81 (0.65 – 1.0)	0.05
Missing ligand	186	0.89 (0.66 – 1.21)	0.46	0.79 (0.6 – 1.03)	0.08
KIR2DL2/L3 – HLA-C1					
No strong 2DL3	949	1		1	
Strong 2DL3	88	0.97 (0.67 – 1.42)	0.89	0.92 (0.66 – 1.27)	0.6
Missing ligand	186	0.93 (0.7 – 1.23)	0.61	0.86 (0.67 – 1.2)	0.23
KIR3DL1 – Bw4 (HLA-B)					

Strong inhibiting	375	1		1	
Weak inhibiting	307	1.7 (1.3 – 2.21)	**< 0.001**	1.44 (1.14 – 1.81)	**0.002**
Non-inhibiting	410	1.4 (1.09 – 1.81)	**0.009**	1.12 (0.89 – 1.4)	0.33
KIR3DL1 – Bw4 (HLA-A and -B)					
Strong inhibiting	375	1		1	
Weak inhibiting	307	1.69 (1.3 – 2.2)	**< 0.001**	1.44 (1.14 – 1.81)	**0.002**
Non-inhibiting	434	1.39 (1.08 – 1.78)	**0.011**	1.11 (0.89 – 1.38)	0.37
HLA-A*24 and A*32	100	0.88 (0.56 – 1.37)	0.56	1.08 (0.77 – 1.52)	0.65
KIR3DL1 and KIR2DS1					
Strong + 2DS1	85	1		1	
Weak + 2DS1	75	1.26 (0.73 – 2.19)	0.41	1.25 (0.77 – 2.03)	0.36
None + 2DS1	112	1.37 (0.83 – 2.27)	0.22	1.07 (0.67 – 1.7)	0.78
Strong without 2DS1	290	0.98 (0.62 – 1.55)	0.94	1.04 (0.7 – 1.56)	0.84
Weak without 2DS1	232	1.82 (1.18 – 2.82)	**0.007**	1.58 (1.06 – 2.35)	**0.025**
None without 2DS1	298	1.04 (0.9 – 2.15)	0.14	1.19 (0.8 – 1.76)	0.4

		Acute GvHD		**Chronic GvHD**		
		Adjusted for disease type, risk score, conditioning regimen, T-cell depletion, source of transplant, transplant center		*Adjusted for age of recipient, disease type, transplant center*		
Variable	*n*	HR (95%-CI)	*p*	*n*	HR (95%-CI)	*p*
KIR genotype						
AA	348	1		377	1	
Bx	750	0.95 (0.74 – 1.21)	0.65	766	1.12 (0.91 – 1.39)	0.28
Cen AA	527	1		563	1	
Cen Bx	571	1.09 (0.87 – 1.37)	0.45	580	1.19 (0.98 – 1.44)	0.08
Tel AA	643	1		682	1	
Tel Bx	455	0.9 (0.72 – 1.14)	0.39	461	1.03 (0.83 – 1.24)	0.8
Centromeric genotype						
AA	508	1		545	1	
cA01/cB01	189	1.24 (0.91 – 1.69)	0.17	287	1.31 (1.0 – 1.7)	**0.047**
cA01/cB02	242	1.06 (0.79 – 1.43)	0.68	253	1.26 (0.99 – 1.6)	0.06
cB01/x	54	0.8 (0.43 – 1.48)	0.47	59	1.01 (0.64 – 1.6)	0.96
cB02/cB02	37	0.85 (0.43 – 1.68)	0.63	31	1.32 (0.79 – 2.21)	0.29
B content score						
Neutral	748	1		797	1	
Better	240	1.08 (0.82 – 1.43)	0.56	237	1.19 (0.95 – 1.5)	0.14
Best	110	0.78 (0.51 – 1.19)	0.25	109	0.95 (0.68 – 1.32)	0.74
KIR2DS1 – HLA-C2						
Absence	829	1		869	1	
Presence	269	1.01 (0.77 – 1.31)	0.96	274	1.07 (0.86 – 1.33)	0.55
KIR2DS1 – HLA-C2						
Absence	829	1		869	1	
2DS1 – C1/x	209	0.99 (0.74 – 1.33)	0.95	213	1.21 (0.96 – 1.52)	0.11
2DS1 – C2/C2	60	1.07 (0.65 – 1.75)	0.8	61	0.63 (0.37 – 1.05)	0.08

