



Ospedale Niguarda

Sistema S



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# Variabilità di espressione delle molecole MHC e trapianto

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AIBT Summer School

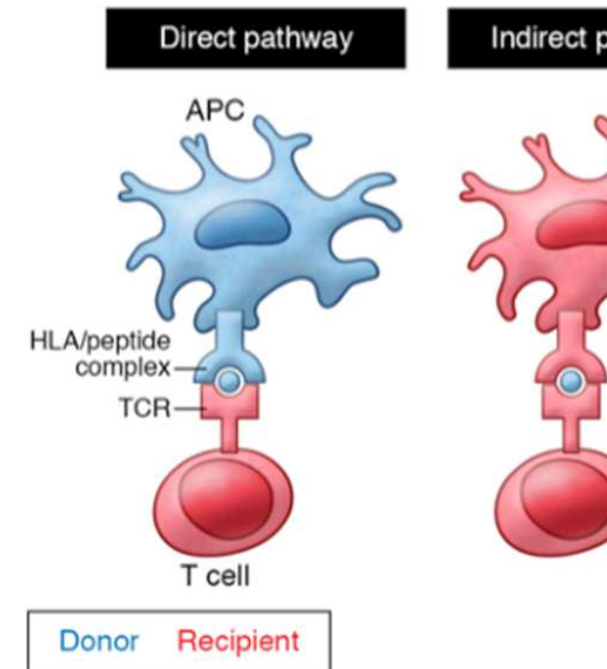
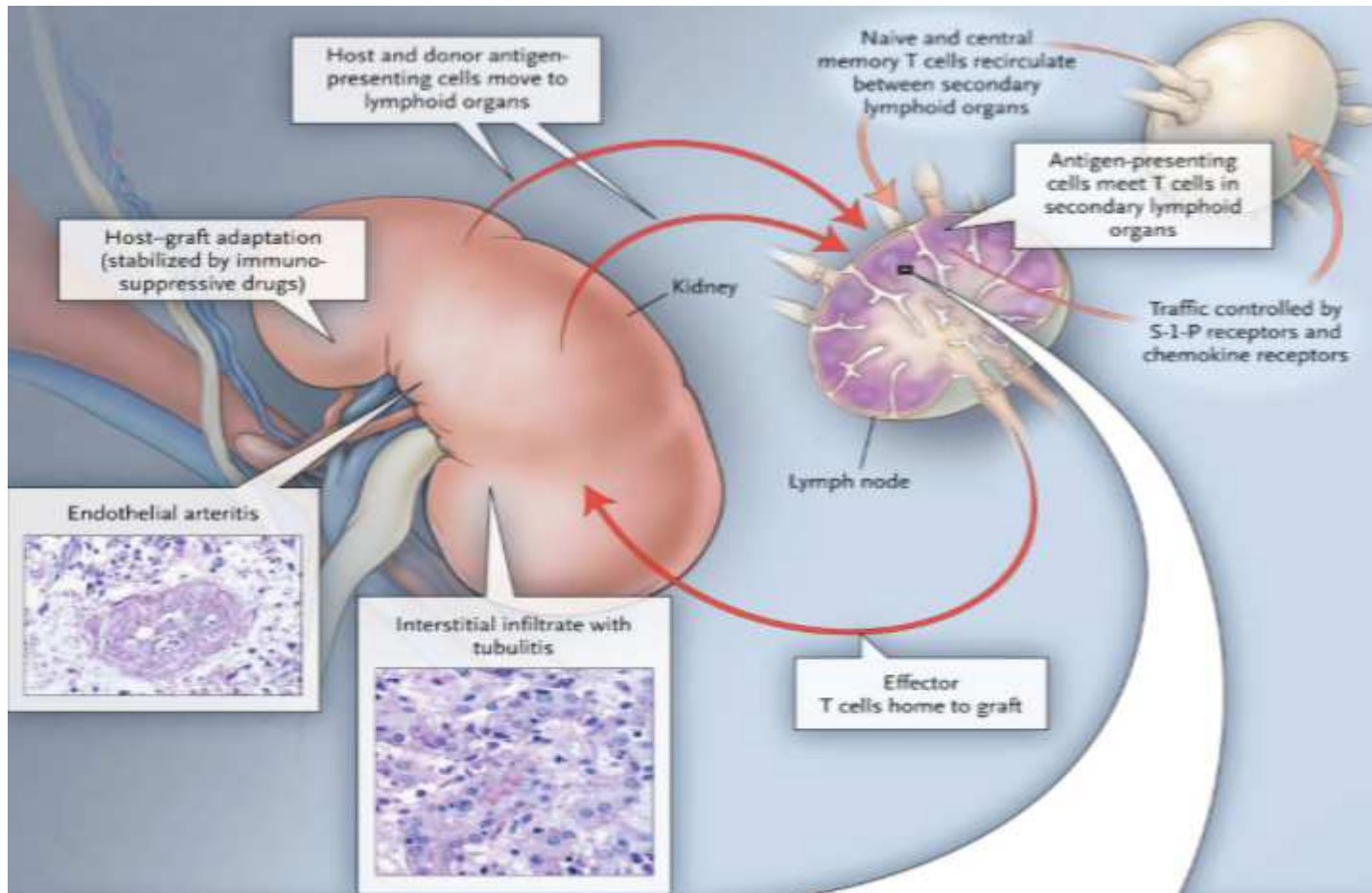
Ercolano, 13-15 giugno 2019



# Overview: topics

- Introduction: HLA and transplantation (solid, alloHSCT), alloreactivity in allogeneic HSCT
- HLA matching and outcome of unrelated HSCT, T-cell epitope models in HLA-
- Variability of HLA expression and unrelated HSCT: SNPs, HLA-C, HLA-DP, EFI abstracts
- HLA loss and impaired expression as mechanisms of immune evasion after HS
- Conclusions/Future directions

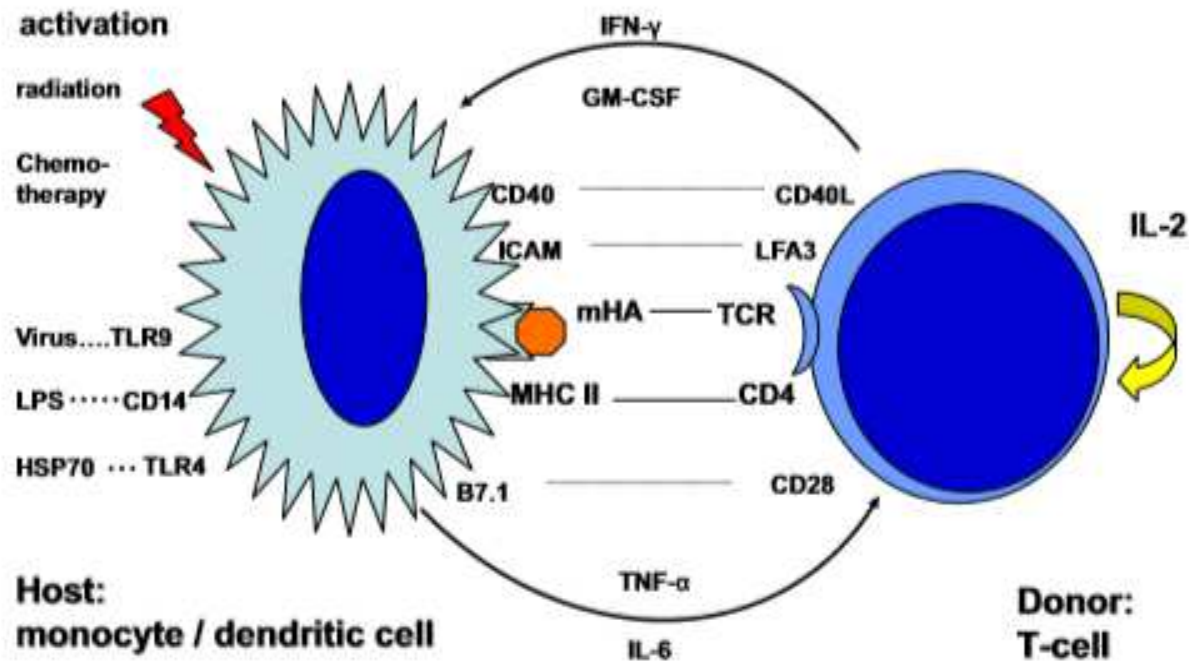
# Rejection in solid organ transplantation



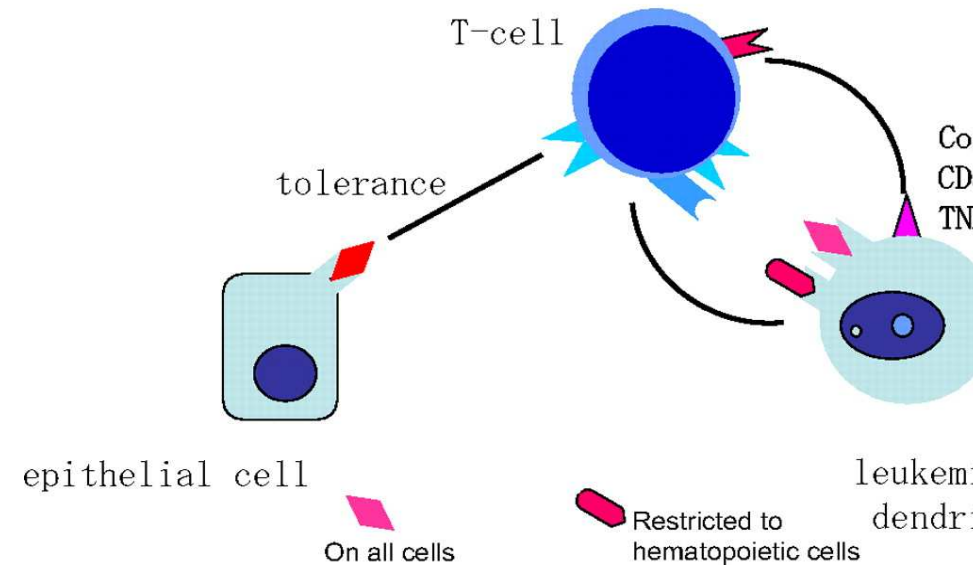
Halloran, NEJM 2004; DeWolf, JCI

# Alloreactivity in HSCT

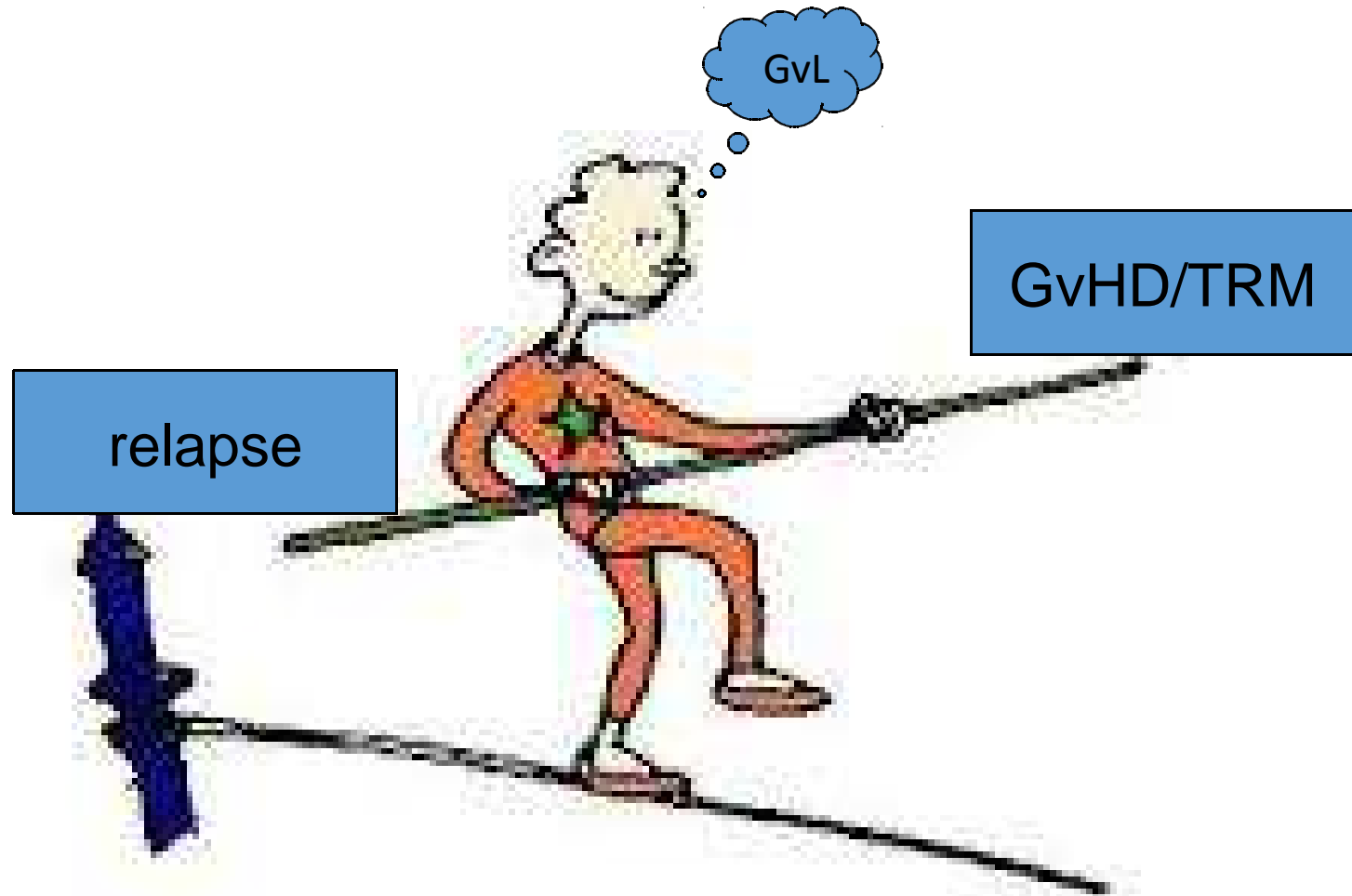
## Pathophysiology of GVHD



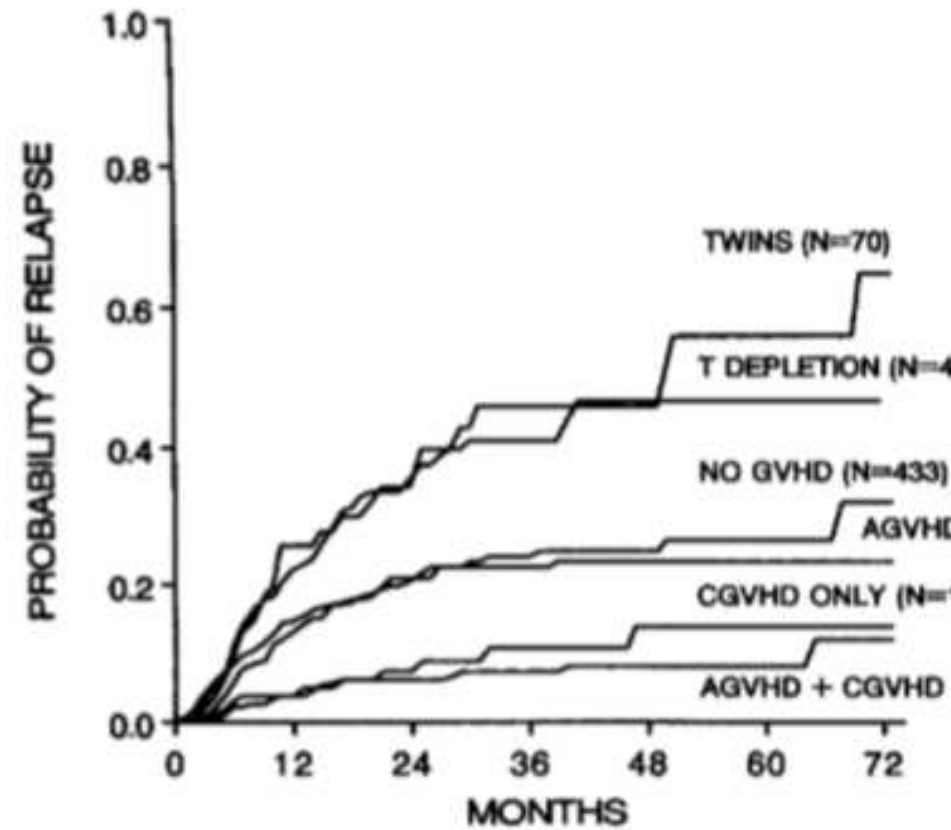
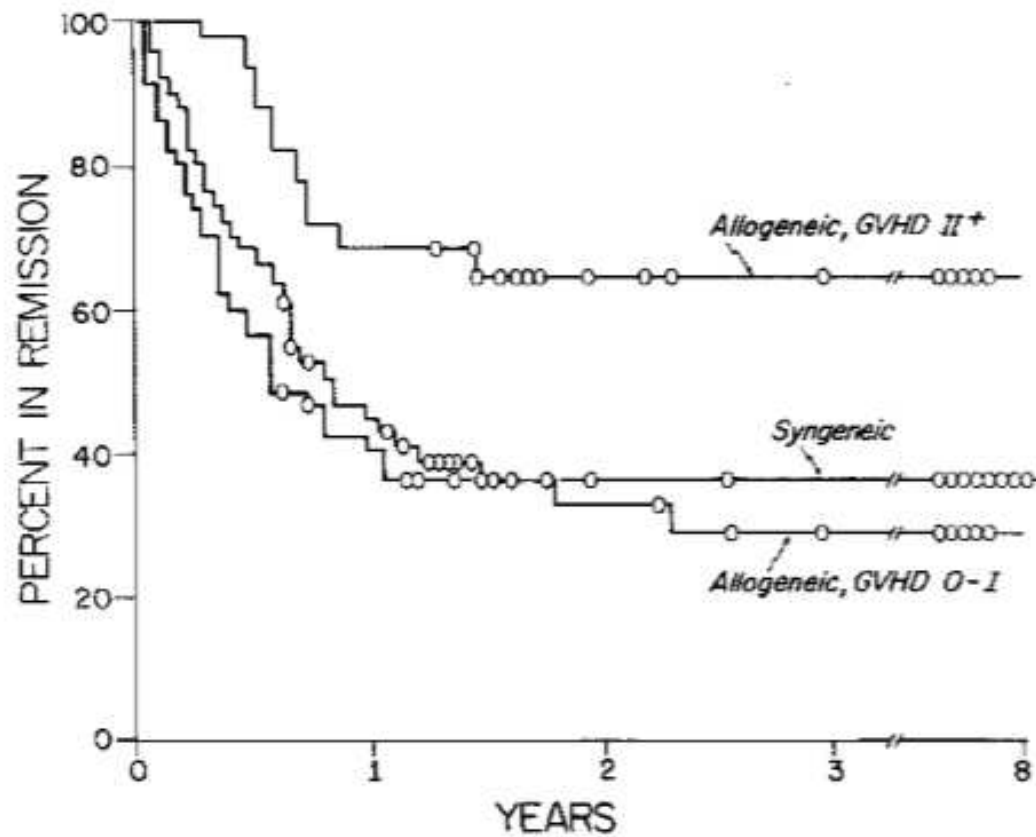
## Adoptive Immunotherapy in Child Leukemia: GVL-Reaction



