Use of killer cell immunoglobulin-like receptor genes as early markers of hematopoietic chimerism after double-umbilical cord blood transplantation

Pauline Rettman,[1] Nolwenn Legrand,[1] Catherine Willem,[1] Laurence Lodé,[2,3] Patrice Chevallier,[4] Anne Cesbron,[5,6] David Senitzer,[7] Christelle Retière,[1,8] and Katia Gagne[1,5,6,8*]*

**CR and KG are equal senior authors.*

[1]Etablissement Français du Sang, Université de Nantes, Immunovirologie et Polymorphisme Génétique, EA4271, France; [2]Laboratoire d'Hématologie Biologique, CHU Hotel Dieu, Nantes, France; [3]current address Laboratoire d'Hématologie, Montpellier, France; [4]Service d'Hématologie Clinique, CHU Hotel Dieu, Nantes, France; [5]Laboratoire d'Histocompatibilité et d'Immunogénétique, EFS Nantes, France; [6]LabEx Transplantex, Université de Strasbourg, France; and [7]Division of Hematology and Bone Marrow Transplantation, City of Hope, National Medical Center, Duarte, CA, USA.

Correspondence: katia.gagne@efs.sante.fr
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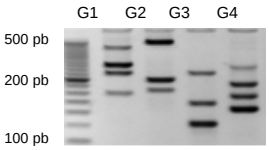
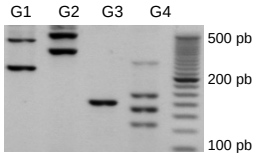
A



Interpretation of UCB1 KIR genotyping

G1			G2			G3			G4		
437pb	2DL1	+	565pb	3DL1	+	231pb	2DS1	+	194pb	2DS5	+
279pb	2DS3	+	334pb	2DL3	-	142pb	3DL2	+	155pb	3DL3	+
232pb	2DL4	+	204pb	2DS2	+	113pb	2DL5	+	130pb	2DS4	+
164pb	2DL2	+	171pb	3DS1	+				108pb	1D	-

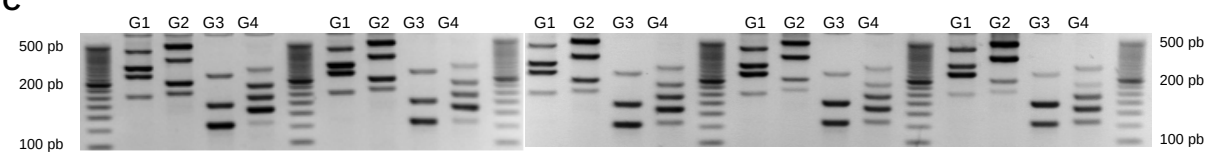
B



Interpretation of UCB2 KIR genotyping

G1			G2			G3			G4		
437pb	2DL1	+	565pb	3DL1	+	231pb	2DS1	-	194pb	2DS5	-
279pb	2DS3	-	334pb	2DL3	+	142pb	3DL2	+	155pb	3DL3	+
232pb	2DL4	+	204pb	2DS2	-	113pb	2DL5	-	130pb	2DS4	+
164pb	2DL2	-	171pb	3DS1	-				108pb	1D	+

C



UCB 1 83% **UCB 2** 17%

UCB 1 67% **UCB 2** 33%

UCB 1 50% **UCB 2** 50%

UCB 1 33% **UCB 2** 66%

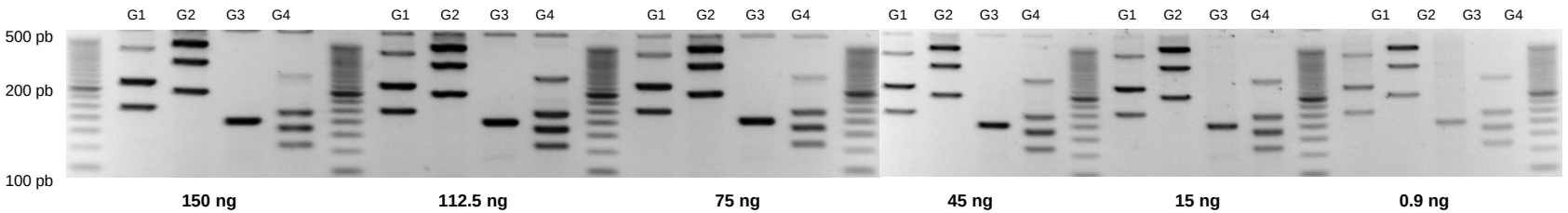
UCB 1 17% **UCB 2** 83%

Interpretation of Mixed UCB1/UCB2 KIR genotyping

G1			G2			G3			G4		
437pb	2DL1	+	565pb	3DL1	+	231pb	2DS1	+	194pb	2DS5	+
279pb	2DS3	+	334pb	2DL3	+	142pb	3DL2	+	155pb	3DL3	+
232pb	2DL4	+	204pb	2DS2	+	113pb	2DL5	+	130pb	2DS4	+
164pb	2DL2	+	171pb	3DS1	+				108pb	1D	+

