HLA: Struttura, Funzione e Polimorfismo

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OSPEDALE SAN RAFFAELE

Summer School AIBT

Pesaro, 10 Giugno 2016

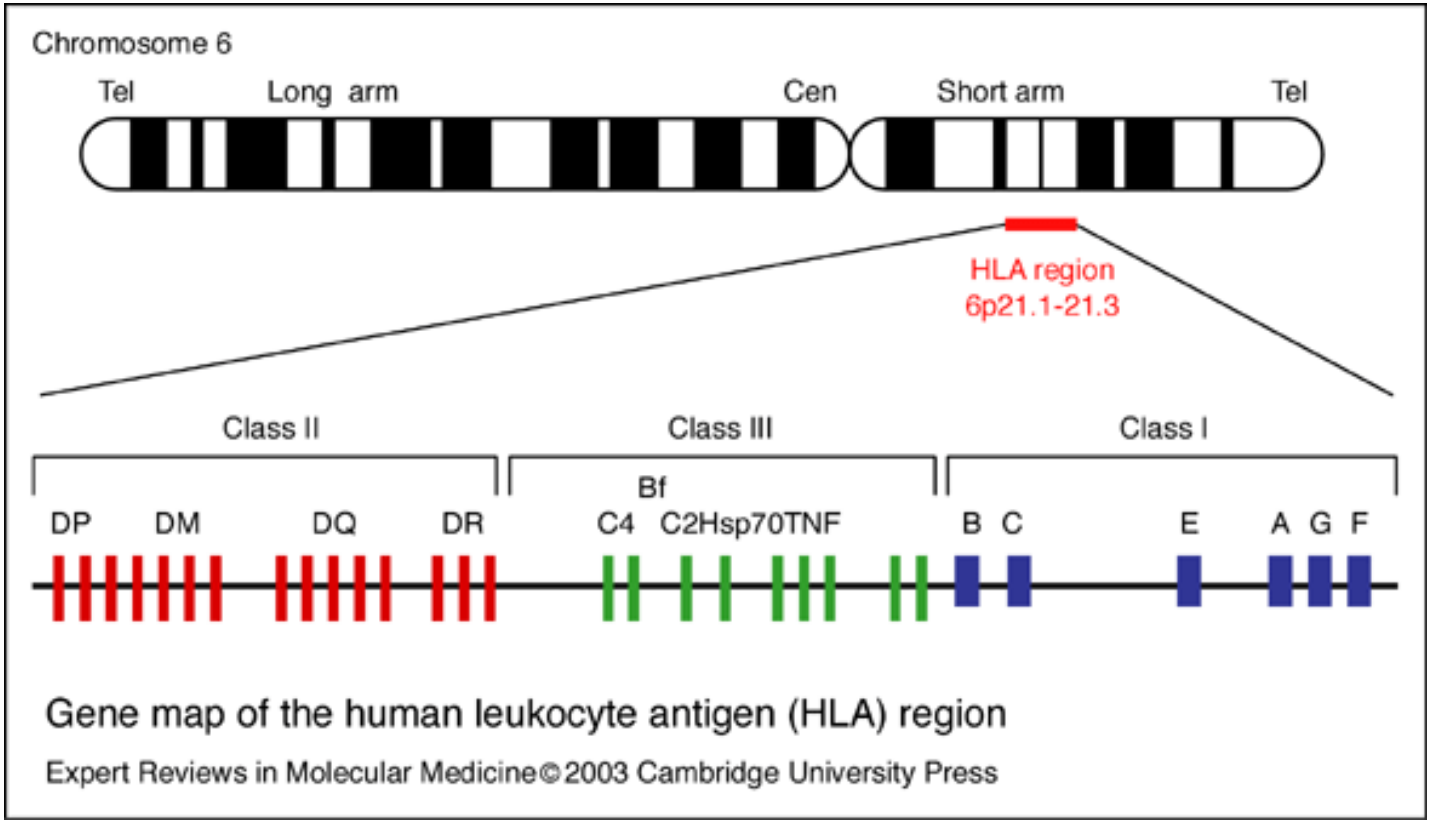
Le molecole HLA

- **Molecole HLA Classiche**
  - ✓ HLA-A, -B, -C – Classe I
  - ✓ HLA-DR, -DQ, -DP – Classe II
  - ✓ Alto grado di polimorfismo (>10’000/>3’500 alleli)
  - ✓ Ruolo nella presentazione dell’antigene; linfociti T e NK

- **Molecole HLA Classe I Non-Classiche**
  - ✓ HLA-E,F,G
  - ✓Poco polimorfici (<100 alleli)
  - ✓ Ruolo nella tolleranza, immunità NK, altro?

- **Molecole HLA Classe I-simili**
  - ✓ MICA, MICB, CD1a, CD1b, CD1d, CD1e
  - ✓Presentazione di antigeni non peptidici (glicolipidi)
  - ✓ Interazione anche con cellule immuni “non convenzionali”: NK, iNKT

