nature medicine

SUPPLEMENTARY INFORMATION

https://doi.org/10.1038/s41591-019-0400-z

In the format provided by the authors and unedited.

Immune signature drives leukemia escape and relapse after hematopoietic cell transplantation

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Supplementary Tables

Supplementary Table 1. Patient and transplant characteristics.

	Discovery Cohort (n=40)		Validation Cohort (n=36)	
	No.	%	No	%
Age at Transplant				
Years, Median (Range)	47 (29 − 74)		55 (21 − 76)	
Sex ratio				
Male/Female	0.6		1.4	
Disease Diagnosis				
De novo Acute Myeloid Leukemia	35	87.5	28	77.8
Secondary Acute Myeloid Leukemia	5	12.5	8	22.2
Cytogenetics				
Favorable	2	5.0	2	5.6
Intermediate	31	77.5	20	55.6
Adverse	6	15.0	14	38.8
Not Available	1	2.5	0	0.0
Molecular Alterations				
FLT3-ITD	19	47.5	7	22.6
NPM1	16	40.0	4	13.8
Status at Transplant				
Complete Remission	13	32.5	22	61.2
Active Disease	27	67.5	14	38.8
Graft Source				
PBSCs	33	82.5	29	80.6
Bone Marrow	5	12.5	7	19.4
Umbilical Cord Blood	2	5.0	0	0.0
Graft Composition				
CD34+ *10e6/Kg, Median (Range)	6 (0.05−9.5)		5.4 (0.7−17.8)	
CD3+ *10e6/Kg, Median (Range)	188 (1.46−439.0)		154.2 (43−457)	
Type of Donor				
HLA-identical Sibling	7	17.5	14	38.8
Matched Unrelated	8	20.0	11	30.6
10/10	5	12.5	9	25.0
9/10	3	7.5	2	5.6
Cord Blood	2	5.0	0	0.0
Haploidentical	23	57.5	11	30.6
Conditioning Regimens				
Myeloablative	28	70.0	18	50.0
Non-myeloablative	12	30.0	18	50.0
***In vivo* T cell Depletion**				
No	5	12.5	12	33.3
Anti Thymocyte Globulin	18	45.0	4	11.2
Post-Transplant Cyclophosphamide	15	37.5	11	30.6
Alemtuzumab	2	5.0	8	22.2
Anti Thymocyte Globulin + Alemtuzumab	0	0.0	1	2.7
***In vivo* B cell Depletion**				
No	24	60.0	27	75.0
Rituximab	14	35.0	0	0.0
Alemtuzumab	2	5.0	9	25.0
Post-transplantation GvHD Prophylaxis				
None	0	0.0	7	19.4
CsA	0	0.0	9	25.0
Tacr/MMF	1	2.5	9	25.0
Sirolimus/MMF	22	55.0	0	0.0
CsA/MTX	12	30.0	2	5.6
CsA/MMF	5	12.5	5	13.8
Other	0	0.0	4	11.2
aGvHD				
No	23	57.5	26	72.2
Grade 1	8	20.0	3	8.3
Grade 2	5	12.5	3	8.3
Grade 3	4	10.0	2	5.6
Grade 4	0	0.0	2	5.6
cGvHD				
No	35	87.5	33	91.7
Limited	1	2.5	2	5.6
Extensive	4	10.0	1	2.7
Relapse				
Time to Relapse (Days), Median (Range)	65 (15-895)		153 (21-2165)	
On IS at the Time of Relapse	24	60.0	15	41.7

Supplementary Table 2. Summary of the analyses carried out in the discovery cohort.