Supplemental Figure 1: Evaluation of mixed UCB chimerism using KIR genotyping by Multiplex SSP-PCR [9] (A) Illustrative 3% agarose gel electrophoresis of KIR multiplex SSP-PCR products and corresponding KIR amplification patterns representing the absence (-) or presence (+) of each KIR gene in the representative cord blood 1 (UCB 1) sample using the four groups of primers (G1, G2, G3, G4) allowing the detection of all functional KIR genes. Discriminating UCB 1 genes are highlighted in light grey (B) Illustrative 3% agarose gel electrophoresis of KIR multiplex SSP-PCR products and corresponding KIR amplification patterns representing the absence (-) or presence (+) of each KIR gene in the representative cord blood 2 (UCB 2) sample using the four groups of primers (G1, G2, G3, G4). Discriminating UCB 2 genes are highlighted in dark grey (C) Illustrative 3% agarose gel electrophoresis of KIR multiplex SSP-PCR products and corresponding KIR amplification patterns representing the absence (-) or presence (+) of each KIR gene in mixed UCB 1/UCB 2 at various ratio using the four groups of primers (G1, G2, G3, G4). Discriminating UCB 1 and UCB 2 genes are highlighted in light grey and dark grey respectively. Simply Load OZYME 20 pb size marker was used. KIR primer pair groups provide short amplicons from 108 to 565 bp.