# GvHD/GvL



# Graft-versus-leukemia & GvHD

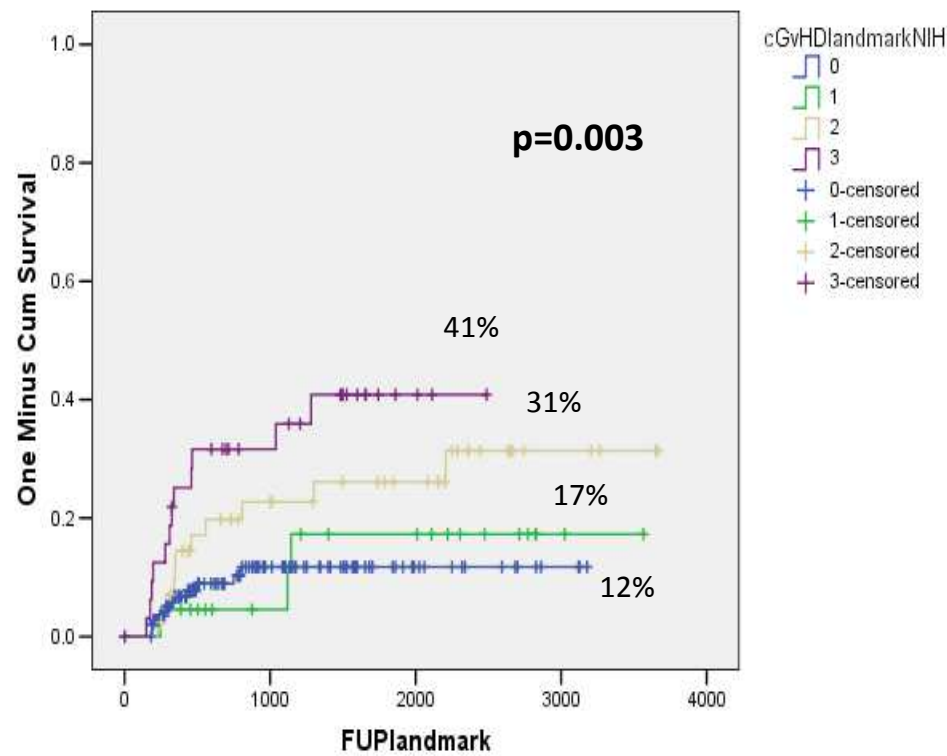


Wieden, NEJM 1979; Horowitz, Blo

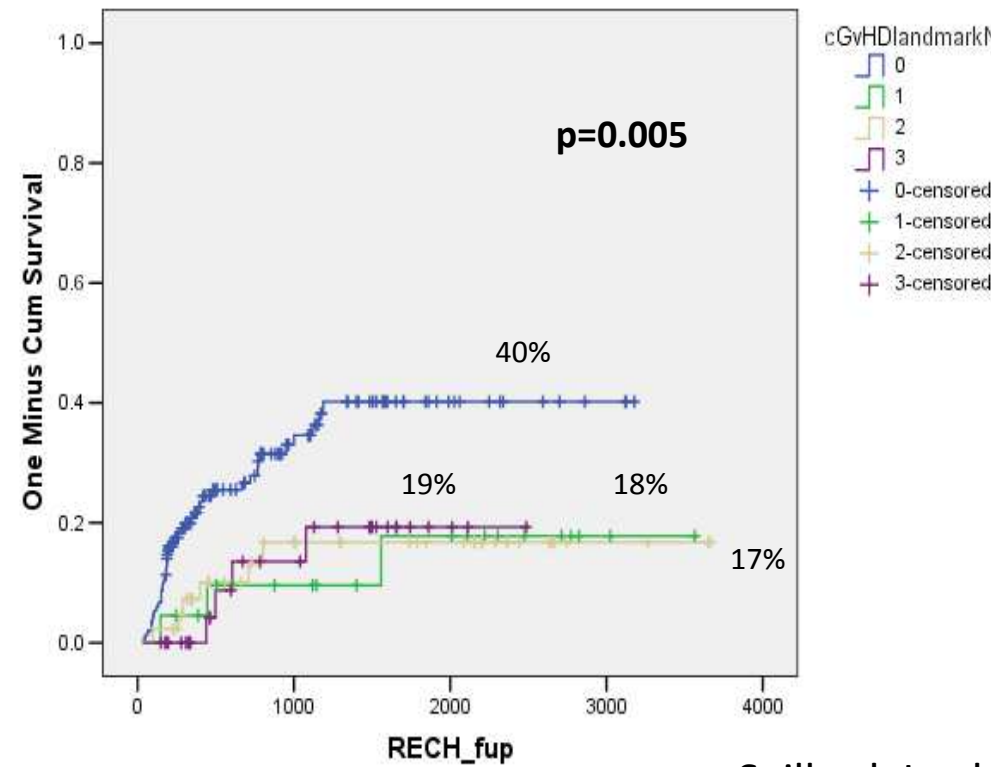
# Graft-versus-leukemia (GvL) & chronic GvHD

Non Relapse Mortality

0: No GVHD; 1: Mild; 2: Moderate; 3: Severe



Relapse





# The «drug T cell»

## **IMPACT OF T-CELL DEPLETION ON OUTCOME OF ALLOGENEIC BONE-MARROW TRANSPLANTATION FOR STANDARD-RISK LEUKAEMIAS**

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However, our data show that T-cell depletion of donor bone marrow, even if it prevents acute and chronic GVHD, promotes recurrence of the malignant disease. Further



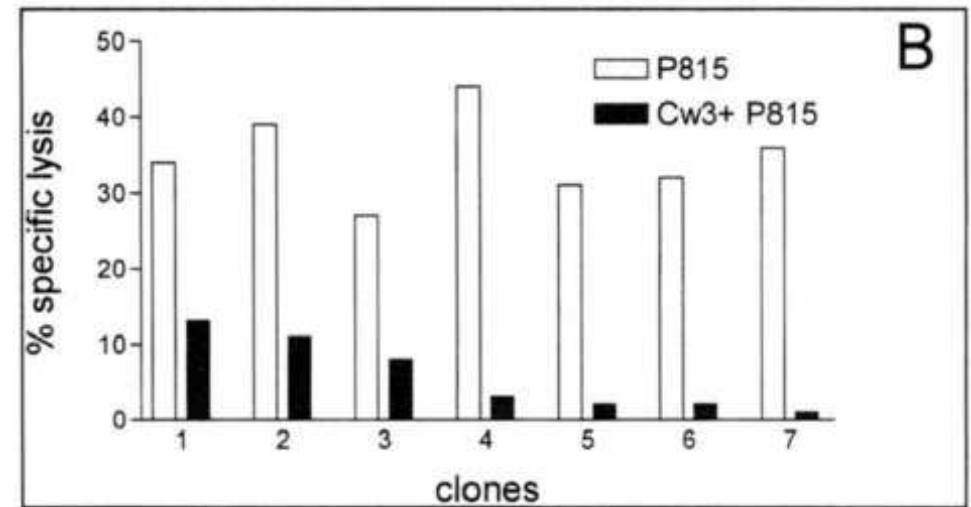
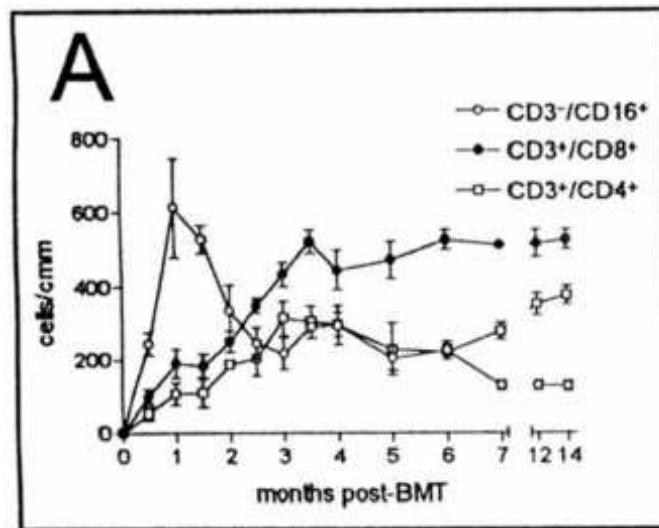
# T-cell depletion and relapse

**Table 6. Outcome**

	ATG dose		<i>P</i>
	High n = 46 (%)	Low n = 55 (%)	
Progression or relapse	24 (52)	16 (29)	.02
Patients surviving in			
Complete remission	10 (22)	14 (25)	
Partial remission	2 (4)	9 (16)	
Stable disease	0	10 (18)	.06
Progressive disease	2 (4)	2 (4)	
Deaths	32 (70)	20 (36)	
Causes of death			
Disease recurrence or progression	21 (46)	13 (65)	
Transplantation-related mortality			
Acute GVHD	5 (16)*	3 (15)*	
Chronic GVHD	0	4 (20)	
Infections	5 (16)	0	NS
Multiorgan failure	1 (3)	0	

# Not only T-cell therapy: the NK effect

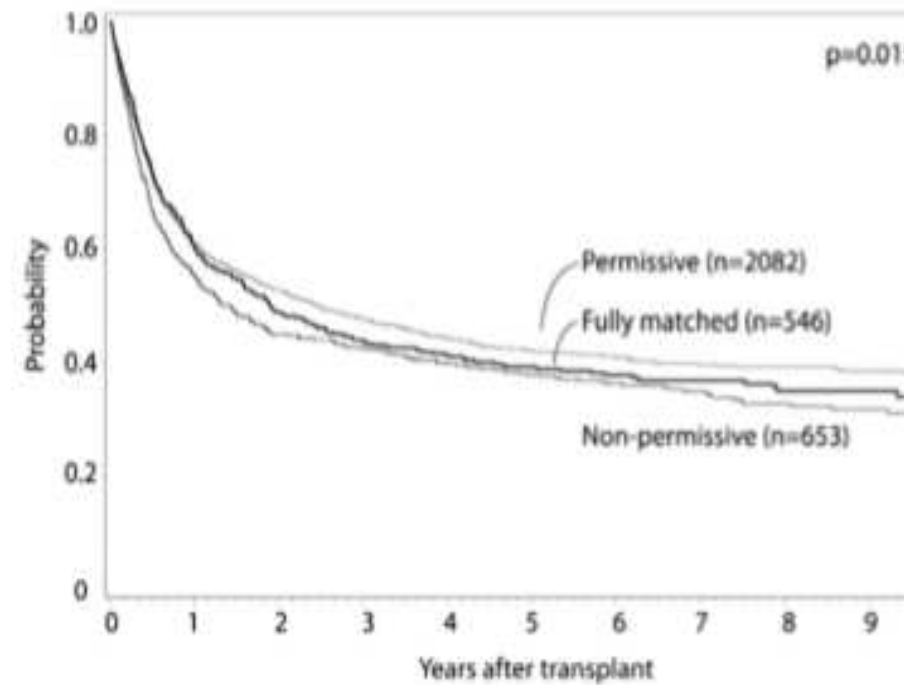
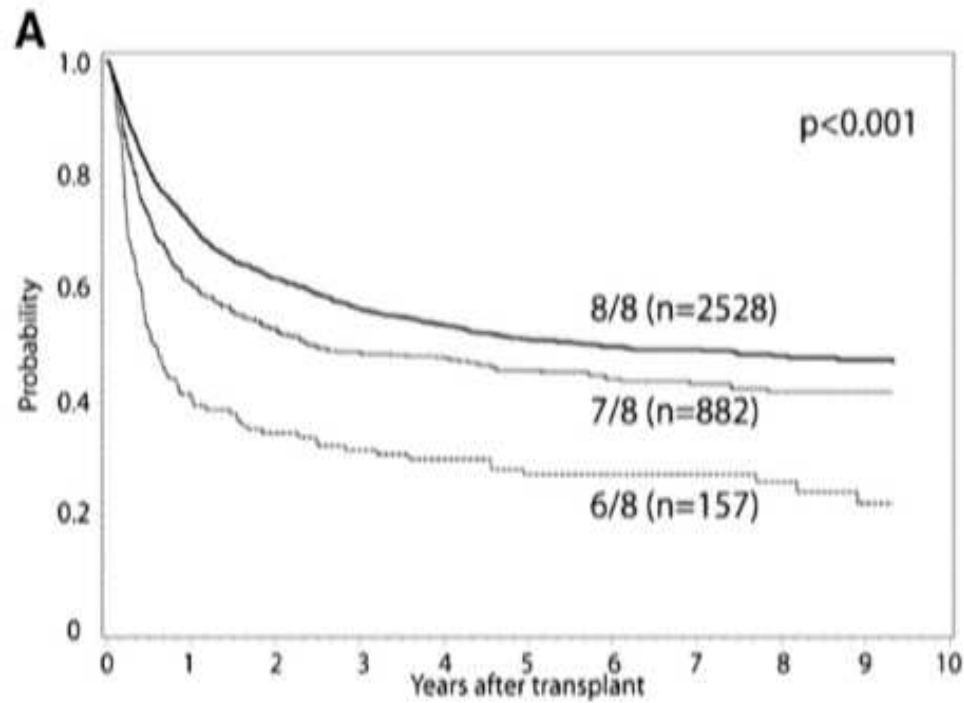
- Haplo TCD = ideal setting to unravel the NK activity (Perugia)




# Take-home messages (1)

- Alloreactivity in HSCT is mainly mediated by T cells
- The optimal immunological balance between host and donor determines the potential cure of leukemia

# HLA matching and outcome after unrelated HSCT



# HLA-DP alloreactivity in vivo: the TCE model

DPB1* alleles	TCE3 group	TCE4 group	Immunogenicity
0901 1001 1701	1	1	
0301 1401 4501	2	2	
0201 0202 0203	3	3	
Others		4	