Patient Code	Type of allo-HCT	Donor-Recipient HLA mismatches	Days from allo-HCT to Relapse	SNP Array	Micro Array	Phenotypic analysis	qPCR	Functional Assays
UPN 1	HLA-identical	-	294	✓	✓			
UPN 2	HLA-identical	-	86		✓	✓	✓	
UPN 3	HLA-identical	-	233	✓	✓			
UPN 4	HLA-identical	-	29	✓	✓	✓	✓	
UPN 5	HLA-identical	-	15	✓	✓	✓	✓	
UPN 6	HLA-Matched Unrelated	B*	58	✓				
UPN 7	HLA-Matched Unrelated	C*	96	✓				
UPN 8	Cord Blood	DRB1§	242	✓				
UPN 9	Cord Blood	A, (C), DQB1	54	✓	✓	✓	✓	
UPN 10	HLA-Haploidentical	(A), DRB1, DQB1, DPB1*	102		✓	✓	✓	✓
UPN 11	HLA-Haploidentical	(A), B, C, DRB1, DQB1, (DPB1)	53	✓				
UPN 12	HLA-Haploidentical	A, B, C, DRB1, DQB1, DPB1	190	✓				
UPN 13	HLA-Haploidentical	A, (B), (C), DQB1, DPB1	26	✓	✓	✓	✓	
UPN 14	HLA-Haploidentical	A, B, C, DRB1, DQB1, DPB1	41	✓	✓	✓	✓	✓
UPN 15	HLA-identical	-	27			✓		
UPN 16	HLA-identical	-	17			✓		
UPN 17	HLA-Matched Unrelated	(DPB1)	33			✓		✓
UPN 18	HLA-Matched Unrelated	DPB1	895			✓		
UPN 19	HLA-Matched Unrelated	DPB1	91			✓		
UPN 20	HLA-Matched Unrelated	DPB1	86			✓		
UPN 21	HLA-Matched Unrelated	-*	29			✓		
UPN 22	HLA-Matched Unrelated	C, DPB1	67			✓		
UPN 23	HLA-Haploidentical	(DRB1), (DQB1), DPB1	62			✓		
UPN 24	HLA-Haploidentical	A, (DRB1), (DQB1)	198			✓		✓
UPN 25	HLA-Haploidentical	A, B, DRB1, DQB1, DPB1	34			✓		
UPN 26	HLA-Haploidentical	A, B, C, DRB1, DPB1	49			✓		
UPN 27	HLA-Haploidentical	A, B, C, DRB1	29			✓		
UPN 28	HLA-Haploidentical	B, C, DRB1, DQB1, DPB1	132			✓		
UPN 29	HLA-Haploidentical	B, C, DRB1, DQB1	60			✓		
UPN 30	HLA-Haploidentical	A, B, C, DRB1, DQB1, (DPB1)	344			✓		
UPN 31	HLA-Haploidentical	B, C, DRB1, DQB1, DPB1	92			✓		
UPN 32	HLA-Haploidentical	A, B, C, DRB1, DQB1, DPB1	62			✓		✓
UPN 33	HLA-Haploidentical	A, (B), C, (DRB1), (DQB1), DPB1	38			✓		
UPN 34	HLA-Haploidentical	A, B, C, DRB1, DQB1, DPB1	551			✓		
UPN 35	HLA-Haploidentical	A, B, C, DRB1, DQB1, DPB1	146			✓		
UPN 36	HLA-Haploidentical	A, B, C, DRB1, DQB1, DPB1	104			✓		
UPN 37	HLA-Haploidentical	A, B, C, DRB1, DQB1, DPB1	90			✓		✓
UPN 38	HLA-Haploidentical	A, B, C, DRB1, DQB1, DPB1	59			✓		
UPN 39	HLA-Haploidentical	A, B, C, DRB1, DQB1, DPB1	856			✓		
UPN 40	HLA-Haploidentical	A, B, C, DRB1, DQB1	63			✓		

* HLA-DPB1 typing not available, § HLA-DQB1 and -DPB1 typing not available

Supplementary Table 3. Summary of genomic alterations detected by SNP array analysis.

	Diagnosis			Relapse after allo-HCT		
UPN#	**Alteration**	**Size**	**Clonality**	**Alteration**	**Size**	**Clonality**
1	None			None		
3	+8	Whole Chr.	Clonal	+8	Whole Chr.	Clonal
	-13(q13.3-14.3)	12.0 Kb	Subclonal (65-75%)	-13(q12.3-21.32)	36.9 Kb	Clonal
				-3(p12.3-14.3)	28 Mb	Subclonal (25-30%)
				-15(q14)	0.11 Mb	Subclonal (25-30%)
4	CN-LOH 13q	85.7 Mb	Subclonal (80-90%)	CN-LOH 13q	85.7 Mb	Clonal
				-6(q23.3)	0.53 Mb	Clonal
				-11(p13-15.1)	17.1 Mb	Clonal
5	+8	Whole Chr.	Clonal	+8	Whole Chr.	Clonal
6	CN-LOH 13q	85.7 Mb	Subclonal (30-45%)	CN-LOH 13	Whole Chr.	Clonal
				-10(p22.3-23.2)	10.9 Mb	Subclonal (20-30%)
7	CN-LOH 11(q13.3-ter)	66.5 Mb	Clonal	CN-LOH 11(q13.3-ter)	66.5 Mb	Clonal
8	None			CN-LOH 13	Whole Chr.	Subclonal (40-50%)
				+21	Whole Chr.	Clonal
9	CN-LOH 3(p21.3)	6.7 Mb	Clonal	CN-LOH 3(p21.3)	6.7 Mb	Clonal
	CN-LOH 5(q13.2)	2.4 Mb	Clonal	CN-LOH 5(q13.2)	2.4 Mb	Clonal
	CN-LOH 7(p12.3-14.3)	40.6 Mb	Clonal	CN-LOH H 7(p12.3-14.3)	40.6 Mb	Clonal
	CN-LOH H 13	Whole Chr.	Subclonal (15-20%)	CN-LOH 13	Whole Chr.	Subclonal (85-95%)
11	CN-LOH 13q	86.8 Mb	Clonal	CN-LOH 13q	86.8 Mb	Clonal
	-16(q23.1)	1 Mb	Subclonal (65-75%)	-16(q23.1)	1 Mb	Clonal
12				+8	Whole Chr.	Clonal
				+21	Whole Chr.	Clonal
13	CN-LOH 13q	95 Mb	Clonal	CN-LOH 13q	87.6 Mb	Clonal
				+8	Whole Chr.	Clonal
				-17(p13.2-p13.1)	0.82 Mb	Clonal
14	CN-LOH 4(p15.32)	7.2 Mb	Clonal	CN-LOH 4(p15.32)	7.2 Mb	Clonal
	-15(q15.1)	1.2 Mb	Clonal	-15(q15.1)	1.2 Mb	Clonal
				CN-LOH 13q	94.1 Mb	Clonal