A

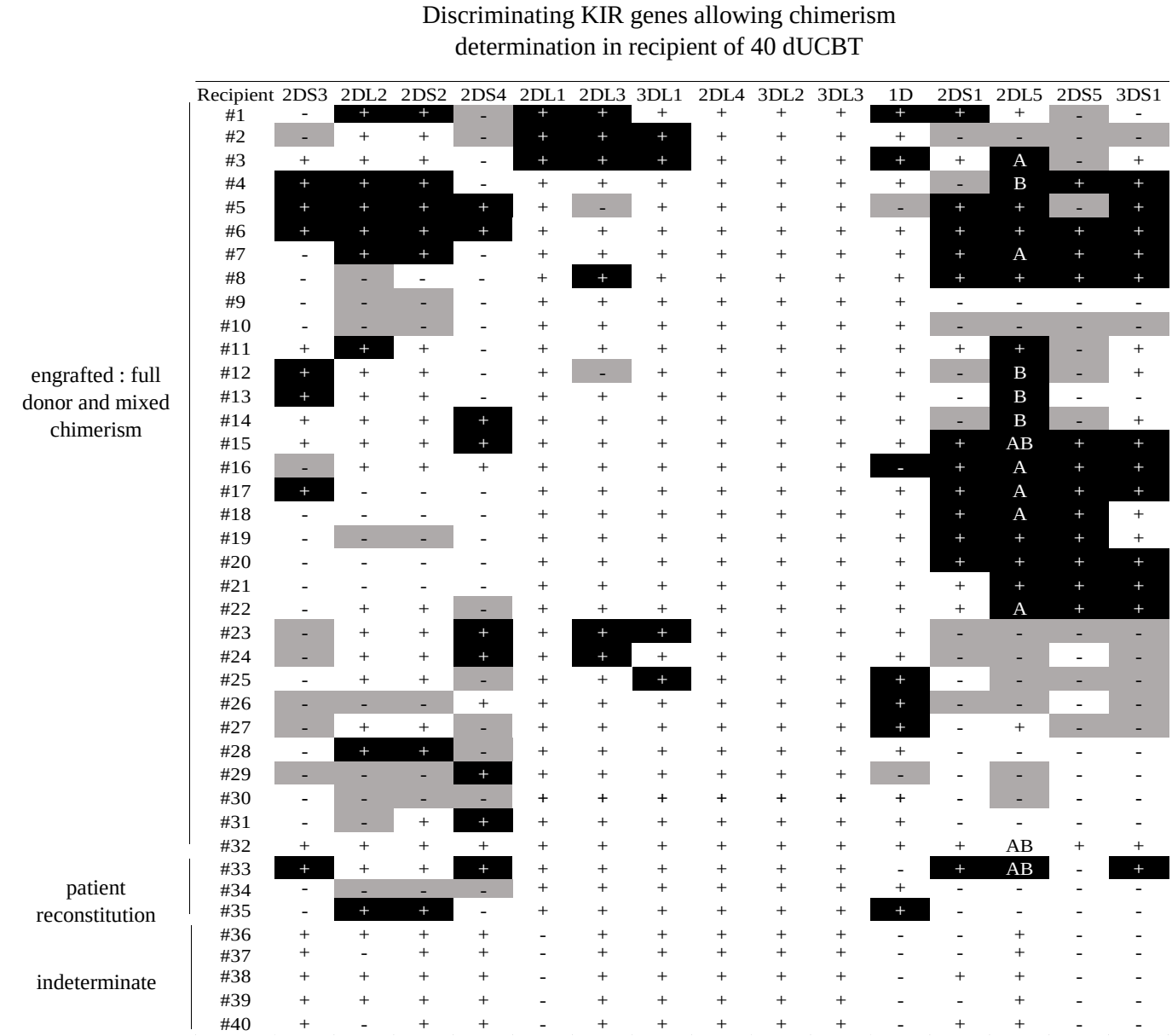


B

Interpretation of UCB KIR genotyping from 150 to 0.9 ng template DNA

G1			G2			G3			G4		
437pb	2DL1	+	565pb	3DL1	+	231pb	2DS1	-	194pb	2DS5	-
279pb	2DS3	-	334pb	2DL3	+	142pb	3DL2	+	155pb	3DL3	+
232pb	2DL4	+	204pb	2DS2	+	113pb	2DL5	-	130pb	2DS4	+
164pb	2DL2	+	171pb	3DS1	-				108pb	1D	+

Supplemental Figure 2: Reproductibility and sensibility of KIR genotyping by Multiplex SSP-PCR [9] (A) Illustrative 3% agarose gel electrophoresis of KIR multiplex SSP-PCR products for one representative cord blood sample (UCB) performed using from 150 to 0.9 ng template DNA. Simply Load OZYME 20 pb size marker was used. (B) Corresponding KIR amplification patterns representing the absence (-) or presence (+) of each KIR gene in the representative cord blood sample using the four groups of primers (G1, G2, G3, G4). Primer pairs provide short amplicons from 108 to 565 bp.



Supplemental Table 1 : Analysis of hematopoietic chimerism status using KIR genotyping in 40 recipients of dUCBT. KIR genotyping were performed in 40 dUCBT recipients at day 90 post-dUCBT for engrafted patients with full or mixed chimerism (#1 to #32), with autologous reconstitution (#33 to #35) and with indeterminate chimerism status (#36 to #40). Hierarchical clustering of KIR genes was performed based on Genesis software analysis reflecting the usefulness of each KIR gene. Discriminating KIR genes allowing hematopoietic chimerism status to be assessed are indicated by their presence (in black) or absence (in grey). Of note, KIR2DL4, KIR3DL2 and KIR3DL3 do not conclusively reveal which UCB unit engrafted, these genes being present in all individuals in comparison with other KIR genes