Mappa dell' MHC umano



3,838,986 bp

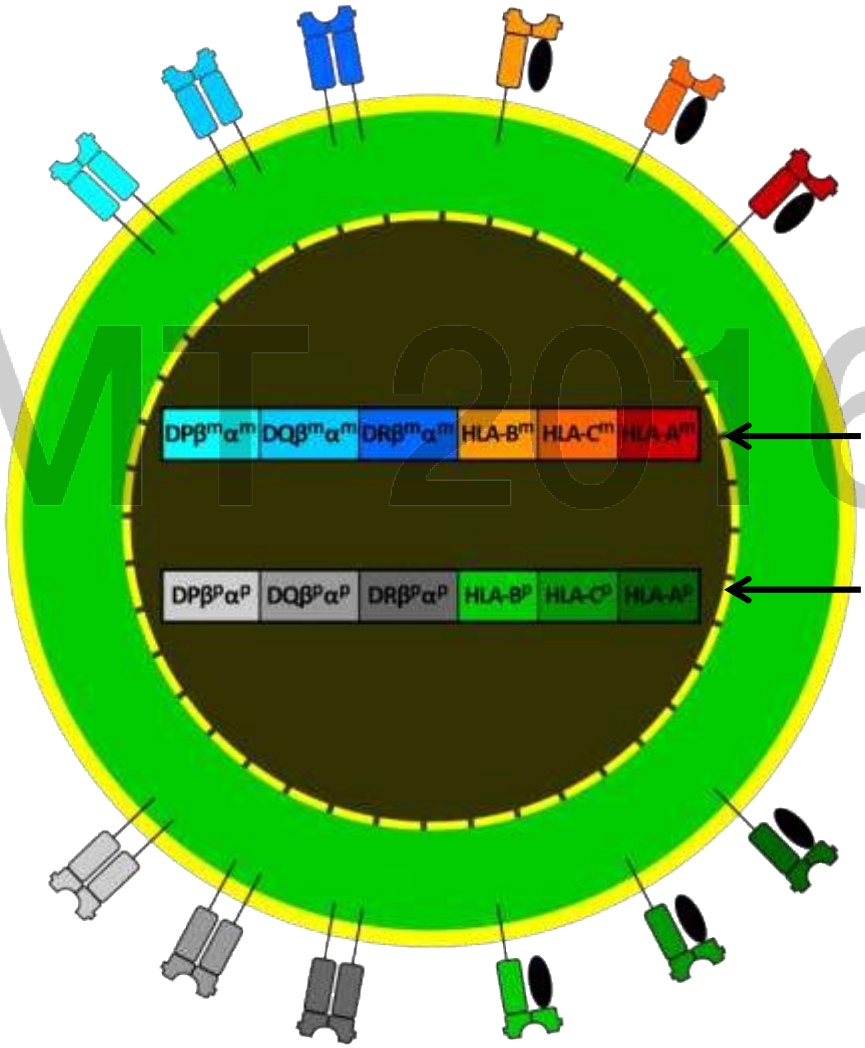
224 geni (128 espressi)

HLA: aplotipi e codominanza

Human Chromosome 6

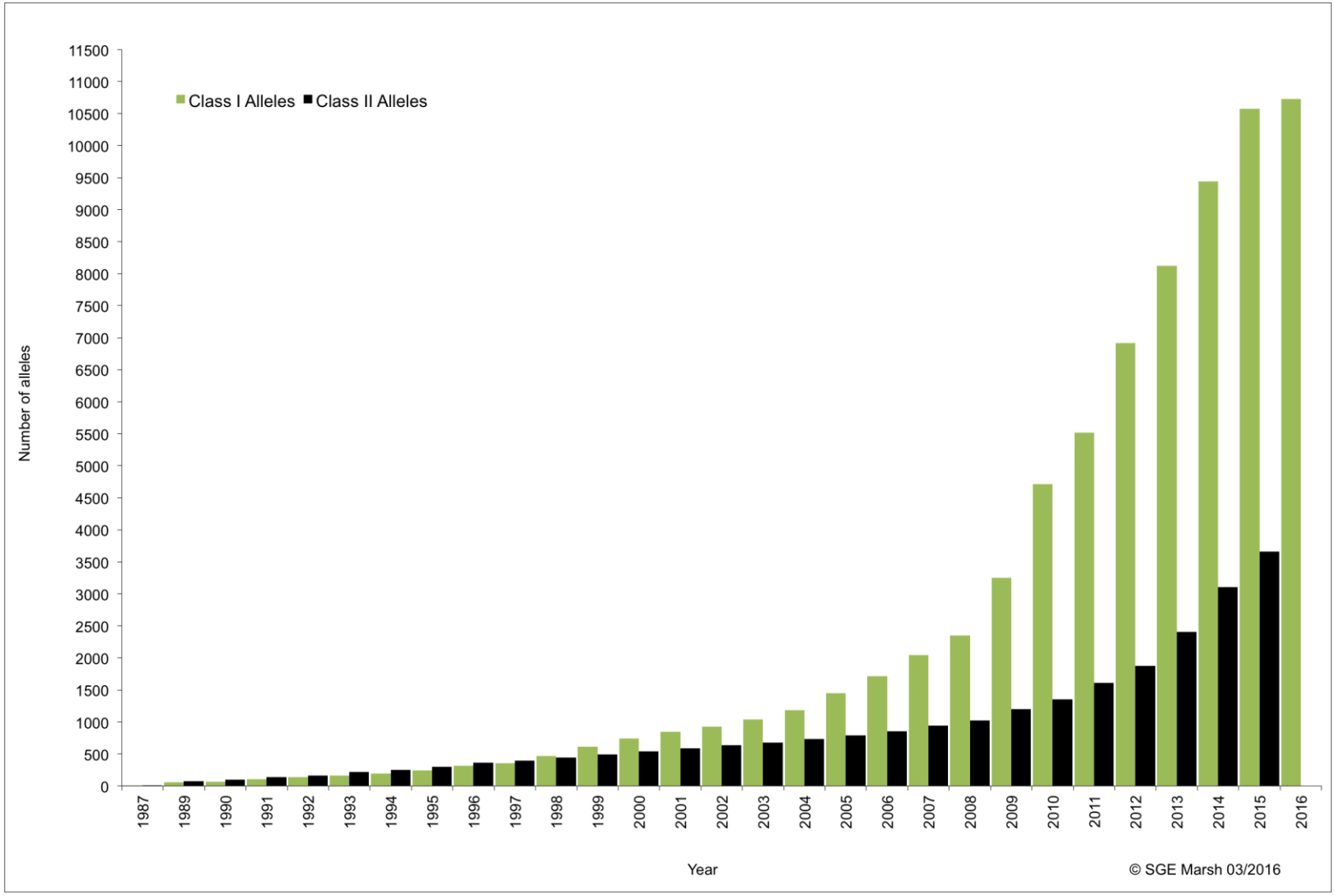
Siblings:

- **HLA-identical (25%)**
  (2/2 haplotypes)
- **HLA-Haploidentical (50%)**
  (1/2 haplotypes)
- **HLA-Diverse (25%)**
  (0/2 haplotypes)

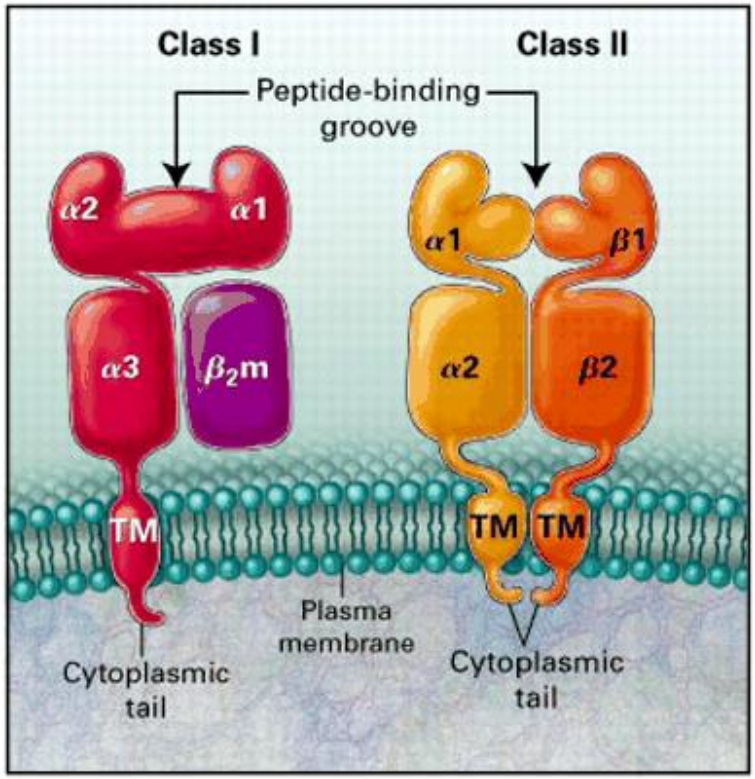


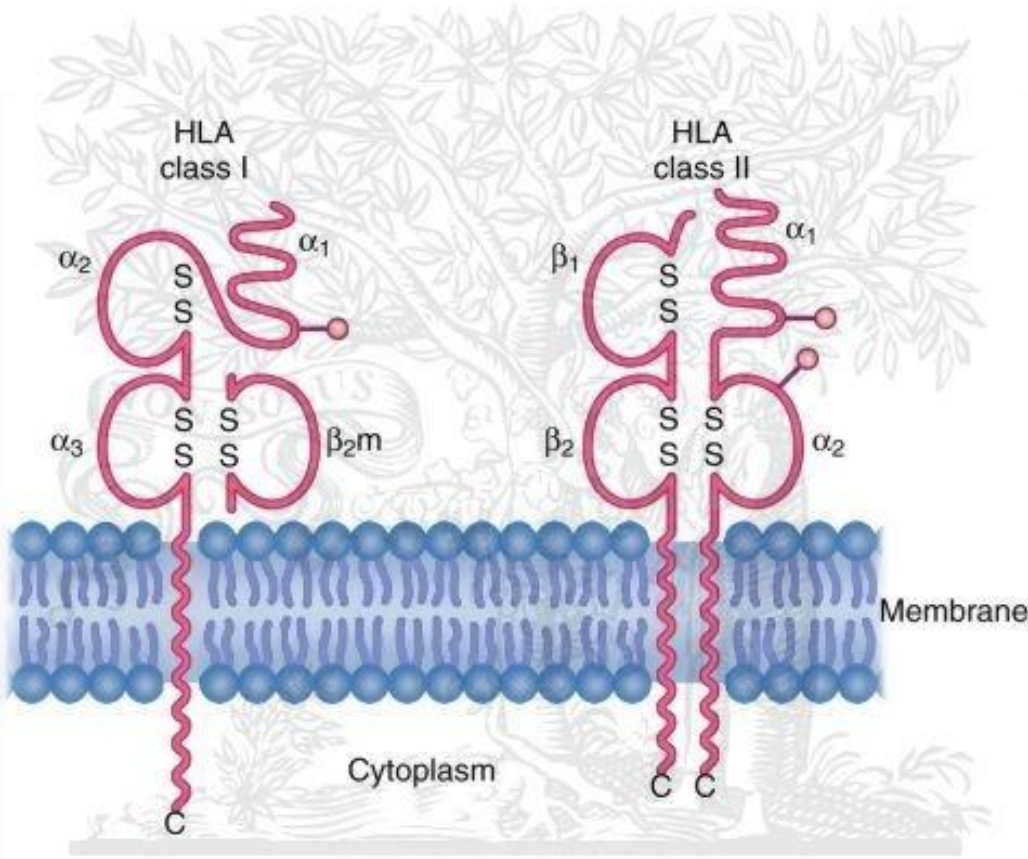
EBMT 2016

Polimorfismo HLA

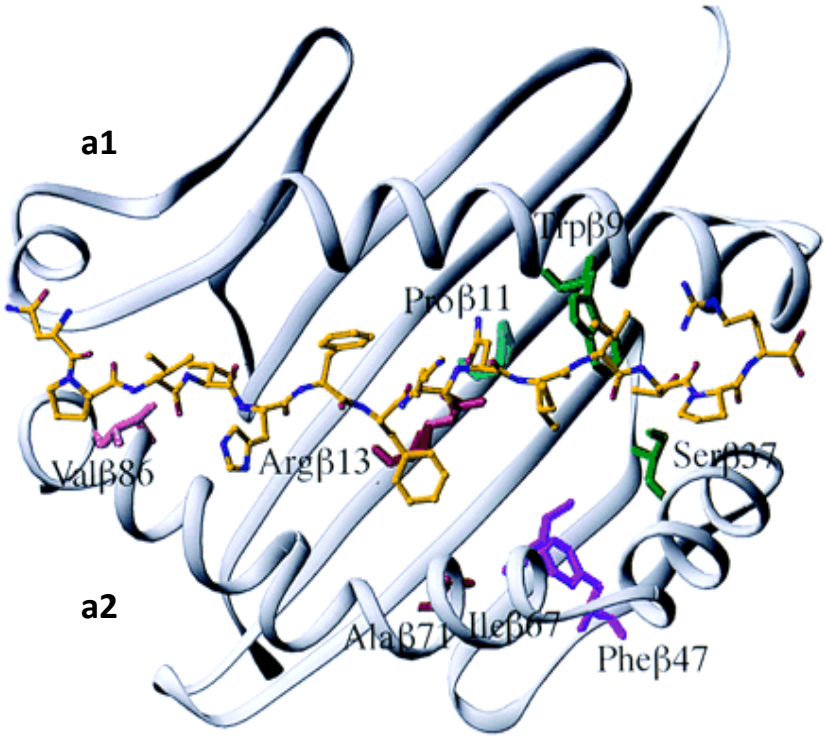


Struttura dele molecole HLA classiche

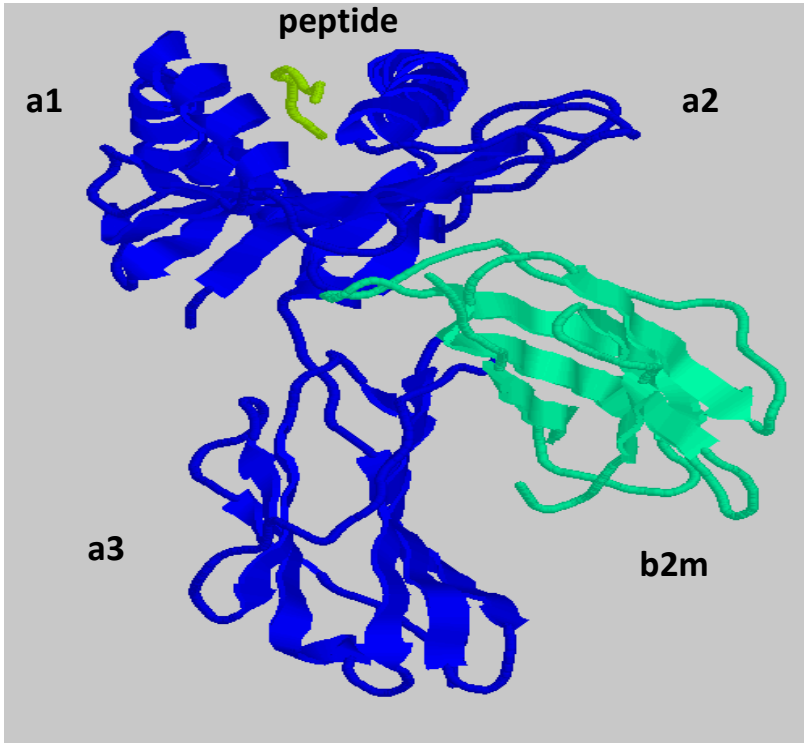