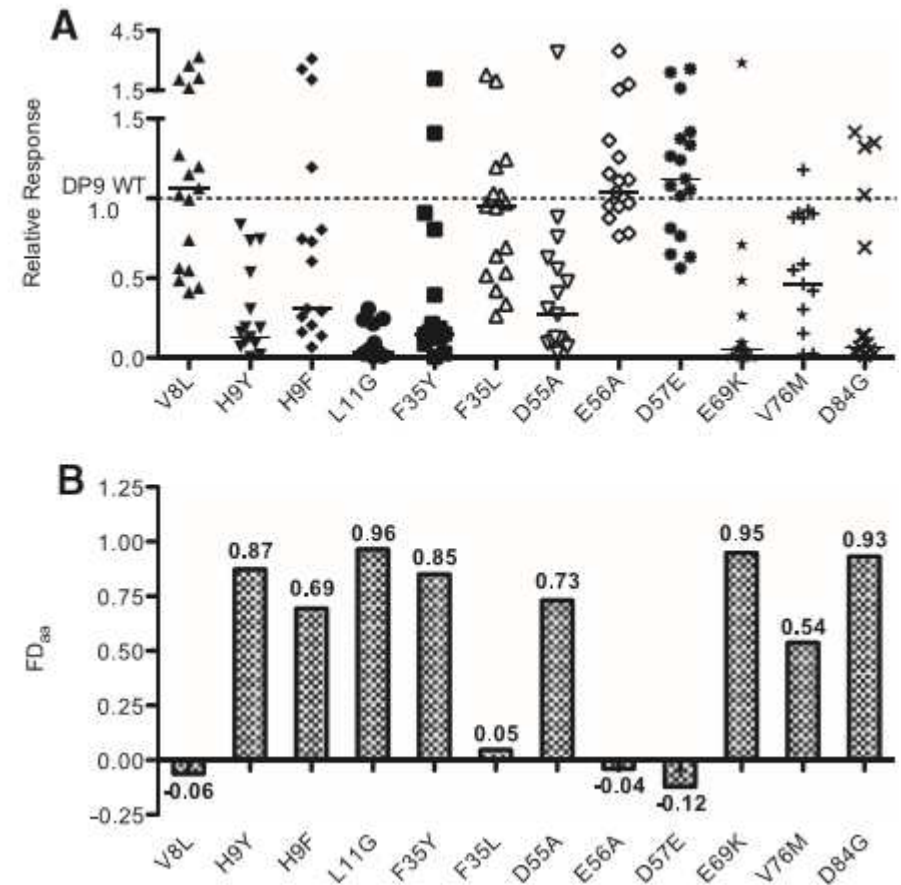
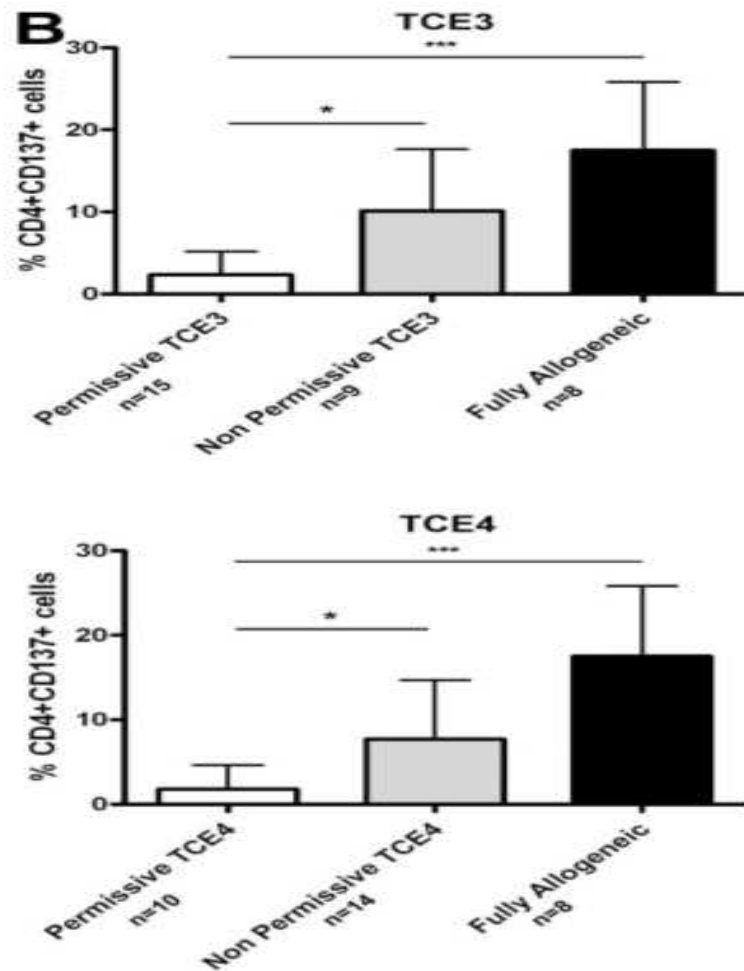
		RECIPIENT DPB1 GROUP								
DONOR DPB1 GROUP	TCE3	→	1/1	1/2	1/3		2/2	2/3		
	↓	TCE4	1/1	1/2	1/3	1/4	2/2	2/3	2/4	3/3
	1/1	1/1	Permissive				Non-permissive			
	1/2	1/2								
	1/3	1/3								
	1/4	1/4								
	2/2	2/2	Non-permissive GvH				Permissive		Non-permissive	
	2/3	2/3								
	2/4	2/4								
	3/3	3/3								
3/4	3/4									
4/4	4/4									
						Permissive		Non-permissive		

Permissive in TCE3 and TCE4

Permissive in TCE3, but not in TCE4

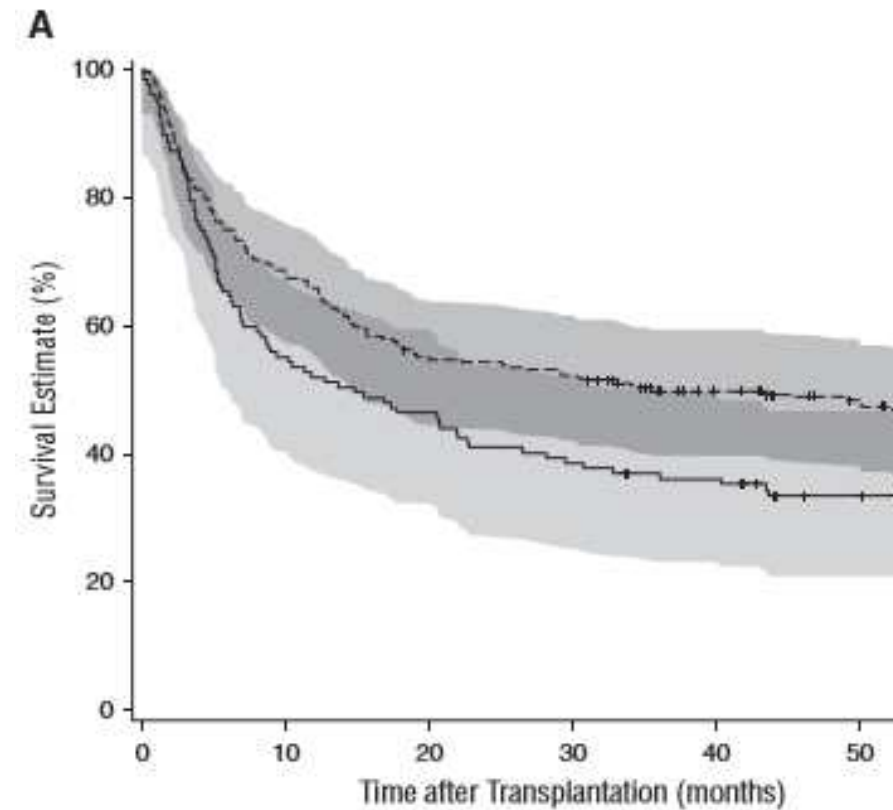
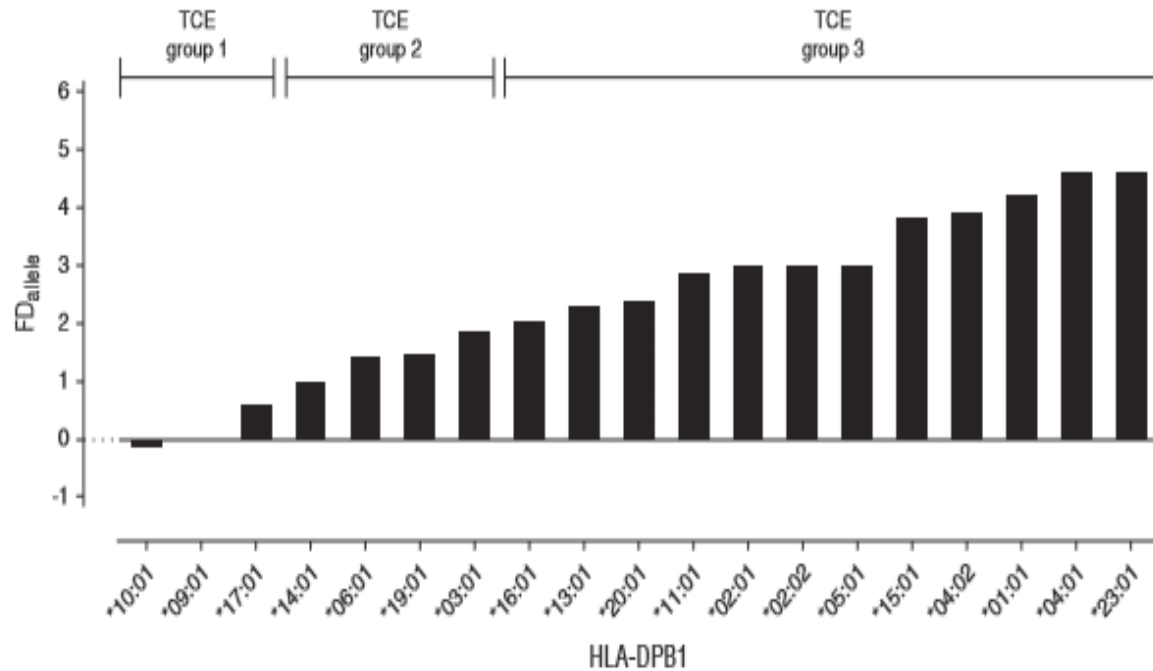
Non-permissive

# HLA-DP alloreactivity in vitro (1)



Sizzano, Blood 2010; Crivello, B

# HLA-DP alloreactivity in vitro (2): FD





# HLA-DP alloreactivity in vitro (3): comparing models

**P152**

## **COMPARATIVE EVALUATION OF HLA-DPB1 MISMATCH MODELS IN HCT IDENTIFIES ASSOCIATION OF TCE4 PERMISSIVENESS WITH SURVIVAL**

8/8 UD-HCT success. In this cohort, TCE4 was superior in  
predicting survival, while aGvHD was predicted by the  
expression model.

## Take-home messages (2)

- Best survival is observed after a 8/8-matched UD (HLA-A, B, C, DRB)
- HLA-DP permissiveness models (TCE3, TCE4, FD, expression model) explain the role of this locus in HSCT

# HLA expression and unrelated HSCT



Contents lists available at [ScienceDirect](#)

Human Immunology

journal homepage: [www.elsevier.com/locate/humimm](http://www.elsevier.com/locate/humimm)



## The MHC in the era of next-generation sequencing: Implications for bridging structure with function

Effie W. Petersdorf<sup>a,\*</sup>, Colm O'hUigin<sup>b</sup>

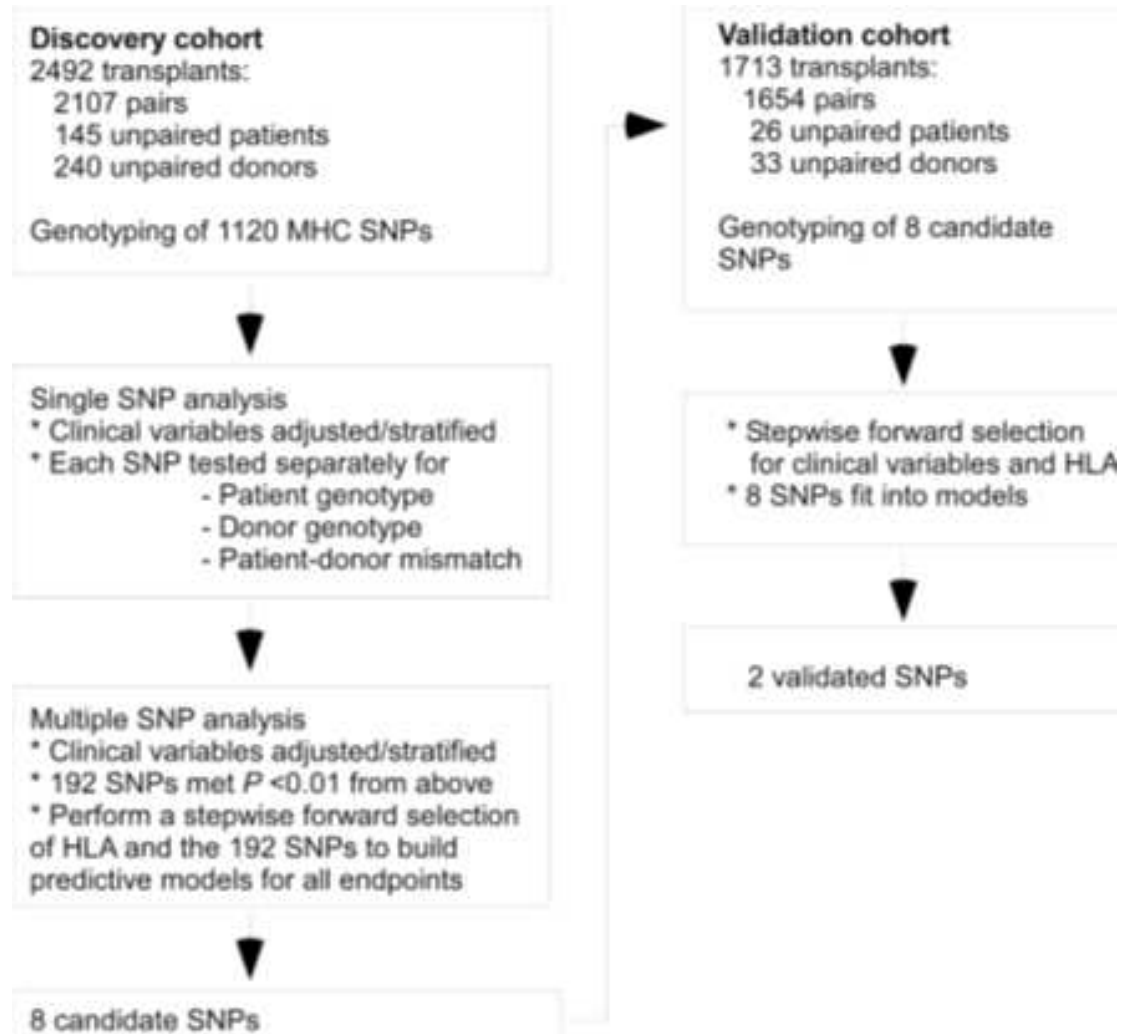
<sup>a</sup> University of Washington, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue North, D4-115, Seattle, WA 98109, United States

<sup>b</sup> Frederick National Laboratory for Cancer Research sponsored by the National Cancer Institute, Microbiome and Genetics Core, Building 37, Room 4140B, Bethesda, MD 20852, United States

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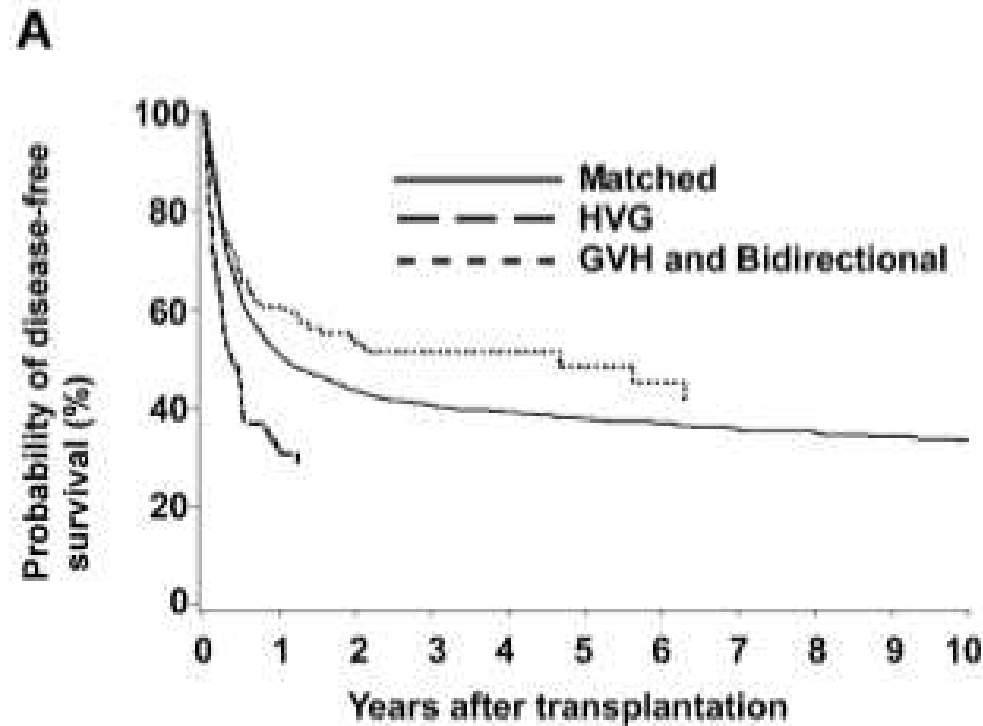
Locus	Sequence Feature/Method	Expression	Clinical Relevance	Citation
Haplotypes	Tiling and splice junction microarray	A1-B8-Cw7-DR2, A3-B7-Cw7-DR15, A26-B18-Cw5-DR3-DQ2 haplotype-specific expression	NE	[7]
	eQTLs	A2-B46-DR9, A33-B58-DR3, A1-B8-DR3 regulatory effects proximate to HLA-A, C, DRB and C4A	NE	[8]
	MicroRNAs	MicroRNAs located within non-coding regions describe local and extended haplotypes	NE	[9]
Class I	Class I regulatory complex ("CRC")	KB1 enhancer conserved in HLA-A and B sequences	NE	[10]
		Locus-specific KB2 divergence	NE	
HLA-A	Methylation	Lineage-specific gradient of expression	NE	[11]
	DNA sequencing of promoter	Increased methylation associated with reduced HLA-A expression	NE	
HLA-A2, B8; C-terminus of $\alpha 2$ and $\alpha 3$ domains		Residue 180 and 239 associated with HLA-A and B expression	NE	[12]
		HLA-A2 Gln <sup>180</sup> and Gly <sup>239</sup> associated with higher cell surface expression compared to HLA-B Glu <sup>180</sup> and Arg <sup>239</sup>	NE	
HLA-A11 promoter		Positions -271 to -263 and -242 to -234 involved in binding zinc finger protein ZFX and impact transcriptional activity of promoter.	NE	[13]
HLA-B	Bw4-80I	KIR3DL1/Bw4 combinations have array of binding strengths and correlate with NK cytotoxicity	NE	[14]
HLA-C	Bw4/Bw6 cell surface expression	Lineage and cell specific expression related to stability and turnover	NE	[15]
	Cell surface expression	Continuous allotype-specific HLA-C expression	HIV Viral load	[16]
	Inference between HLA-C allele and MFI from <del>HLA-C</del>	HLA-C patient/donor mismatches according to level of patient's mismatched allotype	GVHD higher when mismatching against high-expression patient HLA-C-	[17]
	Phylogeny	Ancient escape from miR-148a repression of HLA-C lineages	NE	[18]
	cDNA transfection of HLA-A,B,C	HLA-C mRNA expression lower than HLA-B	NE	[19]
	Rs9264942	Variant upstream of HLA-C; proxy for miR-148a	HIV viral load	[2,20]
	("35 SNP")			
		mRNA levels and cell surface expression for C206 positive samples from analysis cohort	No correlation of HLA-C206 with 35 SNP in analysis	[21]
		Unrelated donor transplants	No correlation of 35 SNP or HLA-C expression levels with unrelated HCT outcomes	[22]
				[23]
		No correlation between 35 SNP and HLA-C mRNA expression	NE	[24]
		HLA-C expression correlated with HLA-A-B-C-DR and B-C haplotype	NE	
	miR-148a of HLA-C 3' UTR	Marked by rs926942	HIV?	[24]
		Downregulation of HLA-C7 and other alleles with intact microRNA; nucleotide deletion within miR-148a binding site leads to higher HLA-C expression		
		Intact miR-148 impacts HIV and Crohn's risks	HIV, Crohn's Disease	[25]
	Rs239541	A allele higher affinity than G allele	NE	[26]
	Within Oct1 binding site in HLA-C promoter	G associated with lower promoter activity	NE	
	Nucleotide sequence of HLA-B, A, C promoter	HLA-B promoter most diverse, but no correlation with mRNA expression	NE	[27]
	Alternative transcript arising from NK-specific	No clear correlation between promoter phylogeny and lineage expression	NE	
		NK intrinsic regulation of HLA-C expression	NE	[28]
Class II	HLA-C*03:03:04 mismatch	Low-expression C*03:03:03:04 mismatch is well-tolerated in HCT	HLA-C mismatched unrelated HCT	[29]
	Low-expression HLA-DRB3, DRB4, DRB5	Additive effect of patient-donor mismatching for low-expression HLA-DRB3, DRB4, DRB5 on transplant outcomes	Unrelated donor HCT	[30]
	HLA-DR-XL9-DQ haplotypes	IRF4 and CTCF binding site variants modified HLA-DRB1/DQA1/DQB1 transcription and HLA-DR/DQ surface expression	SLE	[31]
HLA-DRA	RFX, X2BP, NF-Y, CIITA, Oct-2, Bob-1	CIITA is a molecular switch for class II expression and IFN- $\gamma$ regulation	NE	[32]
	Histone modifications and binding to RFX and CIITA	Network of histone modifying proteins together with multi-subunit complex impact class II expression	NE	[33]
HLA-DRB	Promoter sequence	Two promoter point mutations associated with DR8 and DR10 did not affect promoter activity luciferase assay	NE	[34]
	Vitamin D response element in DRB1 promoter on DRB1*15 haplotypes	DR15 expression modulated by stimulation of response element by Vitamin D	Multiple Sclerosis	[35]
HLA-DQ	Histone quantitative trait loci	Enrichment of loci on autoimmune disease haplotypes and influence gene expression	Systemic Lupus Erythematosus	[36]
	HLA-DQB1 transcripts and promoter footprinting qPCR	Differential expression of HLA-DQB1 alleles	NE	[37]
		Transcription of HLA-DQ associated with polymorphism in promoter of DQB1*03:01 and 03:02	NE	[38]
		Spacing of W and X1 elements in the promoter	NE	
		Differential expression of DQB1*03:01, 05:01, 06:02	NE	[39]
HLA-DP	Rs9277534 in 3' UTR	Sequence analysis of rs9277535 and rs927534	Hepatitis B persistence with rs9277534G associated with high transcription levels and cell surface expression of HLA-DP	[40]
		Cell surface expression	Increased GVHD with mismatching against high-expression HLA-DPB1 mismatch in the transplant recipient	[41]
		Validation of rs9277534A with lower mRNA expression than rs9277534G		
	Rs9277534A and G phylogenetic clades	Patient/donor HLA-DPB1 mismatching for alleles within rs9277534A/G clades	GVHD increased with mismatching for rs9277534G high-expression alleles	[42]
	Rs9277534 and donor-specific antibodies	Rs9277534G high-expression HLA-DP associated with stronger B cell flow crossmatch	Solid organ transplantation	[43]