A

	Patient HLA class I typing						UCB 1 HLA class I typing						UCB 2 HLA class I typing					
	HLA-A	HLA-A	HLA-B	HLA-B	HLA-C	HLA-C	HLA-A	HLA-A	HLA-B	HLA-B	HLA-C	HLA-C	HLA-A	HLA-A	HLA-B	HLA-B	HLA-C	HLA-C
#1	03:01	33:03	07:02	35:01	04:01	07:02	03:XX	11:XX	07:XX	35:XX	04:01	07:02	03:XX	24:XX	07:XX	35:XX	04:01	07:02
#2	02:01	02:06	08:01	44:02	05:01	07:01	02:XX	02:XX	44:XX	51:XX	04:XX	04:XX	02:XX	02:XX	44:XX	51:XX	14:XX	16:XX
#3	03:01	03:01	51:01	18:01	07:01	15:02	03:01	32:01	18:01	55:01	07:01	03:03	03:01	26:01	07:XX	51:01	07:02	14:02
#4	29:02	02:01	35:01	44:02	04:01	05:01	02:01	30:02	35:01	44:02	04:01	05:01	02:01	29:02	07:02	44:03	07:02	16:01
#5	03:01	68:01	18:01	35:01	04:01	07:01	02:XX	02:XX	18:XX	35:XX	01:02	01:02	02:XX	68:01	35:XX	40:XX	03:04	04:01
#6	03:01	31:01	14:01	14:02	08:02	08:02	03:XX	32:XX	07:XX	14:XX	07:XX	08:XX	03:XX	24:XX	07:XX	14:XX	07:XX	08:XX
#7	03:01	24:02	35:01	38:01	03:03	12:03	03:XX	24:XX	35:XX	38:XX	04:XX	12:XX	24:XX	26:XX	35:XX	38:XX	04:01	12:03
#8	26:01	68:02	07:02	38:01	07:02	12:03	26:01	68:01	38:01	40:01	03:04	12:03	03:01	26:01	07:02	38:01	07:02	12:03
#9	24:02	30:02	07:02	51:01	01:02	07:02	02:XX	24:XX	07:XX	51:XX	07:02	14:02	24:XX	30:XX	07:XX	18:XX	07:02	05:01
#10	11:01	11:01	18:01	56:01	01:02	05:01	30:XX	11:XX	18:XX	55:XX	03:03	05:01	02:XX	30:XX	18:XX	56:XX	01:02	05:01
#11	01:01	29:02	27:05	35:03	02:02	04:01	01:XX	11:XX	27:XX	44:XX	02:XX	04:XX	01:XX	02:XX	27:XX	44:XX	02:XX	06:XX
#12	29:02	30:04	44:03	37:01	06:02	16:01	01:01	29:02	37:01	44:03	06:02	16:01	01:01	29:02	37:01	44:03	06:02	16:01
#13	03:01	30:01	27:05	14:01	02:02	08:02	03:01	30:01	08:01	14:01	07:01	08:02	30:02	03:01	18:01	35:01	04:01	05:01
#14	02:05	03:02	08:01	41:01	07:02	17:01	01:01	02:01	08:01	41:01	07:01	07:01	02:01	68:01	08:01	44:02	07:01	07:04
#15	02:01	01:01	08:01	35:01	04:01	07:01	01:XX	32:XX	35:XX	57:XX	04:01	06:02	25:01	32:01	35:01	51:01	04:XX	12:XX
#16	01:01	11:01	55:01	52:01	12:03	03:03	11:01	24:02	55:01	07:02	03:03	07:02	11:01	29:02	44:03	55:01	16:01	03:03
#17	01:01	29:02	08:01	49:01	07:01	07:01	01:01	02:01	08:02	49:01	07:01	07:01	01:01	02:05	37:01	49:01	06:02	07:01
#18	02:01	24:02	35:03	51:05	12:03	04:01	02:01	24:02	35:01	40:01	03:04	04:01	02:01	24:02	38:01	35:01	04:01	12:03
#19	02:05	11:01	50:01	44:03	06:02	16:01	02:XX	68:XX	44:XX	50:XX	06:XX	07:XX	02:XX	11:XX	08:XX	44:XX	07:XX	07:XX
#20	24:02	68:01	08:01	44:02	03:04	15:02	24:XX	68:XX	44:XX	51:XX	07:XX	14:XX	24:XX	68:XX	27:XX	44:XX	01:XX	07:XX
#21	02:01	03:01	07:02	44:05	02:02	07:02	02:XX	03:XX	07:XX	44:XX	07:XX	02:XX	02:XX	03:XX	07:XX	44:XX	07:XX	07:XX
#22	30:02	03:01	18:01	44:03	05:01	16:01	30:XX	29:XX	18:XX	44:XX	05:01	16:01	30:XX	29:XX	18:XX	44:XX	05:01	16:01
#23	01:01	02:01	14:02	35:03	04:01	08:02	01:01	02:01	14:02	40:01	03:04	08:02	01:01	02:01	35:03	44:02	04:01	05:01
#24	02:01	68:01	44:03	51:01	05:01	16:01	23:01	02:01	44:02	51:01	05:01	15:02	02:01	02:01	44:02	51:01	14:02	16:04
#25	32:01	01:01	15:01	58:01	05:01	07:01	01:XX	32:XX	37:XX	34:XX	05:01	05:01	01:XX	32:01	07:02	15:01	07:02	03:XX
#26	02:01	25:01	13:02	44:02	05:01	06:02	02:01	25:01	44:02	44:02	05:01	05:01	02:01	02:01	15:01	44:02	03:03	03:04
#27	02:XX	24:02	18:01	44:02	05:01	07:01	02:XX	24:XX	44:XX	55:XX	03:XX	05:XX	02:XX	24:XX	38:XX	44:XX	05:XX	12:XX
#28	02:01	03:01	49:01	56:01	05:01	07:01	02:XX	03:XX	35:XX	56:XX	01:XX	04:XX	02:XX	03:XX	35:XX	51:XX	04:XX	15:XX
#29	03:01	30:02	15:01	27:05	02:02	03:03	02:01	30:02	15:01	07:02	02:02	07:02	03:01	30:02	35:01	27:05	02:02	03:03
#30	24:02	31:01	08:01	51:01	07:01	15:02	03:XX	24:XX	35:XX	51:XX	05:01	06:02	24:XX	25:XX	08:XX	51:XX	01:02	07:01
#31	11:01	33:01	44:02	14:02	05:01	08:02	11:01	29:02	14:02	44:03	08:02	16:01	11:01	29:02	14:02	44:03	08:01	16:01
#32	03:01	03:01	35:03	39:01	04:01	12:03	03:01	03:01	07:XX	35:XX	04:01	07:02	02:XX	03:XX	07:XX	35:XX	04:01	07:02
#33	02:01	02:01	15:18	18:01	05:01	07:04	02:XX	02:XX	18:XX	13:XX	05:01	06:02	02:XX	02:XX	18:XX	41:XX	07:01	08:02
#34	03:01	03:01	15:01	44:03	03:03	16:01	03:01	03:01	07:02	44:03	04:01	07:02	03:01	29:02	44:03	51:01	14:02	16:01
#35	03:01	24:02	27:05	39:06	07:02	07:02	03:01	24:02	27:05	44:27	01:02	07:04	03:01	24:02	39:01	44:02	05:01	12:03
#36	02:01	68:01	40:01	51:01	03:04	14:02	24:02	24:02	40:01	51:01	03:04	07:01	03:02	31:01	40:01	51:XX	03:04	14:02
#37	01:01	02:05	08:01	58:01	07:01	07:18	01:01	02:01	08:01	35:08	07:XX	04:01	01:01	02:01	08:01	51:01	05:01	07:01
#38	02:01	03:01	14:02	15:16	08:02	16:01	02:XX	03:XX	14:02	51:08	02:02	08:02	02:01	03:01	14:02	38:01	08:02	12:03
#39	02:01	11:01	35:01	41:02	04:01	17:03	02:01	11:01	35:03	14:02	04:01	08:02	02:01	11:01	14:02	35:01	04:01	08:02
#40	11:01	32:01	14:01	35:XX	04:01	08:02	03:XX	32:XX	07:02	35:02	04:01	07:02	32:XX	32:XX	51:XX	35:02	02:02	04:01