Struttura cristallografica HLA Classe I



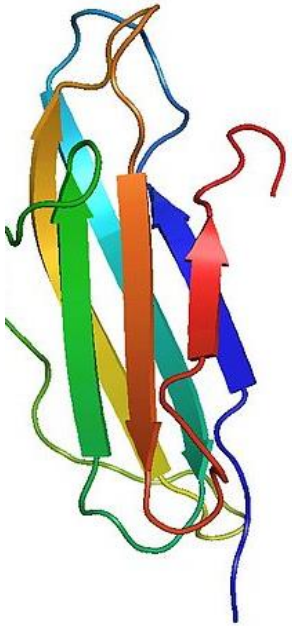
Superficiale



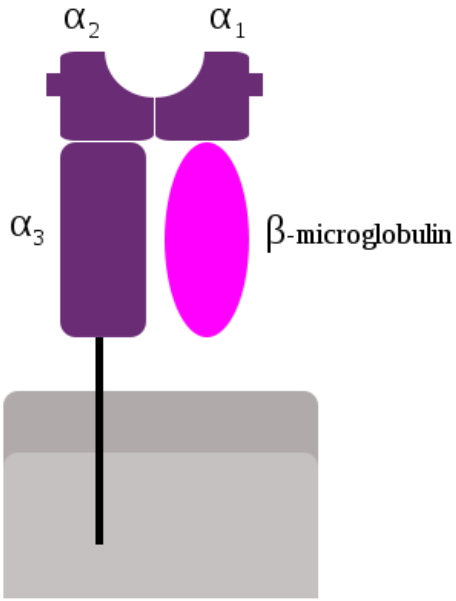
Laterale

β2-microglobulina

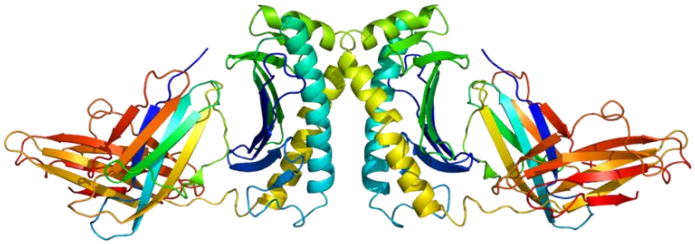
- Human Chromosome 15 – 45-45.01MB
- Limited polymorphism
- Associates with HLA class I (classical and class I-like)
- Associates with Human hemochromatosis protein (HFE)