# HLA expression and unrelated HSCT: SNPs (1

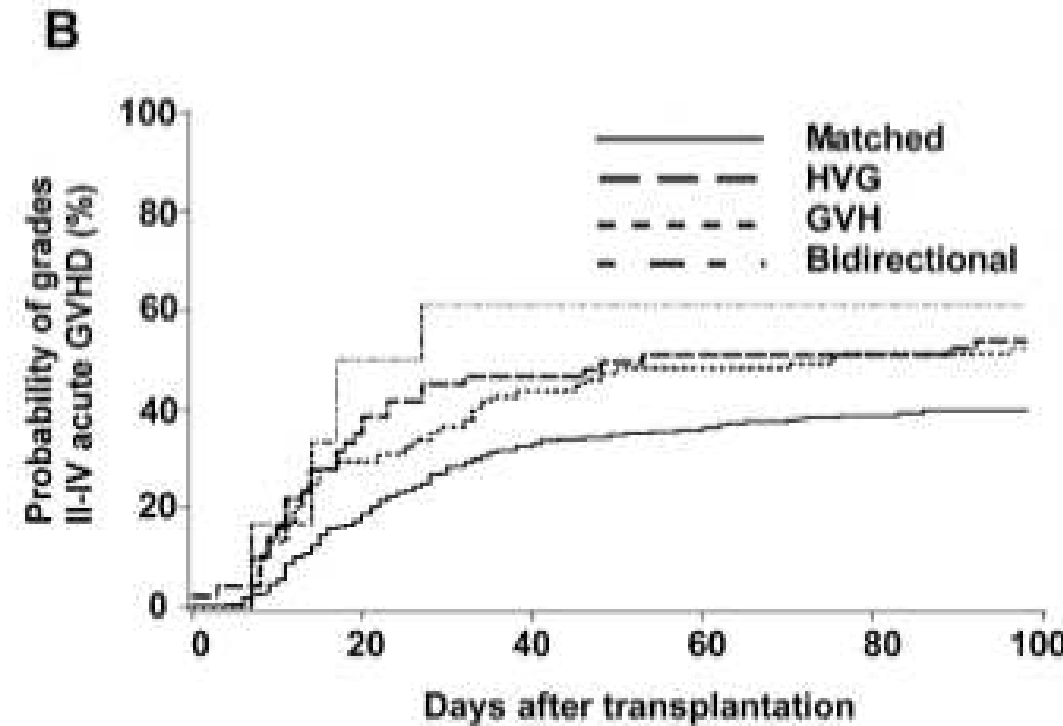


- First study investigating the role of SNPs affecting HLA expression
- A total of 1,120 MHC-region SNPs were tested on 4,205 HSCTs
- rs887464, rs2281389

# HLA expression and unrelated HSCT: SNPs (2)



*rs887464*



*rs2281389*

# HLA expression and unrelated HSCT: HLA-C

## Regular Article

### TRANSPLANTATION

## HLA-C expression level affects GVHD and mortality after HCT from HLA-C-mismatched unrelated donors

Effie W. Petersdorf,<sup>1,2</sup> Theodore A. Gerhard Ehninger,<sup>6</sup> Torstein Egeland,<sup>3</sup> Katharine Hsu,<sup>12</sup> Pavel Jindra,<sup>13</sup> Alvin S. Basch,<sup>14</sup> Stephen R. Spellman,<sup>10</sup> Jean-Marie Hama,<sup>11</sup> and Mary Carrington,<sup>21,22</sup> for the International Bone Marrow Transplant Registry

### Key Points

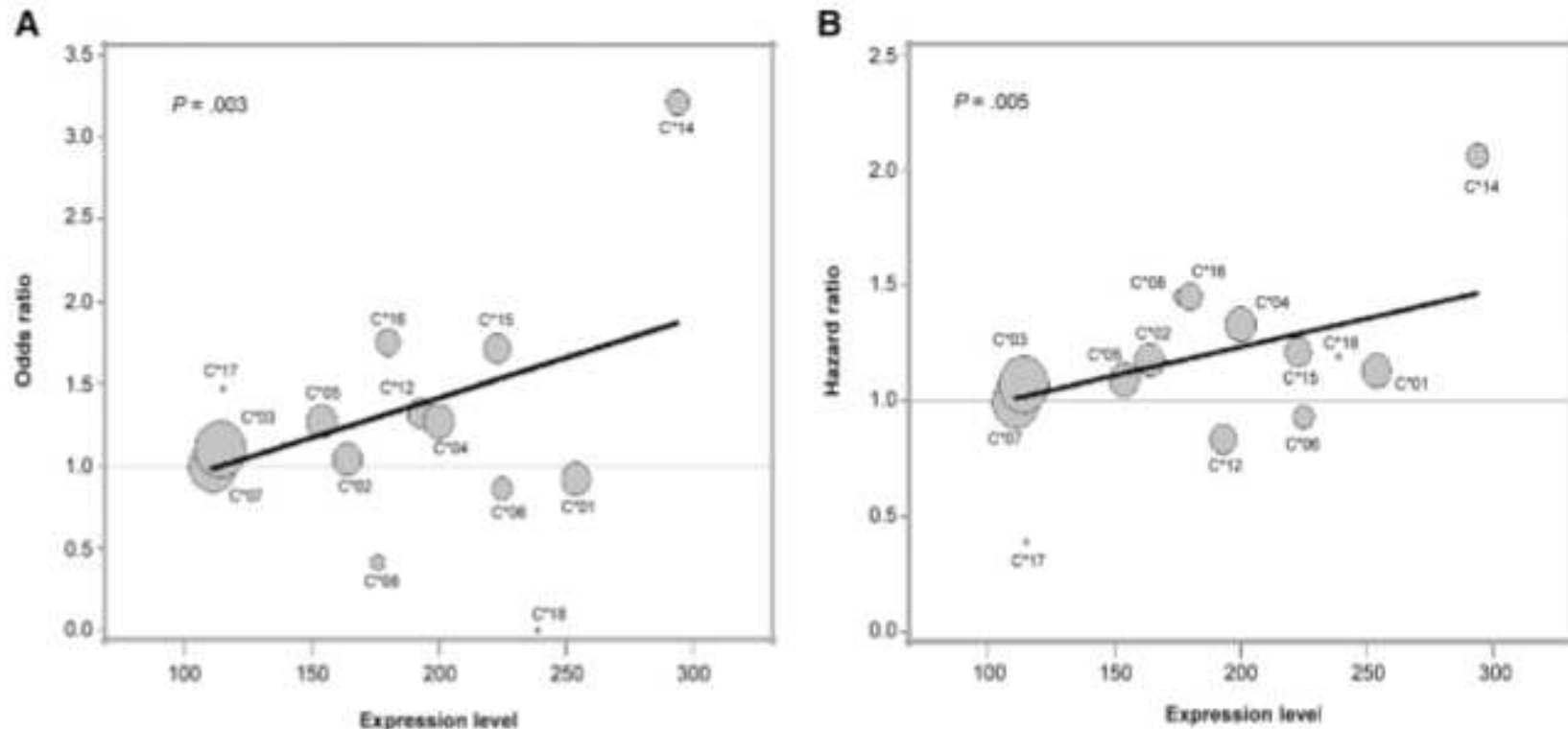
- The expression level of patient HLA-C allotypes affects GVHD and mortality after HCT from HLA-C-mismatched unrelated donors.
- Transplant outcome can be improved by avoiding high-risk HLA-C-mismatched donors when no matched stem cell source is available.

## HLA-C expression level affects GVHD and mortality after HCT from HLA-C-mismatched unrelated donors

Cesbron,<sup>4</sup> Ermette Du Toit,<sup>5</sup> Haagenson,<sup>10</sup> Mary M. Horowitz,<sup>11</sup> J. Hama,<sup>16</sup> Marlis L. Schroeder,<sup>17</sup> O'Huigin,<sup>21</sup> Richard Apps,<sup>21,22</sup> and the International Bone Marrow Transplant Registry



# HLA expression and unrelated HSCT: HLA-C



**Table 2. Level of HLA-C expression influences acute GVHD, nonrelapse mortality, and overall mortality, but not relapse**

Nonshared HLA-C allotype*	Acute GVHD† (N = 453/1861)		Nonrelapse mortality† (N = 709/1727)		Overall mortality† (N = 1246/1975)		Relapse† (N = 501/1727)	
	OR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Patient's mismatch	1.34 (1.10-1.62)	.003	1.22 (1.06-1.39)	.005	1.15 (1.03-1.27)	.009	1.03 (0.86-1.22)	.76
Donor's mismatch	1.07 (0.88-1.30)	.49	1.15 (1.01-1.31)	.04	1.14 (1.03-1.26)	.01	0.97 (0.82-1.16)	.74
Sum of mismatched allotypes	1.16 (1.03-1.32)	.02	1.15 (1.06-1.25)	.002	1.12 (1.05-1.19)	.001	1.00 (0.89-1.12)	.96

# HLA expression and unrelated HSCT: HLA-C

**Table 3. Distribution of HLA-C allotypes according to 3 models of HLA-C mismatching**

Patient's nonshared allotype*	MFI	HLA-C mismatch model for the nonshared patient allotype N (%)					
		Allele vs antigen† (N = 1971)		Residue 116‡ (N = 1955)		Residues 77/80§ (N = 1955)	
		Allele (N = 389)	Antigen (N = 1582)	Matched (N = 847)	Mismatched (N = 1108)	Matched (N = 955)	Mismatched (N = 1000)
C*07	111	104 (27)¶	288 (18)	242 (29)	147 (13)	210 (22)	179 (18)
C*03	114	238 (61)¶	187 (12)	268 (32)	155 (14)	321 (34)	100 (10)
C*17	115	0	3 (<1)	1 (<1)	2 (<1)	1 (<1)	2 (<1)
C*05	154	5 (1)	147 (9)	50 (6)	100 (9)	43 (5)	107 (11)
C*02	164	1 (<1)	146 (9)	31 (4)	114 (10)	43 (5)	102 (10)
C*08	176	5 (1)	28 (2)	10 (1)	22 (2)	15 (2)	17 (2)
C*16	180	10 (3)	91 (6)	43 (5)	57 (5)	29 (3)	71 (7)
C*12	193	4 (1)	118 (7)	84 (10)	36 (3)	86 (9)	34 (3)
C*04	200	5 (1)	161 (10)	10 (1)	155 (14)	39 (4)	126 (12)
C*15	223	11 (3)	111 (7)	4 (<1)	118 (11)	51 (5)	71 (7)
C*06	225	5 (<1)	63 (4)	36 (4)	32 (3)	25 (3)	43 (4)
C*18	239	0	2 (<1)	1 (<1)	1 (<1)	1 (<1)	1 (<1)
C*01	254	0	152 (10)	40 (5)	112 (10)	68 (7)	84 (8)
C*14	294	1 (<1)	85 (5)	27 (3)	56 (5)	23 (2)	59 (6)
Mean MFI of the patient's mismatched HLA-C allotype		123.2	176.7	148.6	179.8	154.2	177.1
		P < .0001		P < .0001		P < .0001	

# HLA expression and unrelated HSCT: HLA-C

## Regular Article

### TRANSPLANTATION

#### Identification of a [unclear] cell transplantation

Marcelo A. Fernandez-Viña,<sup>1</sup> Ta  
Minoo Battiwalla,<sup>7</sup> Lee-Ann Baxt  
Machteld Oudshoorn,<sup>12</sup> Steven C  
Ann Woolfrey,<sup>3</sup> John Umejiego,<sup>4</sup>

## Key Points

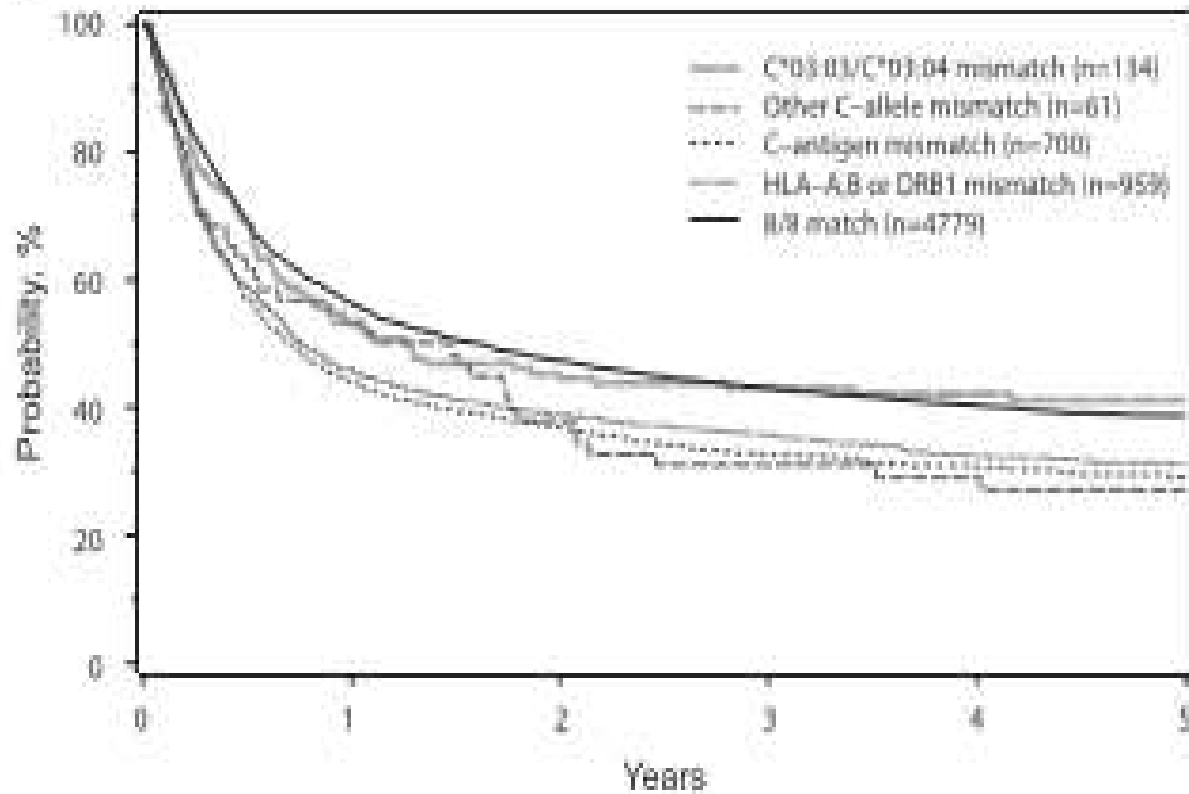
- Mismatches in alleles C\*03:03/C\*03:04 were most frequent (68.7%) among the transplants with a single allele level mismatch in HLA-C.
- The 7/8 C\*03:03/C\*03:04 mismatch group was not significantly different from the 8/8 HLA matched transplants in any transplant outcome.