KIR2DS2*00101 – HLA-C*16,C*01:02, A*11						
Absence	912	1		946	1	
Presence	186	0.99 (0.74 – 1.34)	0.97	197	1.11 (0.87 – 1.42)	0.4
KIR2DS4*00101 – C1/C2/A11						
Absence	874	1		917	1	
Presence	208	1.23 (0.94 – 1.62)	0.13	208	1.29 (1.02 – 1.64)	**0.035**
KIR3DS1						
Absence	679	1		716	1	
Presence	419	0.9 (0.71 – 1.14)	0.38	427	1.06 (0.87 – 1.29)	0.54
KIR3DS1 – Bw4 (HLA-B)						
Absence	832	1		716	1	
Presence	266	0.96 (0.73 – 1.27)	0.79	427	1.03 (0.82 – 1.28)	0.84
KIR2DL1 – HLA-C2						
Strong	586	1		619	1	
Weak	48	0.68 (0.35 – 1.35)	0.27	49	1.13 (0.7 – 1.8)	0.62
Missing ligand	379	0.94 (0.73 – 1.2)	0.62	397	1.05 (0.85 – 1.29)	0.64
KIR2DL2/L3 – HLA-C1						
≥ 1 KIR2DL2 – C1	492	1		497	1	
KIR2DL3 – C1	447	0.92 (0.72 – 1.18)	0.54	475	0.84 (0.68 – 1.03)	0.09
Missing ligand	159	0.94 (0.67 – 1.34)	0.74	171	0.69 (0.5 – 0.94)	**0.018**
KIR2DL2/L3 – HLA-C1						
Weak/weak	380	1		408	1	
Weak/strong	227	1.38 (1.01 – 1.87)	**0.042**	227	1.36 (1.05 – 1.76)	**0.02**
Strong/strong	309	1.08 (0.81 – 1.46)	0.59	310	1.27 (1.0 – 1.62)	**0.049**
Missing ligand	159	1.06 (0.74 – 1.53)	0.75	171	0.87 (0.63 – 1.2)	0.4
KIR2DL2/L3 – HLA-C1						
No strong 2DL3	844	1		869	1	
Strong 2DL3	77	1.13 (0.73 – 1.74)	0.58	81	1.59 (1.14 – 2.22)	**0.006**
Missing ligand	159	0.95 (0.69 – 1.32)	0.78	171	0.77 (0.57 – 1.04)	0.09
KIR3DL1 – Bw4 (HLA-B)						
Strong inhibiting	330	1		342	1	
Weak inhibiting	276	1.06 (0.78 – 1.44)	0.72	279	1.11 (0.86 – 1.43)	0.44

Non-inhibiting	355	1.08 (0.81 – 1.44)	0.62	382	0.99 (0.77 – 1.26)	0.95
KIR3DL1 – Bw4 (HLA-A and -B)						
Strong inhibiting	330	1		342	1	
Weak inhibiting	276	1.05 (0.77 – 1.43)	0.77	279	1.1 (0.86 – 1.42)	0.45
Non-inhibiting	375	1.05 (0.79 – 1.4)	0.73	405	0.99 (0.78 – 1.25)	0.91
HLA-A*24 and A*32	90	1.01 (0.66 – 1.56)	0.95	88	0.92 (0.63 – 1.37)	0.7
KIR3DL1 and KIR2DS1						
Strong + 2DS1	78	1		79	1	
Weak + 2DS1	67	0.91 (0.47 – 1.76)	0.77	68	1.52 (0.92 – 2.51)	0.1
None + 2DS1	98	1.17 (0.66 – 2.08)	0.59	102	1.07 (0.65 – 1.75)	0.8
Strong without 2DS1	252	0.97 (0.59 – 1.58)	0.89	263	1.1 (0.72 – 1.68)	0.67
Weak without 2DS1	209	1.06 (0.65 – 1.75)	0.81	211	1.08 (0.7 – 1.68)	0.73
None without 2DS1	257	1.0 (0.61 – 1.64)	0.99	280	1.07 (0.7 – 1.64)	0.77

Cen; centromeric, CI; confidence interval, GvHD; graft-versus-host disease, HR; hazard ratio, OS; overall survival, PFS; progression-free survival, Tel; telomeric, TRM; transplant-related mortality

Table S6. Multivariable cox regression analysis of KIR3DL1 and KIR2DS1 interactions tested on six different transplant outcomes in the AML subcohort (*n* = 498).

		Acute GvHD		**Chronic GvHD**		
		Adjusted for disease type, risk score, conditioning regimen, T-cell depletion, source of transplant, transplant center		*Adjusted for age of recipient, disease type, transplant center*		
Variable	*n*	HR (95%-CI)	*p*	*n*	HR (95%-CI)	*p*
KIR3DL1 – Bw4 (HLA-B)						
Strong inhibiting	144	1		144	1	
Weak inhibiting	127	1.17 (0.78 – 1.76)	0.87	127	1.17 (0.78 – 1.76)	0.45
Non-inhibiting	165	1.31 (0.9 – 1.91)	0.86	165	1.31 (0.9 – 1.91)	0.16
KIR3DL1 – Bw4 (HLA-A and -B)						
Strong inhibiting	144	1		144	1	
Weak inhibiting	127	0.96 (0.6 – 1.62)	0.88	127	1.1 (0.86 – 1.42)	0.51
Non-inhibiting	172	0.95 (0.58 – 1.56)	0.84	172	0.99 (0.78 – 1.25)	0.23
HLA-A*24 and A*32	42	2.18 (1.19 – 3.99)	**0.01**	42	0.92 (0.63 – 1.37)	0.98
KIR3DL1 and KIR2DS1						
Strong + 2DS1	38	1		38	1	
Weak + 2DS1	32	0.65 (0.25 – 1.7)	0.38	32	1.33 (0.62 – 2.85)	0.46
None + 2DS1	47	0.7 (0.29 – 1.66)	0.41	47	1.63 (0.82 – 3.24)	0.16
Strong without 2DS1	106	0.58 (0.27 – 1.23)	0.15	106	0.97 (0.51 – 1.84)	0.93
Weak without 2DS1	95	0.66 (0.32 – 1.38)	0.27	95	1.07 (0.56 – 2.06)	0.84
None without 2DS1	118	0.64 (0.31 – 1.31)	0.22	118	1.14 (0.61 – 2.14)	0.67