B2M

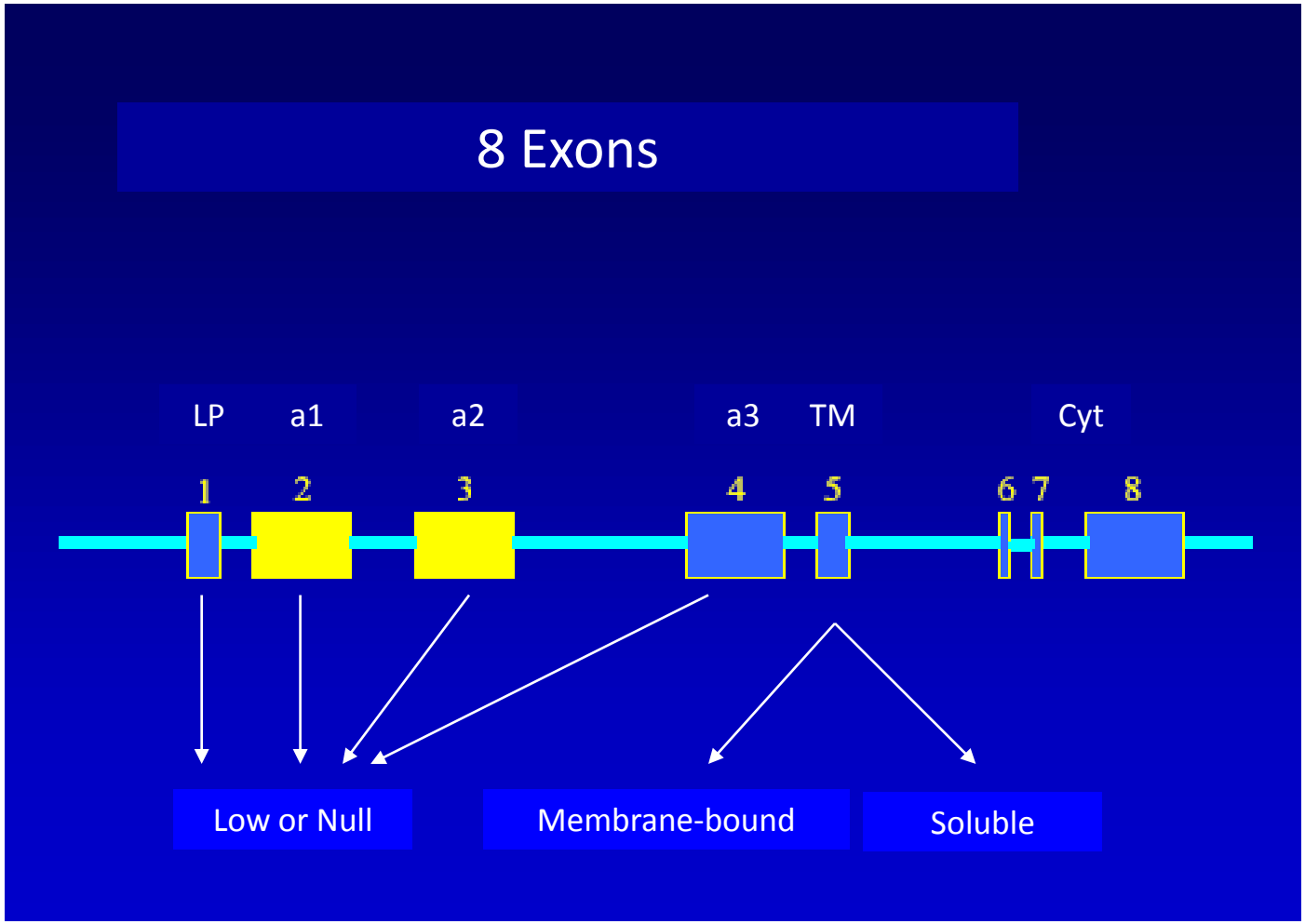


HLA Class I