#### hematopoietic stem

oud Aljurf,<sup>5</sup> Medhat Askar,<sup>6</sup>  
a Marino,<sup>11</sup>  
ia Turner,<sup>15</sup> Edmund K. Waller,<sup>16</sup>

# HLA expression and unrelated HSCT: HLA-C

A



- Similar OS between 8/8-matched and 7/8 with MM only in C\*03:03/C\*03:04
- These alleles may have equivalent peptide presentation properties (differ by a single AA at residue 91 which is not in contact with any peptide or TCR)
- Their serologic epitopes have a low immunogenicity score

# HLA expression and unrelated HSCT: DQ, DRB3/4/5, DP (low expression loci)

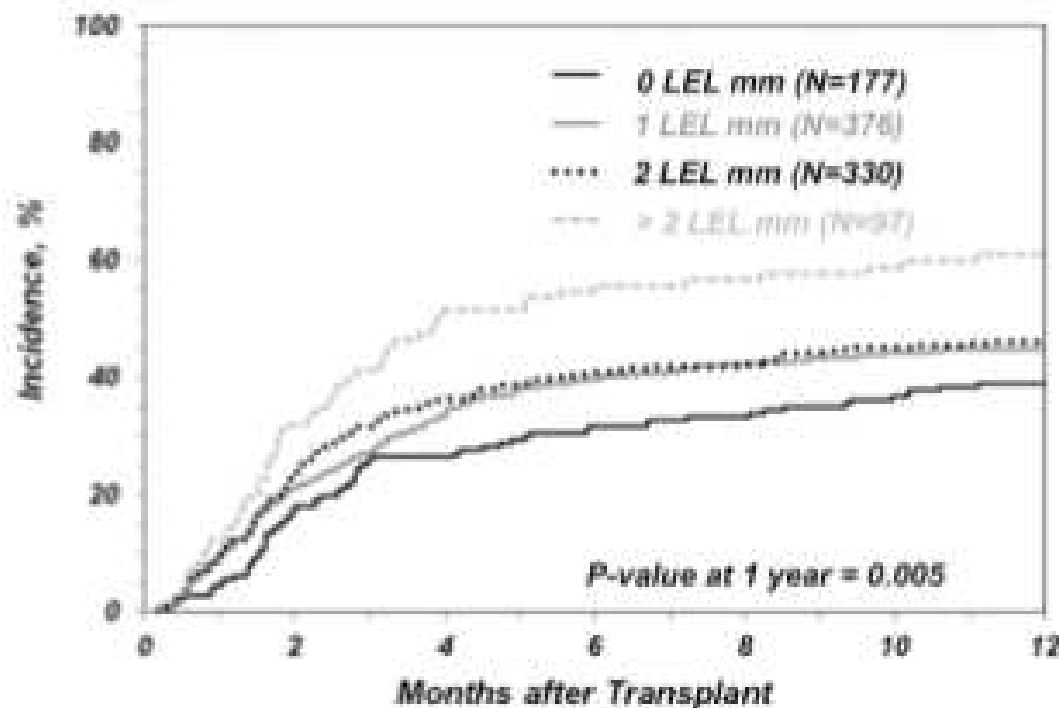
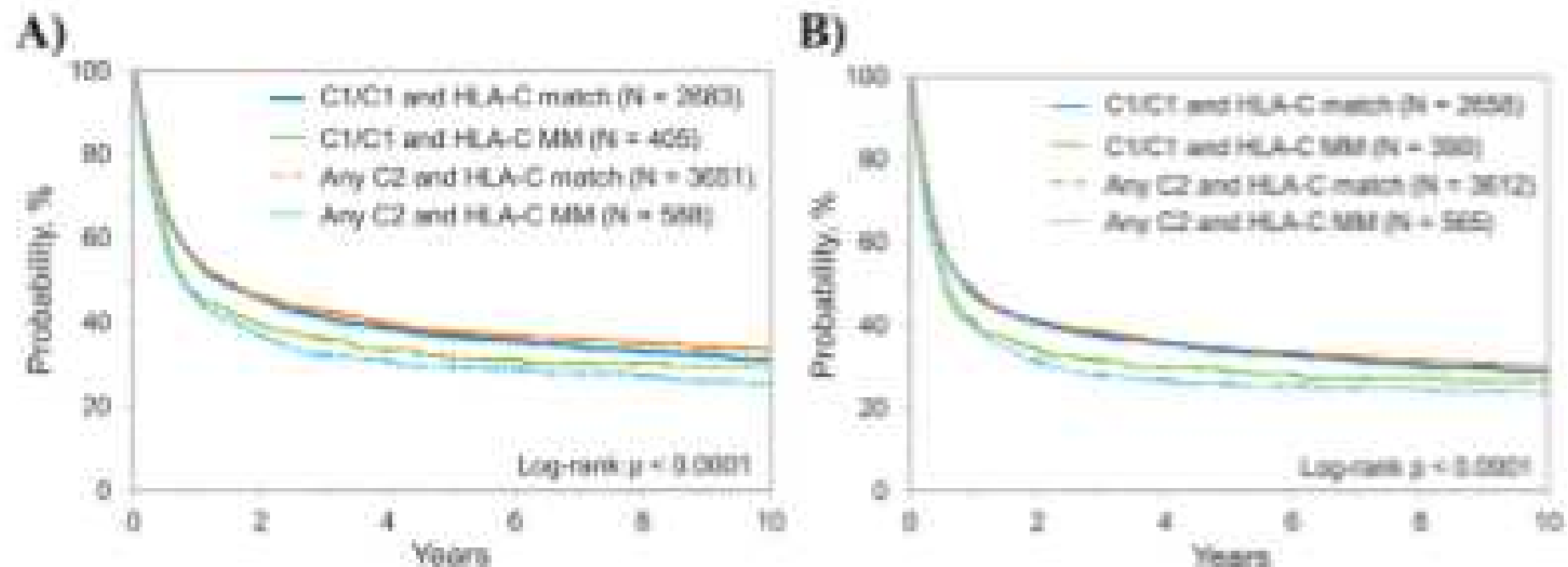


Figure 2. Incidence of TRM as a function of degree of mismatching at HLA-DRB3/4/5, DQ and DP (LEL) loci in transplants matched in 7/8 alleles of HLA HLA-A, -B, -C, and -DRB1 loci.

- In 8/8-matched HSCTs, any M at LEL did not impair outcome whereas they did in 7/8 HSC
- MMs at DQ were found in 8. of 8/8 HSCTs (no impact on outcome)

## Recipient HLA-C Haplotypes and microRNA 148a/b Binding Sites Have No Impact on Allogeneic Hematopoietic Cell Transplantation Outcomes



# HLA expression and unrelated HSCT: HLA-DP (



## High HLA-DP Expression and Graft-versus-Host Disease

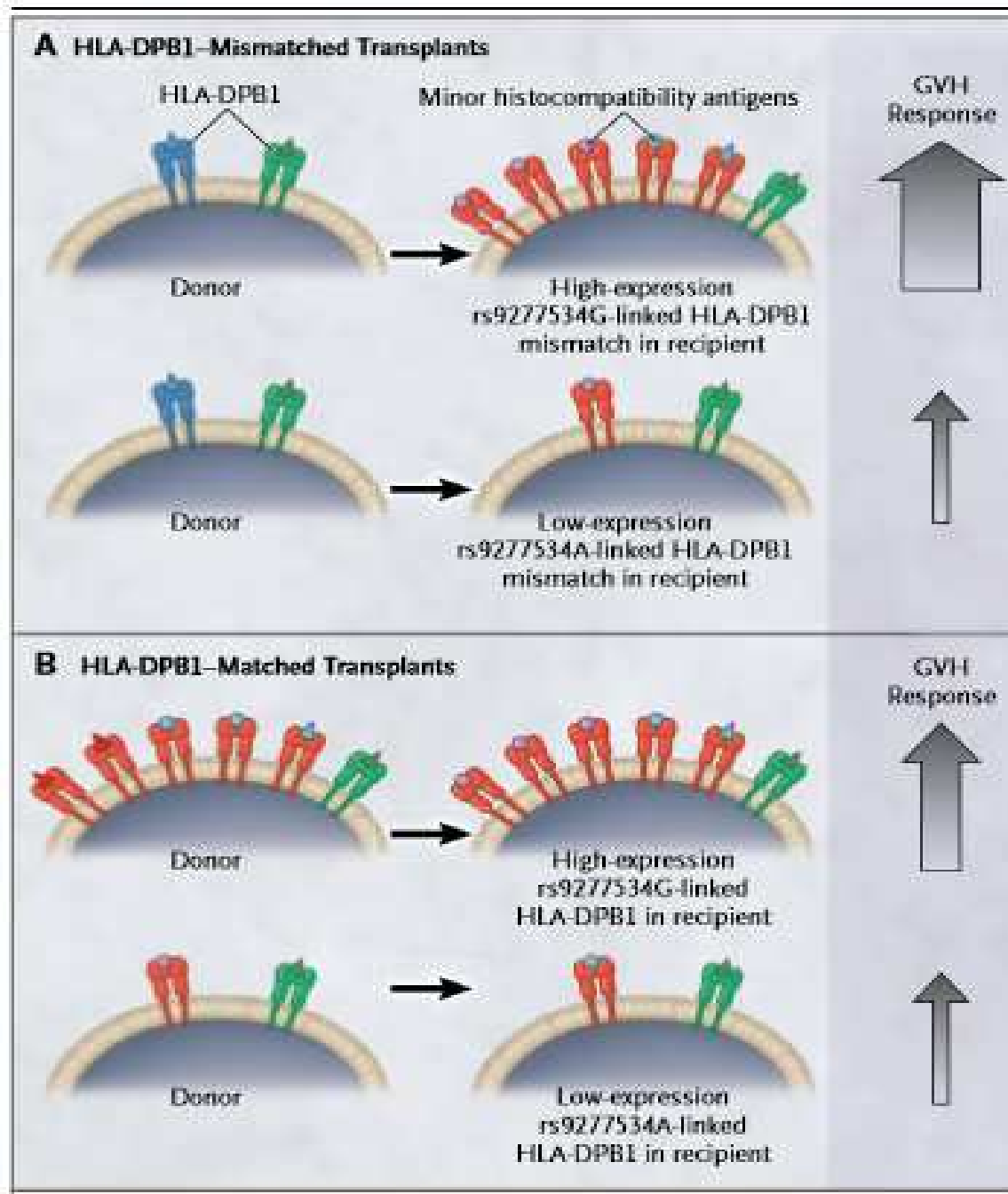
Effie W. Petersdorf, M.D., Mari Malkki, Ph.D., Colm O'hUigin, Ph.D., Mary Carrington, Ph.D., Ted Gooley, Ph.D.,  
Michael D. Haagenson, M.S., Mary M. Horowitz, M.D., Stephen R. Spellman, M.B.S., Tao Wang, Ph.D.,  
and Philip Stevenson, M.S.

### CONCLUSIONS

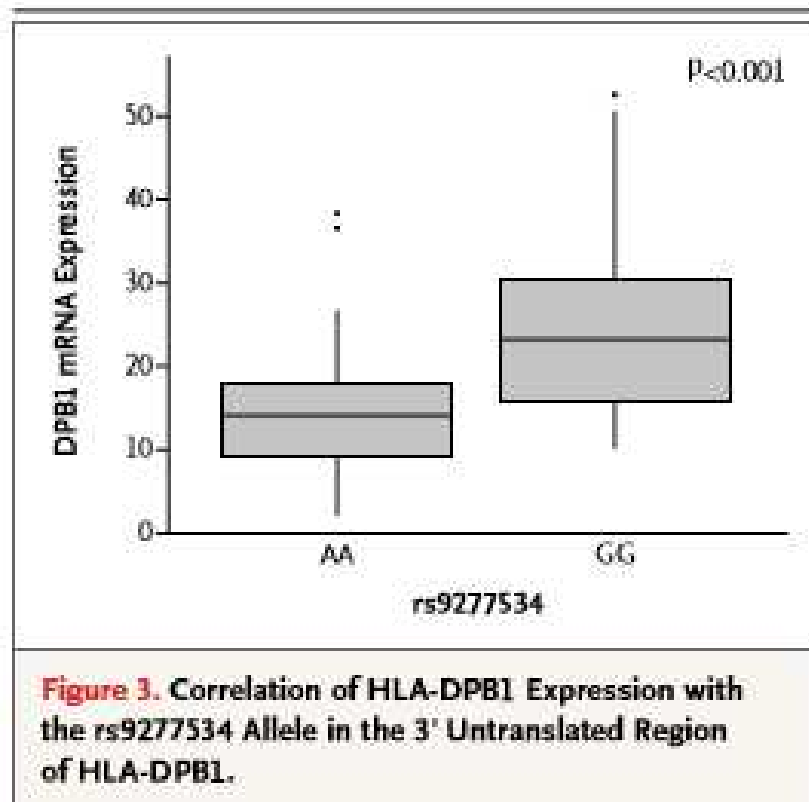
The risk of GVHD associated with HLA-DPB1 mismatching was influenced by the HLA-DPB1 rs9277534 expression marker. Among recipients of HLA-DPB1–mismatched transplants from donors with the low-expression allele, recipients with the high expression allele had a high risk of GVHD. (Funded by the National Institutes of Health and others.)