		Relapse/progression		**PFS**		
		Adjusted for age of recipient, disease type, disease state, risk score, source of transplant, transplant center		*Adjusted for Karnofsky score, disease state, disease type, presence of comorbidities, transplant center*		
Variable	*n*	HR (95%-CI)	*p*	*n*	HR (95%-CI)	*p*
KIR3DL1 – Bw4 (HLA-B)						
Strong inhibiting	144	1		144	1	
Weak inhibiting	127	1.8 (1.21 – 2.6)	**0.004**	127	1.37 (0.96 – 2.0)	0.08
Non-inhibiting	165	1.7 (1.16 – 2.4)	**0.006**	165	1.28 (0.92 – 1.8)	0.15
KIR3DL1 – Bw4 (HLA-A and -B)						
Strong inhibiting	144	1		144	1	
Weak inhibiting	127	1.77 (1.2 – 2.6)	**0.004**	127	1.37 (0.96 – 2.0)	0.08
Non-inhibiting	172	1.63 (1.13 – 2.4)	**0.009**	172	1.24 (0.89 – 1.7)	0.2
HLA-A*24 and A*32	42	0.59 (0.28 – 1.3)	0.168	42	0.73 (0.4 – 1.3)	0.31
KIR3DL1 and KIR2DS1						
Strong + 2DS1	38	1		38	1	
Weak + 2DS1	32	1.2 (0.49 – 3.1)	0.659	32	0.9 (0.42 – 1.9)	0.78
None + 2DS1	47	2.0 (0.91 – 4.4)	0.086	47	1.16 (0.59 – 2.3)	0.66
Strong without 2DS1	106	1.6 (0.75 – 3.3)	0.23	106	1.14 (0.63 – 2.1)	0.66
Weak without 2DS1	95	3.1 (1.53 – 6.3)	**0.002**	95	1.84 (1.01 – 3.4)	**0.046**
None without 2DS1	118	2.5 (1.25 – 5.2)	**0.01**	118	1.54 (0.86 – 2.8)	0.15

		OS		**TRM**		
		Adjusted for Karnofsky score, disease state, disease type, presence of comorbidities		*Adjusted for Karnofsky score, disease type, transplant center, source of transplant*		
Variable	*n*	HR (95%-CI)	*p*	*n*	HR (95%-CI)	*p*
KIR3DL1 – Bw4 (HLA-B)						
Strong inhibiting	144	1		144	1	
Weak inhibiting	127	1.39 (0.95 – 2.0)	0.09	127	0.8 (0.43 – 1.5)	0.49
Non-inhibiting	165	1.23 (0.85 – 1.8)	0.26	165	0.72 (0.4 – 1.28)	0.26
KIR3DL1 – Bw4 (HLA-A and -B)						
Strong inhibiting	144	1		144	1	
Weak inhibiting	127	1.4 (0.95 – 2.0)	0.09	127	0.8 (0.43 – 1.48)	0.47
Non-inhibiting	172	1.21 (0.84 – 1.7)	0.3	172	0.68 (0.38 – 1.2)	0.18
HLA-A*24 and A*32	42	0.82 (0.43 – 1.6)	0.57	42	0.69 (0.26 – 1.81)	0.45
KIR3DL1 and KIR2DS1						
Strong + 2DS1	38	1		38	1	
Weak + 2DS1	32	0.71 (0.32 – 1.6)	0.4	32	1.43 (0.46 – 4.47)	0.54
None + 2DS1	47	0.8 (0.39 – 1.6)	0.54	47	0.94 (0.31 – 2.82)	0.91
Strong without 2DS1	106	0.79 (0.42 – 1.5)	0.46	106	1.37 (0.55 – 2.43)	0.5
Weak without 2DS1	95	1.39 (0.75 – 2.6)	0.29	95	0.88 (0.32 – 2.47)	0.8
None without 2DS1	118	1.16 (0.63 – 2.1)	0.64	118	0.9 (0.34 – 2.37)	0.83

CI; confidence interval, GvHD; graft-versus-host disease, HR; hazard ratio, OS; overall survival, PFS; progression-free survival, TRM; transplant-related mortality

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