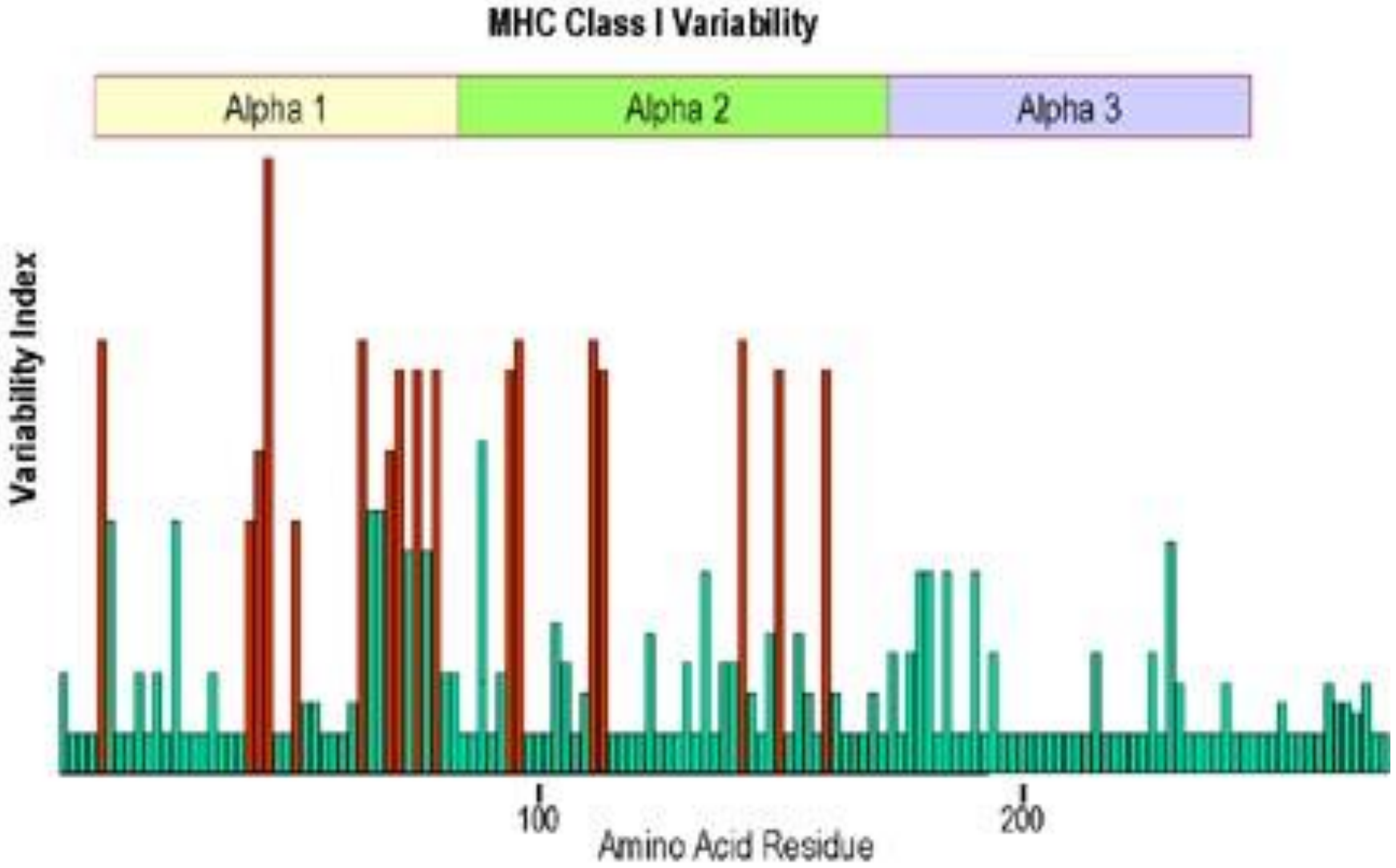
HFE (iron metabolism)