Petersdorf

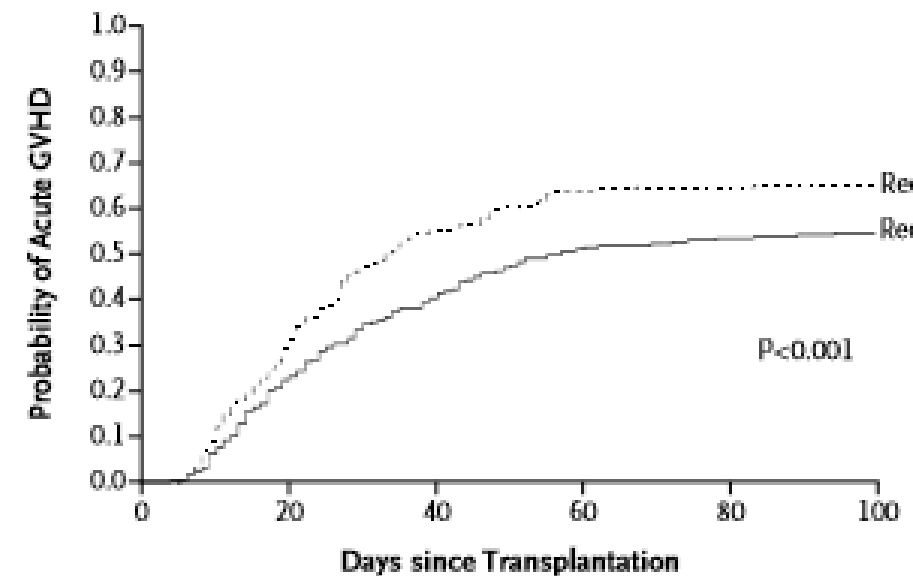




# HLA expression and unrelated HSCT: HLA-DP (



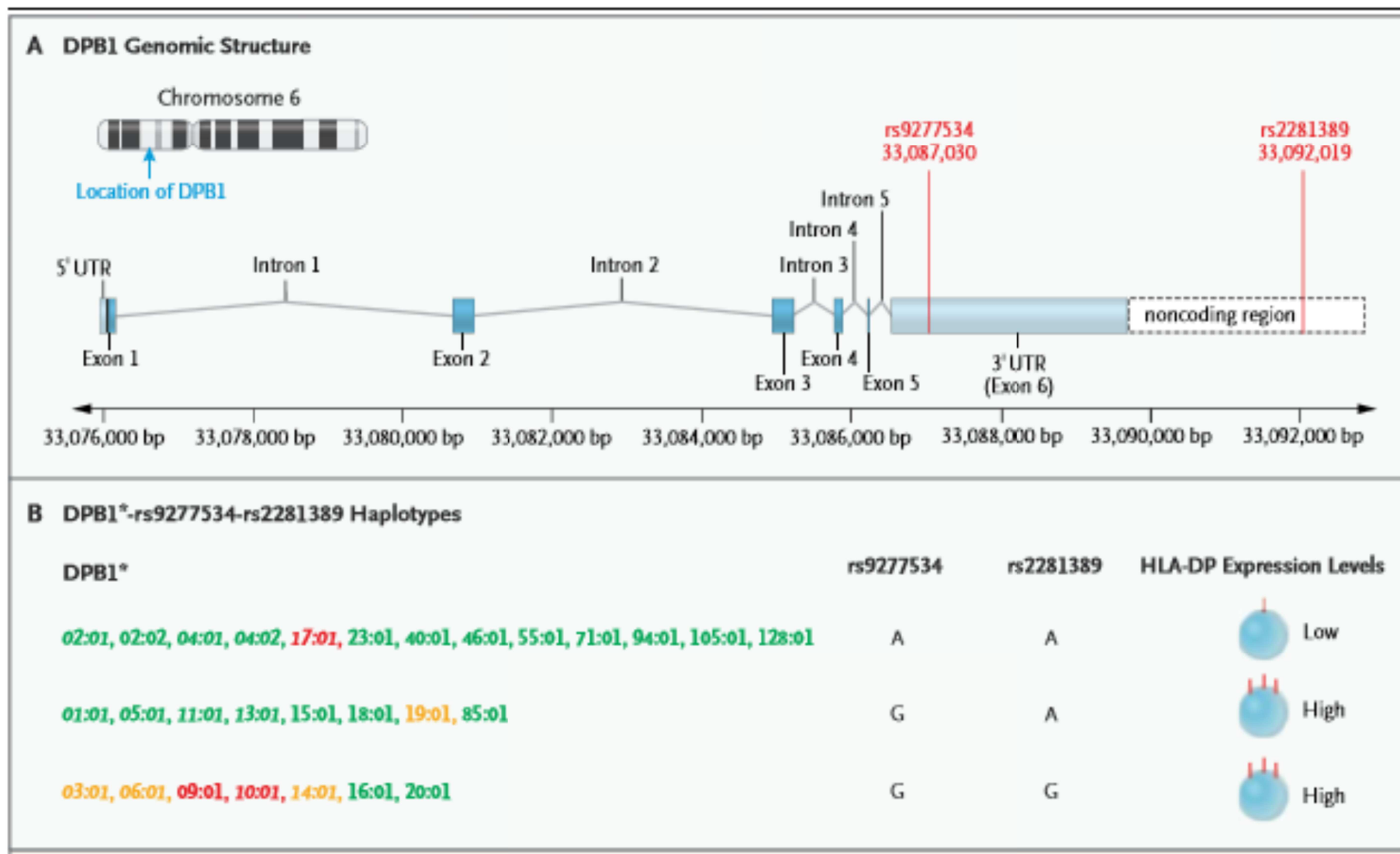
**A** Donor rs9277534A



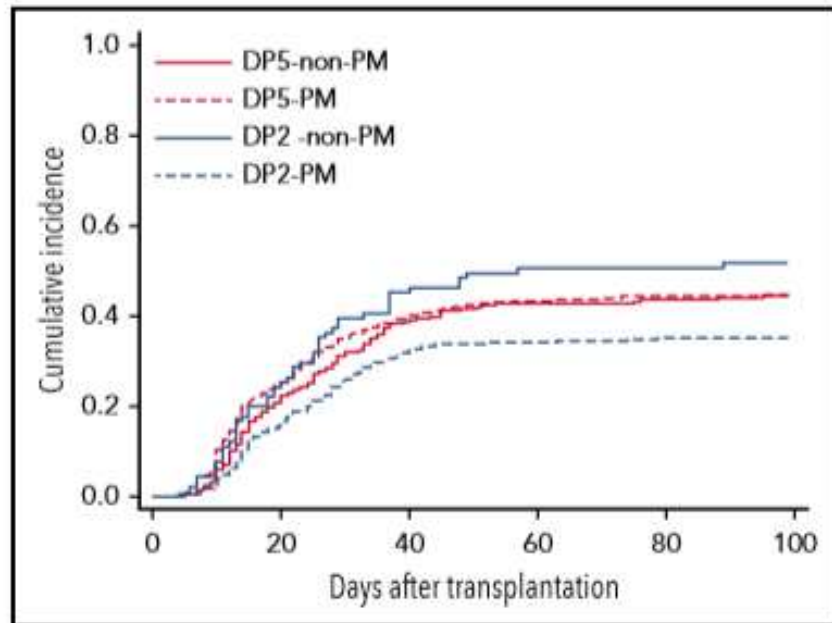
**No. of Recipients at Risk**

Recipient rs9277534A	413	335	267	230	219	211
Recipient rs9277534G	481	343	245	201	193	184

# SNPs and TCE model for HLA-DP



# HLA expression and unrelated HSCT: HLA-DP (



**Table 3. Effect of patient mismatch HLA-DP group on transplant outcome**

Clinical outcome	Patient mismatch HLA-DP group	HR	95% CI	P	
Acute GVHD 2-4					
All patients	DP2 DP5	1.00 1.28	1.07-1.52	.005	
Donor HLA-DP2 mismatch	DP2 DP5	1.00 1.32	1.01-1.72	.040	
Donor HLA-DP5 mismatch	DP2 DP5	1.00 1.35	1.06-1.72	.013	

**Table 2. HLA DPB1 allele information used for this study**

Allele name at field 2 level	Allele frequency (n = 2966 alleles),* %	Allele name at field 4 level	Accession no.	Predicted Immunogenicity†	rs9277534 allele	Grouping for DP5‡
DPB1*05:01	38.40	DPB1*05:01:01:01	LC257912	Group 3	G	DP5
DPB1*02:01	24.11	DPB1*02:01:02:01	LC257894	Group 3	A	DP2
DPB1*09:01	9.95	DPB1*09:01:01	LC257921	Group 1	G	DP5
DPB1*04:02	9.78	DPB1*04:02:01:02	LC257910	Group 3	A	DP2
DPB1*04:01	5.06	DPB1*04:01:01:01	LC257908	Group 3	A	DP2
DPB1*03:01	3.98	DPB1*03:01:01	LC257905	Group 2	G	DP5
DPB1*02:02	3.41	DPB1*02:02:01:05	LC257902	Group 3	A	DP2
DPB1*13:01	1.96	DPB1*13:01:01	LC257922	Group 3	G	DP5
DPB1*14:01	1.48	DPB1*14:01:01	LC257924	Group 2	G	DP5
DPB1*19:01	0.74	DPB1*19:01	LC257926	Group 3	G	DP5
DPB1*06:01	0.57	DPB1*06:01:01	LC257920	Group 3	G	DP5
DPB1*17:01	0.14	DPB1*17:01	LC257925	Group 1	A	DP2
DPB1*36:01	0.14	DPB1*36:01	LC257929	Unknown	G	DP5
DPB1*41:01	0.10	DPB1*41:01:01	LC257931	Group 3	A	DP2
DPB1*38:01	0.07	DPB1*38:01	LC257930	Group 3	G	DP5
DPB1*25:01	0.03	DPB1*25:01	LC257928	Unknown	G	DP5
DPB1*47:01	0.03	DPB1*47:01	LC257932	Group 3	A	DP2
DPB1*21:01	Unknown	DPB1*21:01	LC257927	Unknown	G	DP5
DPB1*48:01	Unknown	DPB1*48:01	LC257933	Group3	A	DP2

Morishima,

# HLA class II upregulation and GvHD

## Key Points

- GVHD after HLA-DPB1-mismatched CD4<sup>+</sup> DLI after TCD-alloSCT is mediated by allo-reactive HLA-DPB1-directed CD4<sup>+</sup> T cells.
- Viral infections after TCD-alloSCT can induce HLA class II on nonhematopoietic tissues, making them targets for CD4<sup>+</sup> T cells in GVHD.

«Ongoing viral infections (CMV) resulting in upregulated HLA class II expression on nonhematopoietic tissues increase the likelihood of GvHD development»

«...these donor-derived anti-CMV T-cell responses may have further stimulated the inflammatory environment and HLA class II expression....thereby enhancing GvHD»

# HLA expression and unrelated HSCT: EFI 201

**O6**

**HLA-DM-MEDIATED PEPTIDE EDITING  
IMPACTS T CELL RECEPTOR DIVERSITY  
AGAINST PERMISSIVE HLA-DPB1  
MISMATCHES**

**O45**

**PEPTIDE REPERTOIRE OR EXPRESSION  
LEVELS: WHAT IS THE DRIVING FORCE FOR T  
CELL ALLOREACTIVITY TO HLA-DP?**

**O36**

**HLA-DM-MEDIATED PEPTIDE EDITING  
REGULATES SELF-HLA-DP RESTRICTED  
CELL ALLOREACTIVITY TO MINOR  
HISTOCOMPATIBILITY ANTIGENS**

**O47**

**CYTOKINE INDUCED UPREGULATION OF  
ALLELIC HLA CLASS I EXPRESSION  
ANALYZED BY RNA SEQUENCING AND  
EFFECT ON ALLOGENIC T CELL RESPONSE**



# Take-home messages (3)

- The advent of NGS allows the investigation of non-coding regions and their impact on the level of expression of HLA allotypes
- Many studies now demonstrate the impact of HLA expression (HLA-A, HLA-B, HLA-C, HLA-DP) on GvHD, non-relapse mortality and survival (not relapse) after unrelated HSCT

# HLA loss as a mechanism of immune escape (

*The NEW ENGLAND JOURNAL of MEDICINE*

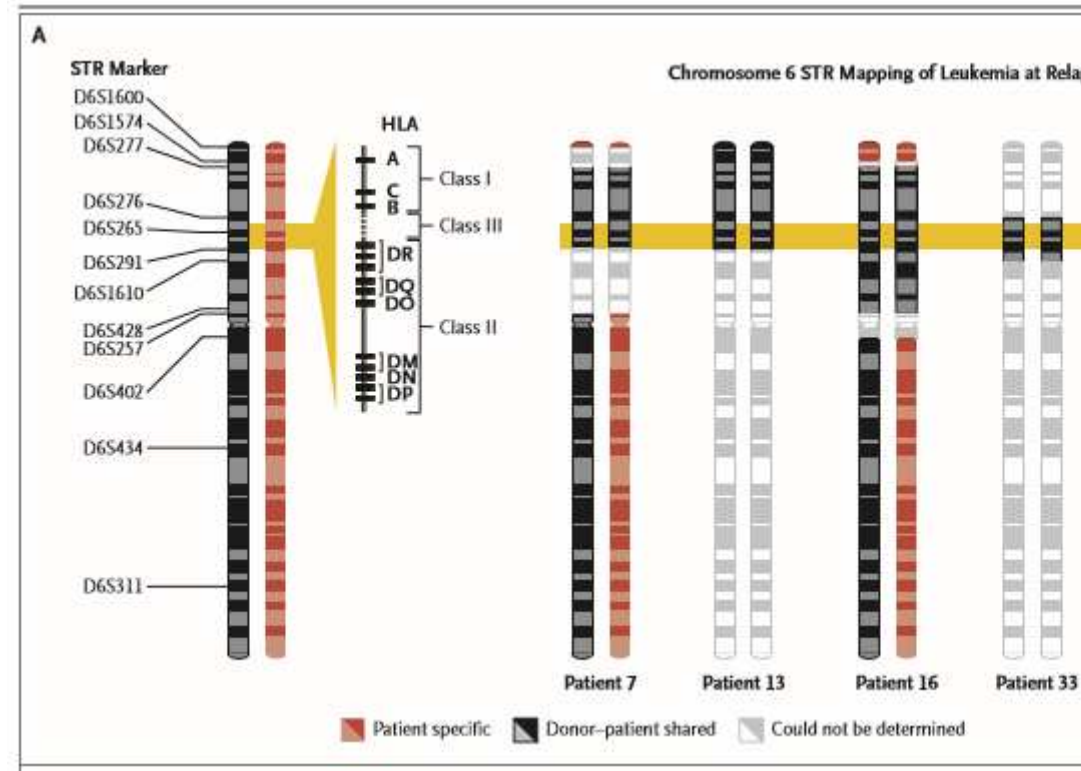
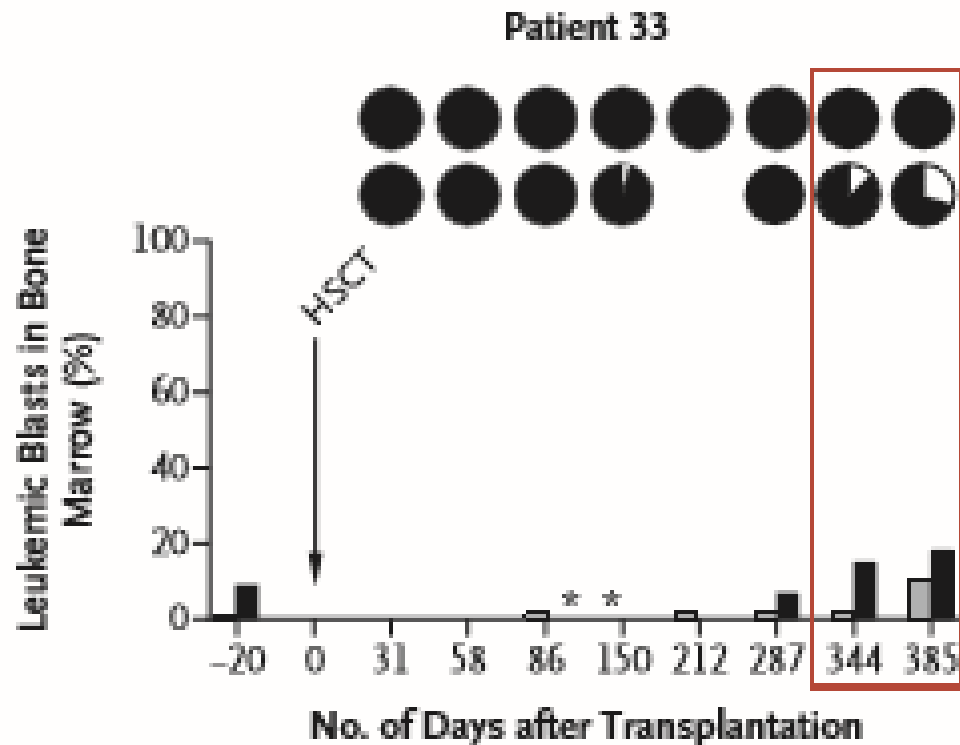
ORIGINAL ARTICLE

## Loss of Mismatched HLA in Leukemia after Stem-Cell Transplantation

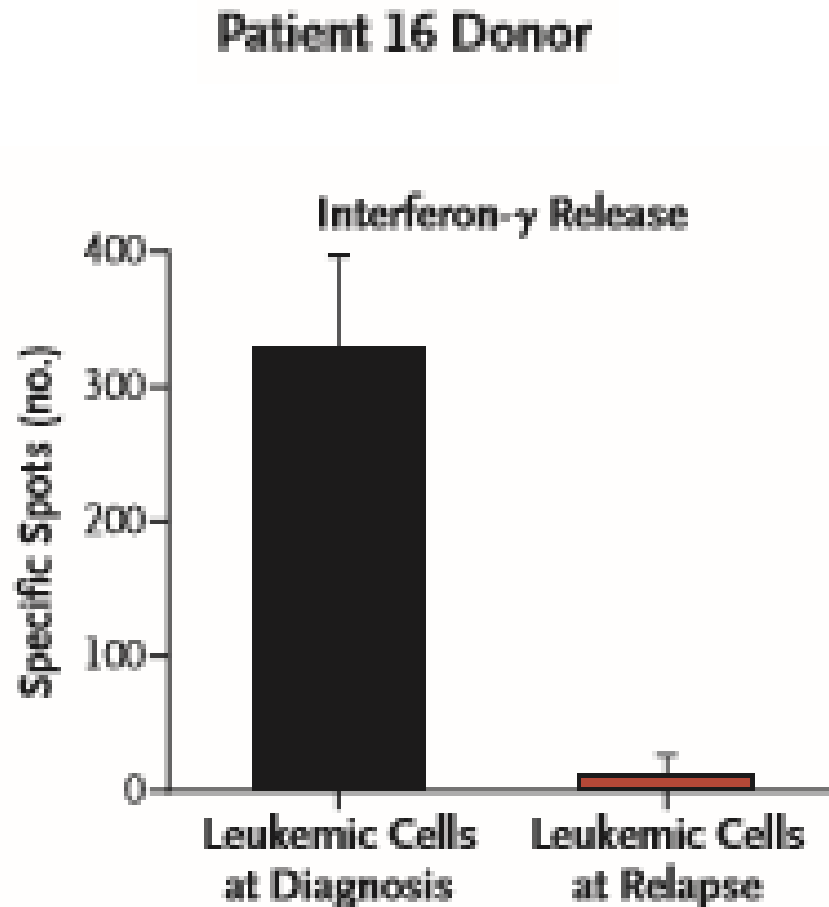
Luca Vago, M.D., Ph.D., Serena Kimi Perna, M.D., Monica Zanussi, B.Sc.,  
Benedetta Mazzi, B.Sc., Cristina Barlassina, B.Sc., Maria Teresa Lupo Stanghellini, M.D.,  
Nicola Flavio Perrelli, B.Sc., Cristian Cosentino, B.Sc., Federica Torri, B.Sc.,  
Andrea Angius, Ph.D., Barbara Forno, M.D., Monica Casucci, B.Sc.,  
Massimo Bernardi, M.D., Jacopo Peccatori, M.D., Consuelo Corti, M.D.,  
Attilio Bondanza, M.D., Ph.D., Maurizio Ferrari, M.D., Silvano Rossini, M.D.,  
Maria Grazia Roncarolo, M.D., Ph.D., Claudio Bordignon, M.D.,  
Chiara Bonini, M.D., Fabio Ciceri, M.D., and Katharina Fleischhauer, M.D.