B

% of HLA class I matching (Low resolution)				
	Patient/UCB 1	Patient/UCB 2	UCB 1/UCB 2	Mean
0 HLA-A MM	47.5	45	45	45.8
1 HLA-A MM	47.5	50	50	49.2
2 HLA-A MM	5	5	5	5
0 HLA-B MM	35	27.5	32.5	31.7
1 HLA-B MM	62.5	67.5	55	61.7
2 HLA-B MM	2.5	5	12.5	6.6
0 HLA-C MM	17.5	20	22.5	20
1 HLA-C MM	67.5	60	45	57.5
2 HLA-C MM	15	20	32.5	22.5

C

% of HLA class I matching (High resolution)				
	Patient/UCB 1	Patient/UCB 2	UCB 1/UCB 2	Mean
0 HLA-A MM	40	33	36.8	36.6
1 HLA-A MM	50	52.4	57.9	53.5
2 HLA-A MM	10	14.3	5.3	9.9
0 HLA-B MM	20	10	16.7	15.6
1 HLA-B MM	75	70	55.5	66.8
2 HLA-B MM	5	20	27.8	17.6
0 HLA-C MM	16.7	26.7	17.9	20.4
1 HLA-C MM	66.6	43.3	42.8	50.9
2 HLA-C MM	16.7	30	39.3	28.7

Supplemental Table 2: A) HLA class I typing of patients and corresponding UCB 1 and UCB 2 units performed on 40 local dUCBT. High resolution typing for HLA-A, HLA-B and HLA-C loci was carried out by Sequence Based Typing kit (Abbott Molecular Park, LL, USA) prospectively on all patients and UCB units before dUCBT. Level of HLA class I matching (%) at low (B) and high (C) resolution level observed between patient and corresponding UCB units encountered in the 40 dUCBT included in the study. MM, Mismatches. XX no available HLA class I allelic typing