Supplementary Table 4. List of the 110 genes deregulated between AML collected at disease diagnosis and relapse after allo-HCT, with respective gene IDs and symbols (provided as a separate excel file).

Supplementary Table 5. Summary of the analyses carried out in the validation cohort.

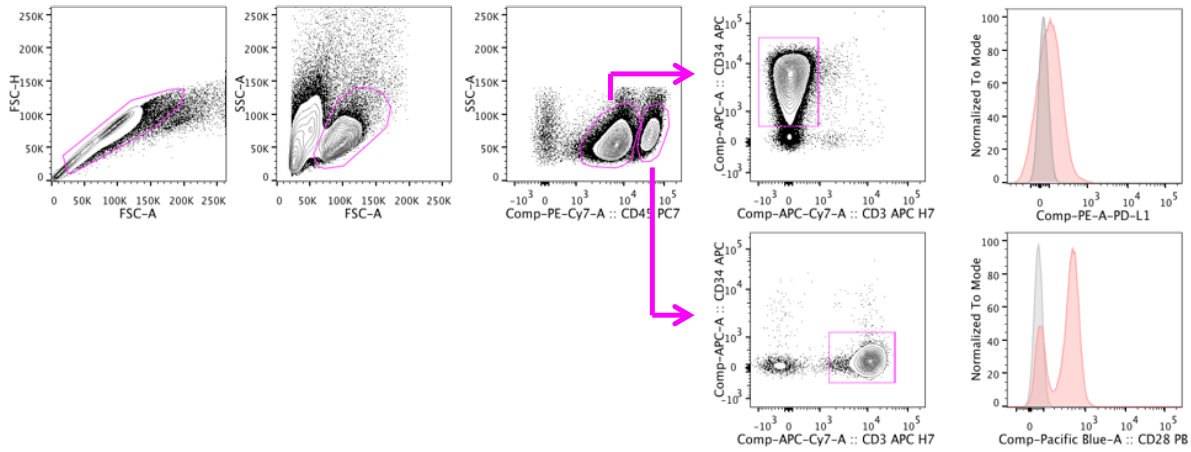
Patient Code	Type of allo-HCT	Donor-Recipient HLA mismatches	Days from allo-HCT to Relapse	RNA-Seq	Phenotypic analysis
Milano 1	HLA-identical	-	62	✓	✓
Milano 2	HLA-identical	-	702	✓	✓
Essen 1	HLA-Matched Unrelated	-*	304		✓
Essen 2	HLA-identical	-	2165		✓
Freiburg 1	HLA-Matched Unrelated	(DQB1)*	65		✓
Marseille 1	HLA-Haploidentical	(B), C*	838	✓	✓
Marseille 2	HLA-Haploidentical	A, B, C, DRB1, DQB1*	92	✓	✓
Marseille 3	HLA-Matched Unrelated	-*	94		✓
Marseille 4	HLA-Matched Unrelated	C*	890		✓
Marseille 5	HLA-Matched Unrelated	-*	139		✓
Marseille 6	HLA-Matched Unrelated	-*	597		✓
Marseille 7	HLA-Matched Unrelated	-*	404		✓
Marseille 9	HLA-Haploidentical	(A), B, C*	153		✓
Marseille 12	HLA-identical	-	558	✓	✓
Marseille 13	HLA-identical	-	128		✓
Baltimore 1	HLA-Haploidentical	(A), B, C, DRB1, DQB1*	166		✓
Baltimore 2	HLA-identical	-	163	✓	✓
Baltimore 3	HLA-Haploidentical	A, B, C, DRB1, (DQB1)*	99		✓
Baltimore 4	HLA-identical	-	59	✓	✓
Baltimore 5	HLA-Haploidentical	A, B, C, DRB1, (DQB1)*	114		✓
Leiden 1	HLA-Matched Unrelated	-*	537		✓
Leiden 2	HLA-identical	-	349		✓
Leiden 3	HLA-Matched Unrelated	(DPB1)	267		✓
Leiden 4	HLA-identical	-	108		✓
Leiden 5	HLA-Matched Unrelated	DPB1	307	✓	✓
Leiden 6	HLA-identical	-	141	✓	✓
Leiden 7	HLA-identical	-	184	✓	✓
Leiden 8	HLA-Matched Unrelated	(DPB1)	351	✓	✓
Leiden 9	HLA-identical	-	78	✓	✓
Leiden 10	HLA-identical	-	153	✓	✓
Sapporo 1	HLA-Haploidentical	A, B, C, DRB1[§]	142		✓
Sapporo 2	HLA-Haploidentical	A, B, C, DRB1[§]	30	✓	✓
Sapporo 4	HLA-identical	-	109	✓	✓
Sapporo 5	HLA-Haploidentical	B, C, DRB1[§]	21		✓
Sapporo 6	HLA-Haploidentical	A, B, C, DRB1[§]	88		✓
Sapporo 7	HLA-Haploidentical	A, B, (C), DRB1[§]	55		✓