Vago

# HLA loss as a mechanism of immune escape (

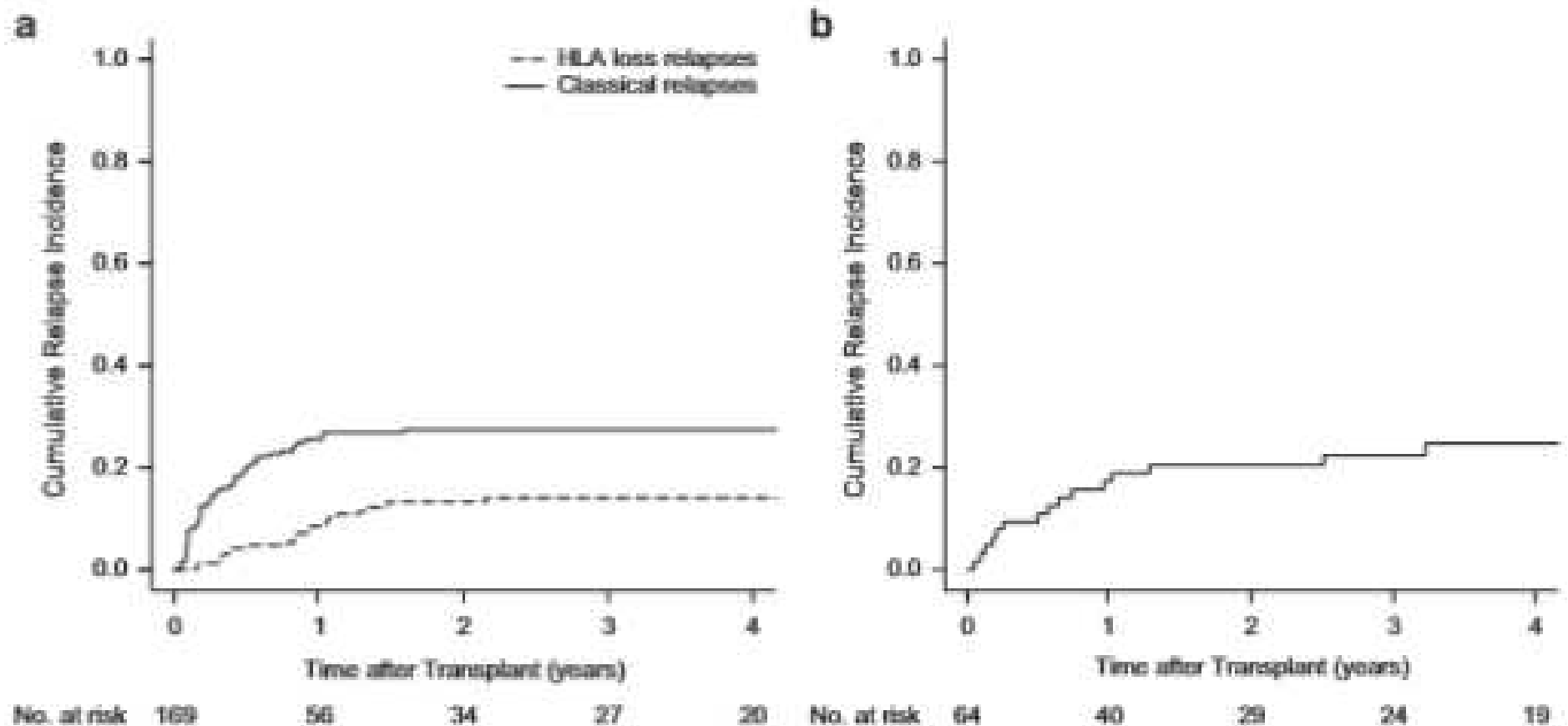


# HLA loss as a mechanism of immune escape (

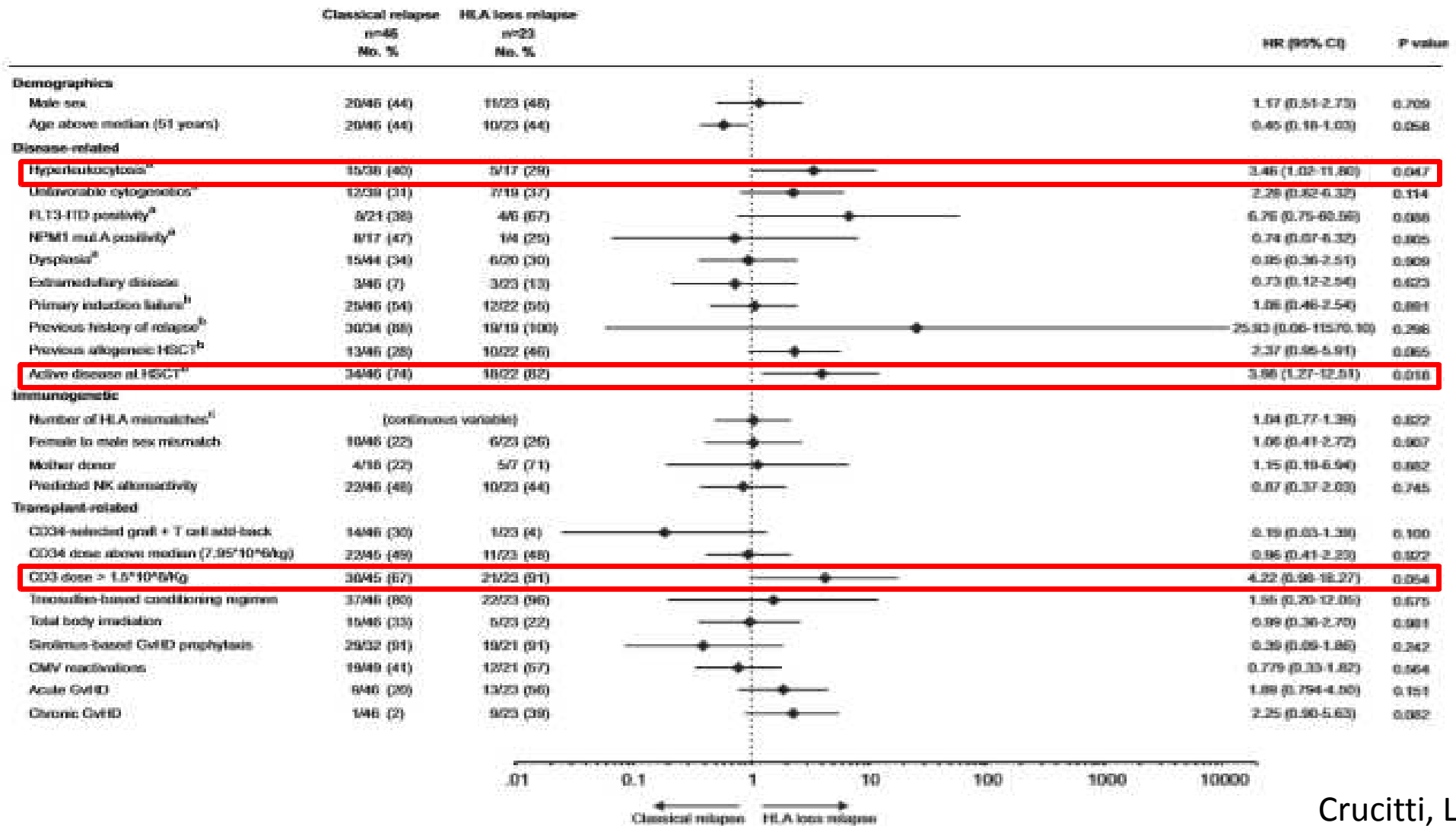


- HLA loss in 5 out of 17 pts (29%)
- No change in class I HLA expression (no NK activation)
- Characterisation of this mechanisms essential to identify alternative donors (with a distinct haplotype)

# HLA loss as a mechanism of immune escape (



# HLA loss as a mechanism of immune escape (



# Impaired HLA expression after HSCT (1)

*The NEW ENGLAND JOURNAL of MEDICINE*

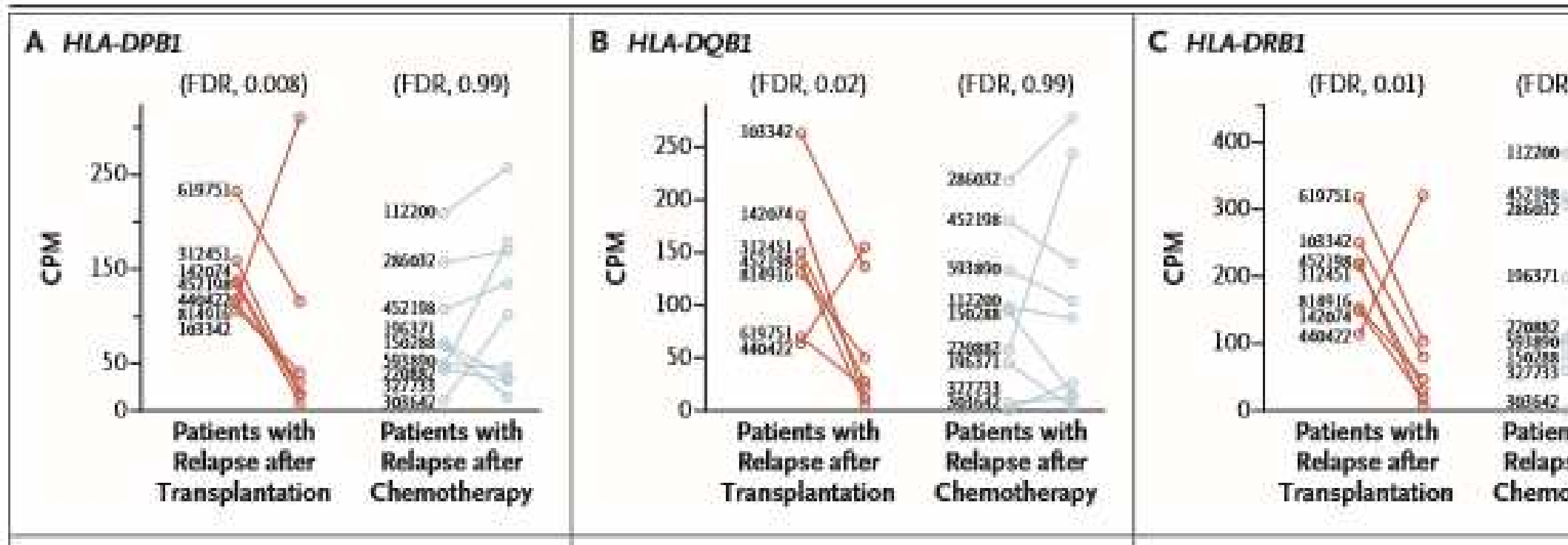
ORIGINAL ARTICLE

## Immune Escape of Relapsed AML Cells after Allogeneic Transplantation

M.J. Christopher, A.A. Petti, M.P. Rettig, C.A. Miller, E. Chendamarai,  
E.J. Duncavage, J.M. Klco, N.M. Helton, M. O'Laughlin, C.C. Fronick, R.S. Fulton,  
R.K. Wilson, L.D. Wartman, J.S. Welch, S.E. Heath, J.D. Baty, J.E. Payton,  
T.A. Graubert, D.C. Link, M.J. Walter, P. Westervelt, T.J. Ley, and J.F. DiPersio

Cristopher,

# Impaired HLA expression after HSCT (2)

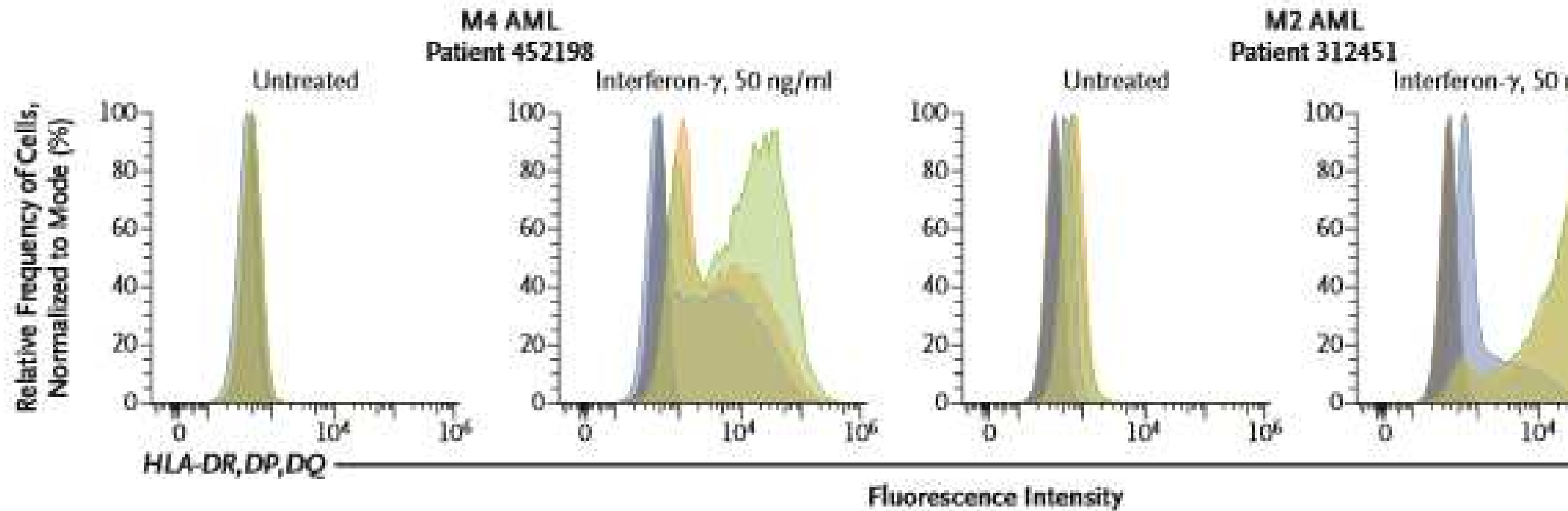




# Impaired HLA expression after HSCT (3)

## B Flow Cytometry after Treatment with Interferon- $\gamma$

72-hr culture 48-hr culture 24-hr culture Negative control



Cristopher,

(Nihil novi sub sole)

Exp. Hematol. 13:782-790 (1985)  
© International Society for Experimental Hematology

Experimental  
Hematology

## **Relapse of Host Leukemic Lymphoblasts following Engraftment by an HLA-mismatched Marrow Transplant: Mechanisms of Escape from the "Graft versus Leukemia" Effect**

Paul M. Sondel,<sup>1,2,3\*</sup> Jacquelyn A. Hank,<sup>2\*\*</sup> James Molenda,<sup>2</sup> Judith Blank,<sup>4</sup> Wayne Borcharding,<sup>1</sup> Walter Longo,<sup>2,5</sup> Michael E. Trigg,<sup>1†</sup> Richard Hong,<sup>1</sup> and Marek J. Bozdech<sup>2†</sup>

Departments of <sup>1</sup>Pediatrics, <sup>2</sup>Human Oncology, <sup>3</sup>Genetics, <sup>4</sup>Pathology and Laboratory Medicine, and <sup>5</sup>Medicine, University of Wisconsin, Madison, Wisconsin, USA

(Received 1 October 1984; in revised form 28 January 1985; accepted 4 March 1985)

Sondel, Exp

(...continues)

populations (Table 2). Designation of host origin was clearly demonstrated for the uncultured leukemia cells by the presence of strong reactivity to HLA-A26 and no reactivity with any reagent detecting HLA-A24. HLA-DR typing was attempted on these cells, but was not interpretable; this was consistent with the nonreactivity of these cells with the monoclonal anti-Ia reagent. HLA typing of the

This patient's recurrent leukemia was HLA-compatible with his coexisting chimeric hematopoietic immune system. Thus, "immune escape as a potential mechanism for his relapse could be examined by studying well-characterized alloimmune responses to foreign HLA antigens. These

# Immune escape from a graft-versus-leukemia effect may play a role in the relapse of myeloid leukemias following allogeneic bone marrow transplantation

**Table 4** Correlation of changes in phenotype with immunogenicity of leukemia and outcome of immune manipulation with donor lymphocytes or withdrawal of cyclosporine

<i>Diagnosis at relapse</i>		<i>P1 (029) AML M4</i>	<i>P2 (005) MDS<sub>t</sub></i>	<i>P3 (025) CML AP</i>	<i>P4 (019) CML CP</i>	<i>P5 (017) CML BC</i>	<i>P6 (026) CML MF</i>
Phenotype	MHC class I	↓	↓	↓	0	0	↑
	HLA DR	↓	↓	↓	0	0	↑
	HLA DQ	L	L	↓	0	0	0
	ICAM-1 (CD54)	↑	↑	↑	0	↓	↓
	B7.1 (CD80)	L	↑	↑	↓	↓	↓
	fas (CD95)	0	↑	0	0	L	↑
Functional tests	stimulation	↓	↓	↓	L	↓	NT
	CTL lysis	↓	↓	0	↓	↑	NT
	NK lysis	0	0	NT	0	↓	NT
Response to treatment		No	No	No	No	Yes Donor lymphocytes	Yes Cyclosporine withdrawal

At relapse: ↑ = increased; ↓ = decreased; 0 = no change; L = no change but low; NT = not tested.