Gene Organization of HLA Class I

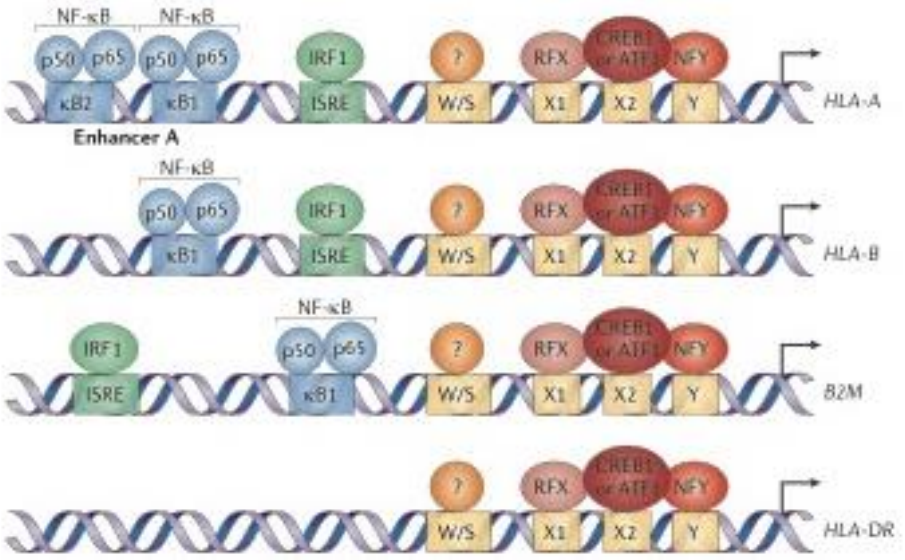


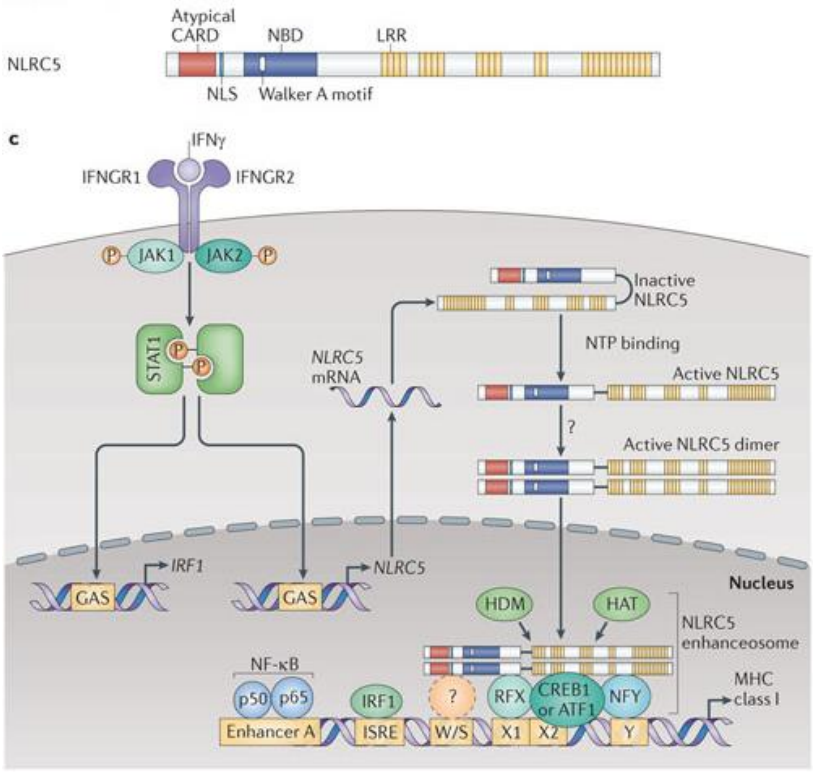
Cortesia del Prof. C. Caracassi

HLA Class I Polymorphism

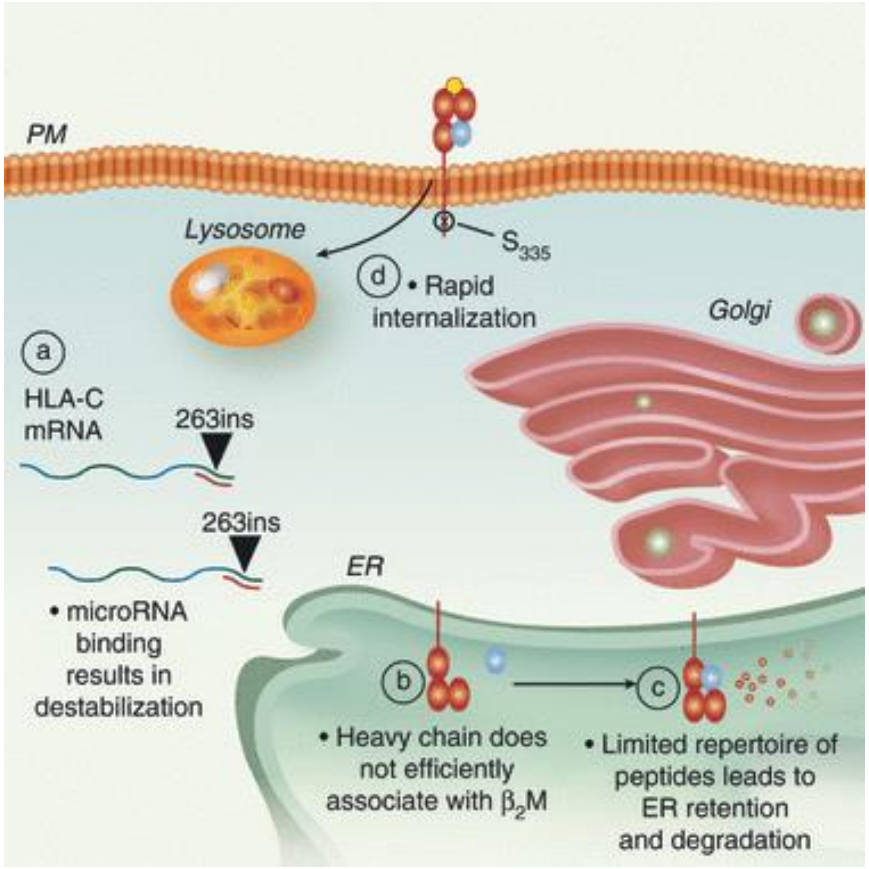