* HLA-DPB1 typing not available, [§] HLA-DQB1 and -DPB1 typing not available

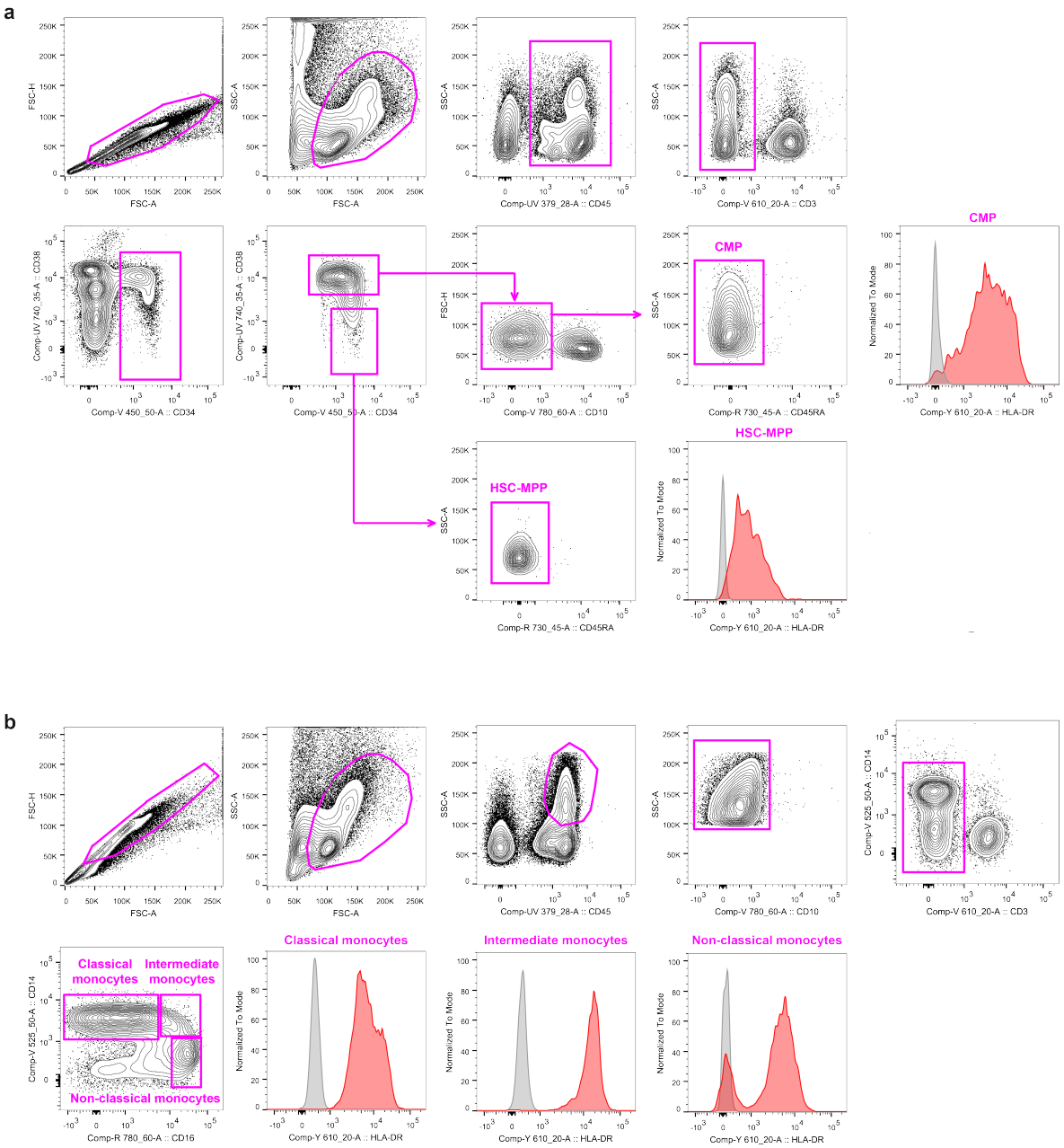
Supplementary Table 6. Primer sequences for qPCR assays.