Clonal Evolution Including Partial Loss of Human  
Leukocyte Antigen Genes Favoring Extramedullary  
Acute Myeloid Leukemia Relapse After Matched Related  
Allogeneic Hematopoietic Stem Cell Transplantation

**Genome-wide Profiling in AML Patients Relapsing after  
Allogeneic Hematopoietic Cell Transplantation**

*Miguel Waterhouse,<sup>1</sup> Dietmar Pfeifer,<sup>2</sup> Milena Pantic,<sup>2</sup> Florian Emmerich,<sup>3</sup>  
Hartmut Bertz,<sup>1</sup> Jürgen Finke<sup>1</sup>*

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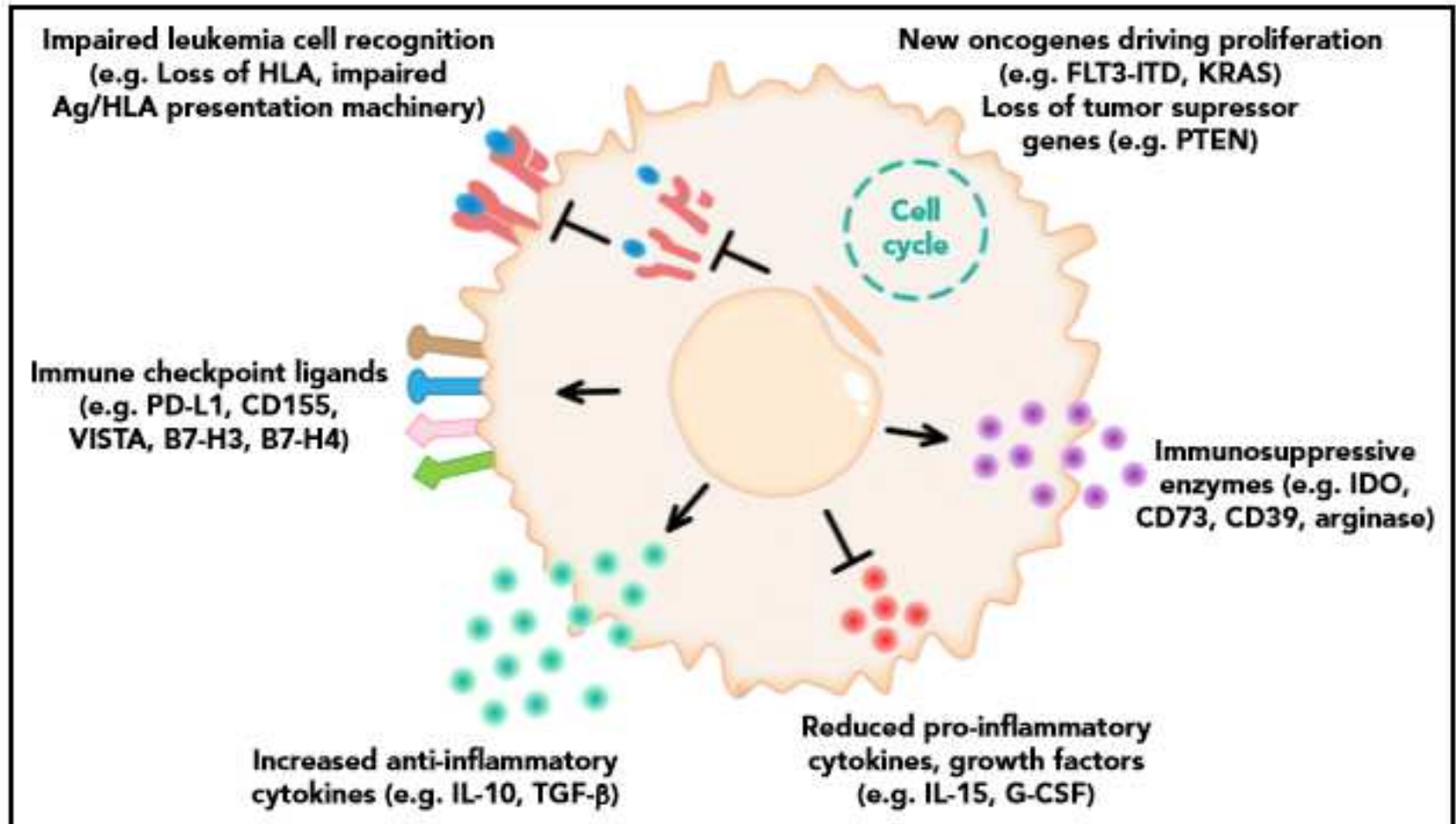
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To the editor:

Genomic loss of patient-specific HLA in acute myeloid leukemia relapse after well-matched  
unrelated donor HSCT

Stolzel, Transplantation 2012; Waterhouse, BBMT 2011; Toffal

# Mechanisms of immune escape after HSCT (1)

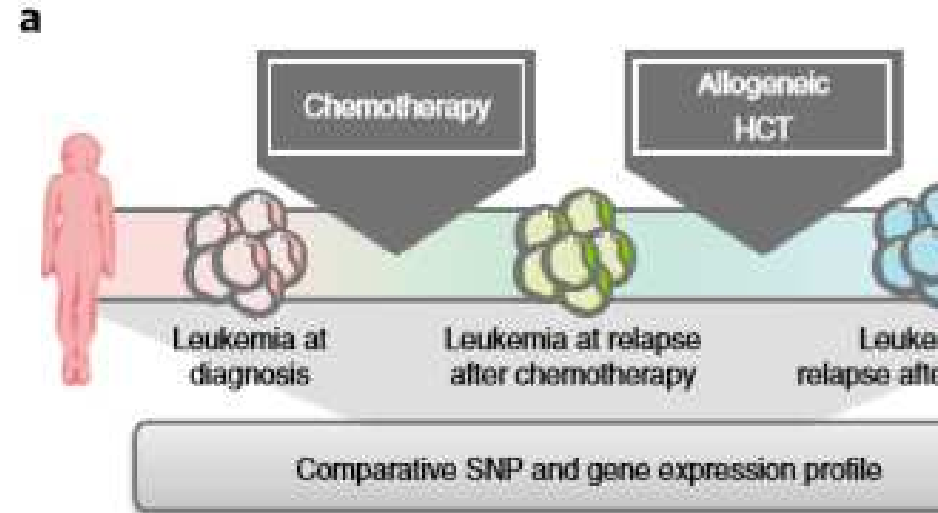


# Mechanisms of immune escape after HSCT (2)

- Two deregulated «macro-clusters», encompassing:

- genes linked to T-cell costimulation
- Ag processing and presentation via HLA class II molecules

- Including cases with genomic loss of HLA, a defined pattern of escape can be identified in more than 2/3 of post-HSCT relapses



# Take-home messages (4)

- HLA loss and impaired HLA expression represents two mechanisms of immune evasion of leukemic cells after HSCT
- Genomic alterations and epigenetic changes occur during these phenomena



# Conclusions: HLA expression in HSCT

- Barrier for optimal outcome → rejection, GF, acute/chronic GvH, non-relapse mortality, OS
- Mechanism of immune evasion → HLA loss, impaired HLA expression
- Opportunity (cell therapy) → GvL, OS, «CURE»

# Envision the near future: AI and HLA matching

HOME PROGETTO SEZIONI ▾ FACULTY

## TRAPIANTO BASATO SULL'EVIDENZA

*Discussione e interpretazione dei risultati degli studi clinici sul trapianto nelle diverse patologie*

## MACHINE LEARNING E DATA MINING NEL TRAPIANTO: COSA C'È DI NUOVO?

Il *Machine learning* e il *Data mining* sono aree di ricerca relativamente recenti della *Computer Science*. Ecco un esempio di applicazione in ambito trapiantologico, in press sul prossimo numero di BBMT

Background e disegno dello studio

Risultati

Cosa cambia nella pratica?

### Bibliografia essenziale

- 1) Fuernkranz J et al. Cognitive Technologies 2012
- 2) Buturovic L et al. BBMT 2018



### TRAPIANTO BASATO SULL'EVIDENZA

### LE SEZIONI

- Trapianto basato sull'evidenza
- Trapianto da donatore alternativo
- Complicanze del trapianto

# Envision the near future: PM and relapse management



## THE PRECISION MEDICINE INITIATIVE

*January 30th, 2015*



*"Doctors have always recognized that every patient is unique, and doctors have always tried to tailor the best they can to individuals. You can match a blood transfusion to a blood type — that was an important step. Matching a cancer cure to our genetic code was just as easy, just as standard? What if figuring out the best treatment was as simple as taking our temperature?"*

*- President Obama, January 30, 2015*

# Envision the near future: Gene Editing

## Plenary Paper

### TRANSPLANTATION

**Toward eliminating HLA class I expression to generate universal cells from allogeneic donors**

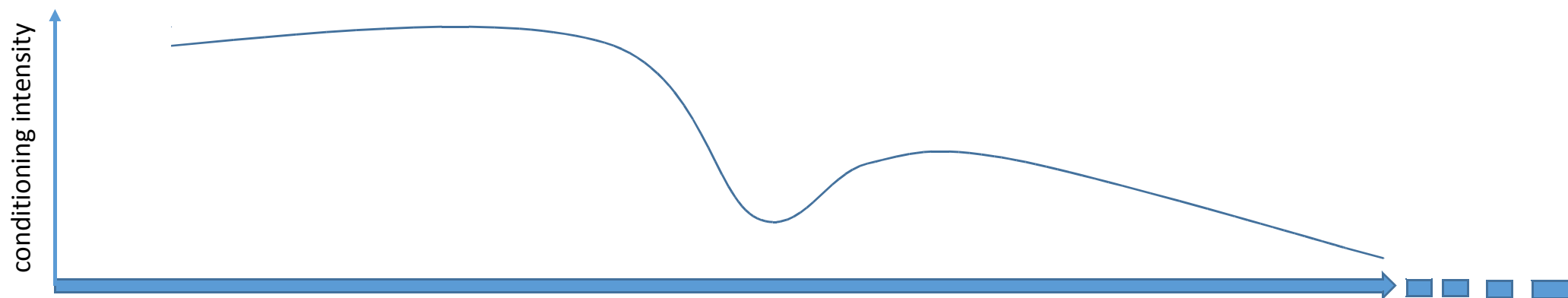
**O1**

**GENERATION OF IMMUNOLOGICALLY  
INVISIBLE TRANSGENIC PORCINE  
PANCREATIC ISLET CELL CLUSTERS AFTER  
SINGLE CELL ENGINEERING AND POST-  
TRANSDUCTION ISLET REASSEMBLING TO  
SUPPORT XENOGRAFT SURVIVAL**

**ORAL PRESENTATION**

**Best Abstracts**

Torikai H, Blood 2013; Carvalho-Oliveira



1957 -  
First BMT

1959 - BMT for  
radiation accident

1979 – Clinical evidence  
of GvL effect

1990 - Donor lymphocyte  
infusions induce disease remission

1990's – Advent of RIC

2000's – «Personalized»  
conditioning regimens

2015 - New era of  
Precision Medicine

Small m  
Cell



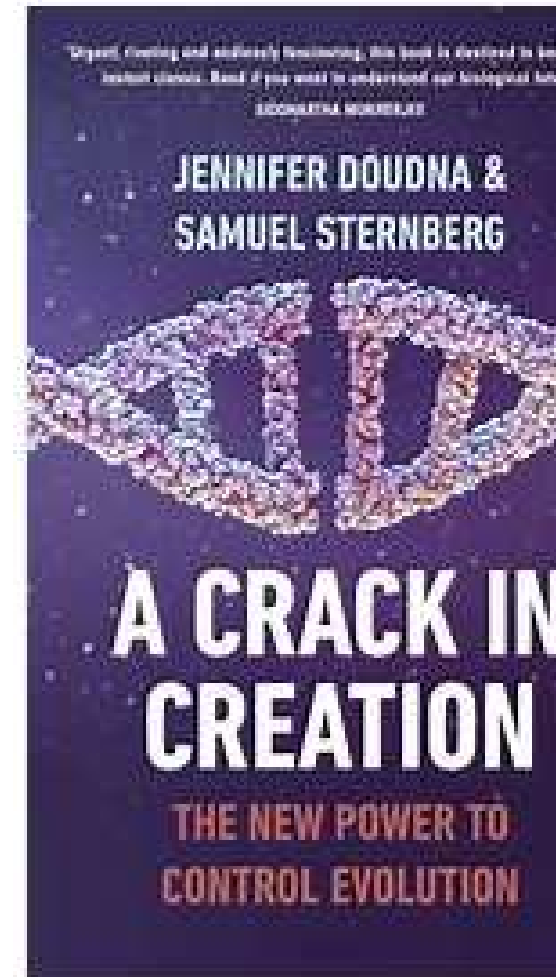
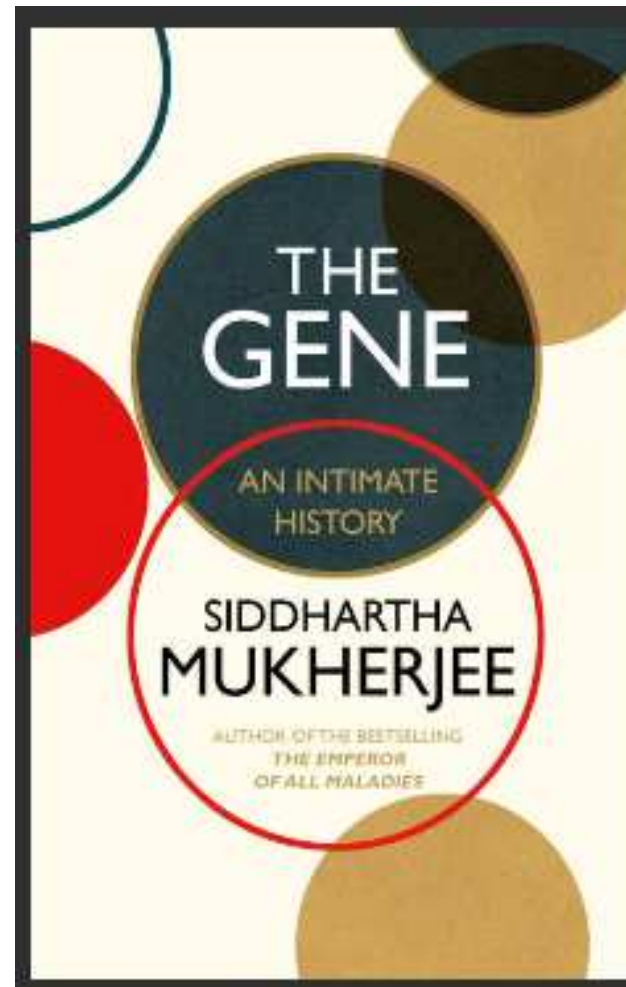
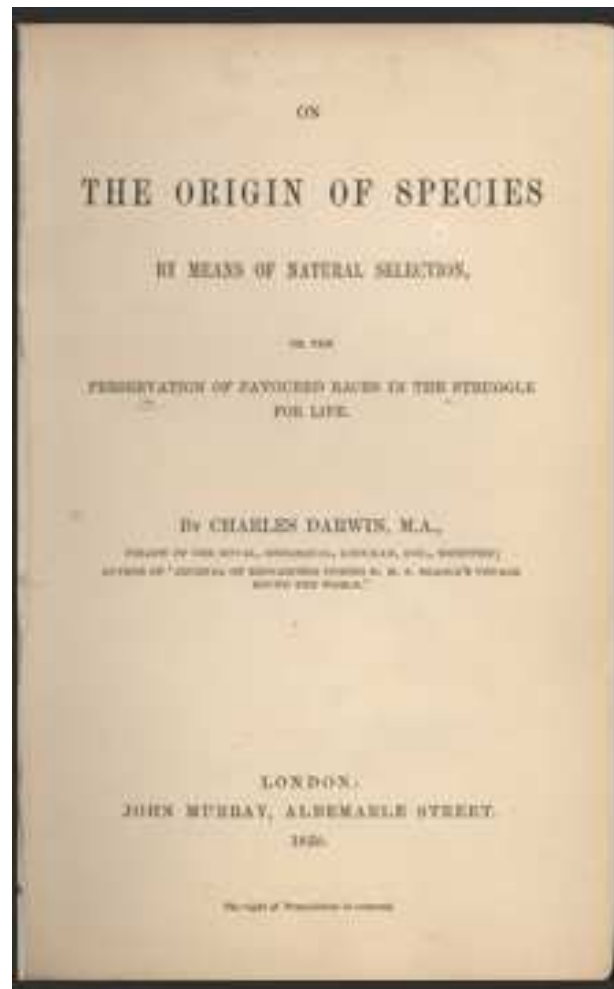
C, Darwin, 1859



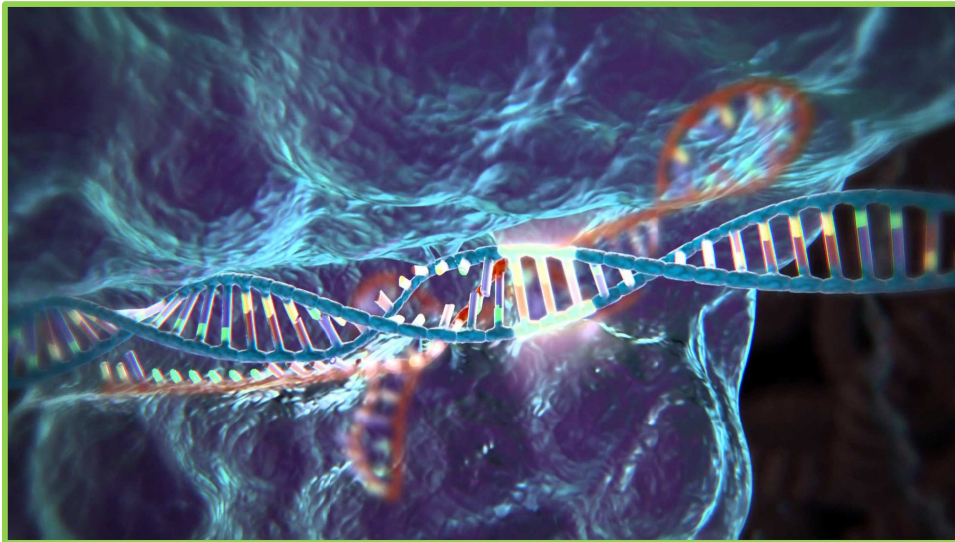
S. Mukherjee, 2016



J. Doudna, 2017



# Gene editing, HLA & Cell Therapy



## **Multiple Knockout of Classical HLA Class $\beta$ -Chains by CRISPR/Cas9 Genome Editing Driven by a Single Guide RNA**

Pietro Crivello, Müberra Ahci, Fabienne Maaßen, Natalie Wossidlo, Esteban Arrieta-Bolaños, Andreas Heinold, Vinzenz Lange, J. H. Frederik Falkenburg, Peter A. Horn, Katharina Fleischhauer and Stefan Heinrichs

cancer immunotherapy. Finally, in a longer term perspective our method might also prove useful in clinical protocols for personalized cellular therapy, for instance, for the generation of HLA-II-compatible hematopoietic stem cells for transplantation (45) or for the reduction of organ graft rejection by knockout endothelial cells as previously suggested (9, 4



Ospedale Niguarda

Sistema S



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L

# GRAZIE !

contatto: [roberto.crocchiolo@ospedaleniguarda.it](mailto:roberto.crocchiolo@ospedaleniguarda.it)



AIBT Summer School  
Ercolano, 13-15 giugno 2019