	Forward Sequence	Reverse Sequence	Reference
CIITA	5'-AGCCTTTCAAAGCCAAGTCC-3'	5'-TTGTTCTCACTCAGCGCATC-3'	*Ulbricht T., et al., 2012*[48]
HLA-A	5'-TCCTTGGAGCTGTGATCACT-3'	5'-AAGGGCAGGAACAACTCTTG-3'	*García-Ruano A.B., et al., 2010*[47]
HLA-C	5'-TCCTGGYTGTCCTAGCTGTC-3'	5'-CAGGCTTTACAAGTGATGAG-3'	*García-Ruano A.B., et al., 2010*[47]
HLA-DRB	5'-CGTGACAAGCCCTCTCACAG-3'	5'-TGTGCAGATTCAGACCGTGC-3'	*Wang J. et al., 2007*[49]
HLA-DPB1	5'-GCAGGGCCACTCCAGAGAA-3'	5'-CCATTAAACGCGTAGCATTCC-3'	Developed in house

Supplementary Figures



Supplementary Figure 1. Gating strategy for the immunophenotypic analysis of the expression of HLA molecules, costimulatory ligands or receptors on AML blasts and T cells. Shown are representative flow cytometry plots with the sequential logical gates. According to physical parameters, live cells are gated upon the inclusion of singlets only. Based on the level of CD45 expression, immature myeloid cells ($CD45^{dim}$) are discriminated from lymphocytes and monocytes ($CD45^{high}$). The bulk $CD45^{dim}$ leukemia population was defined as CD34 or CD33 or CD117-positive and CD3-negative. The $CD45^{high}$ T cell population was identified as CD34 or CD33 or CD117-negative and CD3-positive subset. To determine the autofluorescence of the cell surface markers of interest in leukemia blasts and T cells, for each sample a negative control was generated using only linage markers (CD45, CD33 or CD34 or CD117, and CD3).



Supplementary Figure 2. Gating strategies for the immunophenotypic analysis of the expression of HLA molecules and inhibitory ligands on bone marrow progenitors (panel **a**) and different subsets of peripheral blood monocytes (panel **b**). Shown are representative flow cytometry plots with the sequential logical gates. **a**, According to physical parameters, live cells are gated upon the inclusion of singlets only. Based on the expression of CD45, hematopoietic cells are selected, followed by exclusion of $CD3^+$ T cells and by gating on $CD34^+$ progenitors. Amongst these, hematopoietic stem cells/multipotent progenitors (HSCs/MPPs) are identified as $CD38^-$ and $CD45RA^-$ and common myeloid progenitors (CMPs) as $CD38^+CD10^-$. **b**, According to physical parameters, live cells are gated upon the inclusion of singlets only. $CD45^{high}SSC^{high}$ cells are selected, followed by exclusion of $CD10^+$ neutrophils and $CD3^+$ lymphocytes. In the resulting subpopulation, classical monocytes are identified as $CD14^{high}CD16^-$, non-classical monocytes as $CD14^{dim}CD16^+$, and intermediate monocytes as $CD14^{bright}CD16^{dim}$. To determine the autofluorescence of the cell surface markers of interest (HLA-DR, -DP, PD-L1, B7-H3, Vista) in the different cell subsets, for each sample a negative control was generated using only linage markers (CD3, CD10, CD14, CD16, CD34, CD38, CD45, CD45RA).