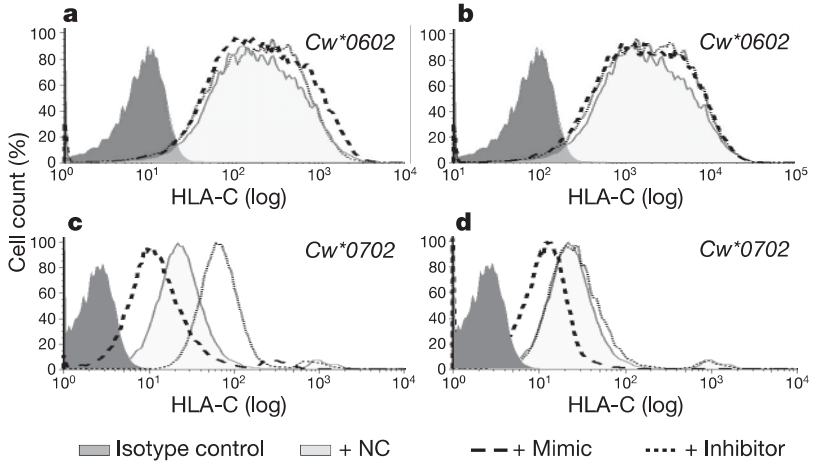
HLA Class I Transcriptional Regulation

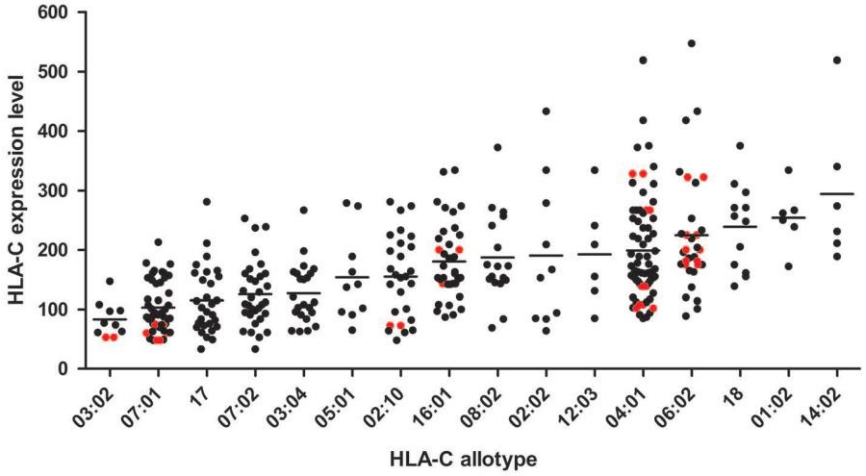




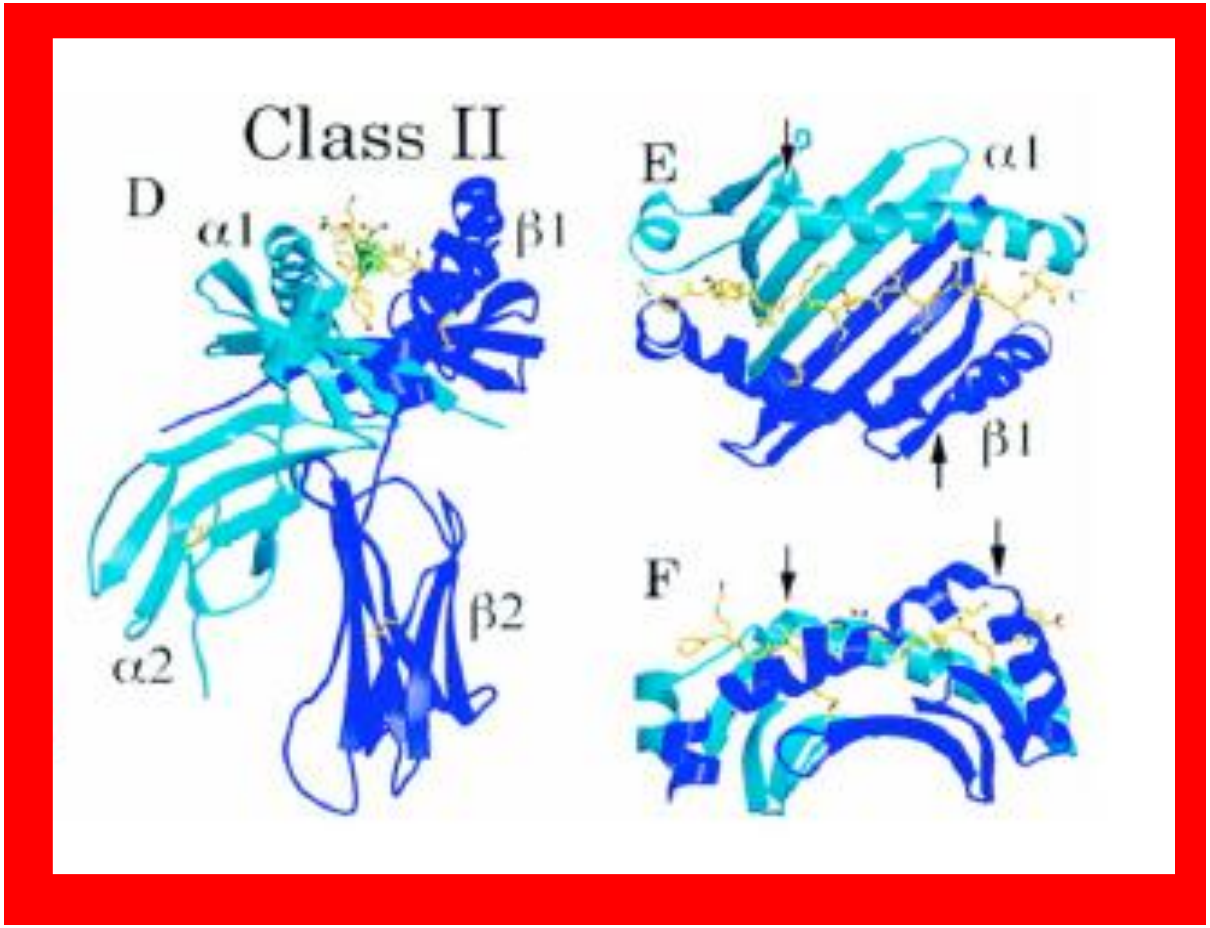
HLA-C Post-Transcriptional Regulation



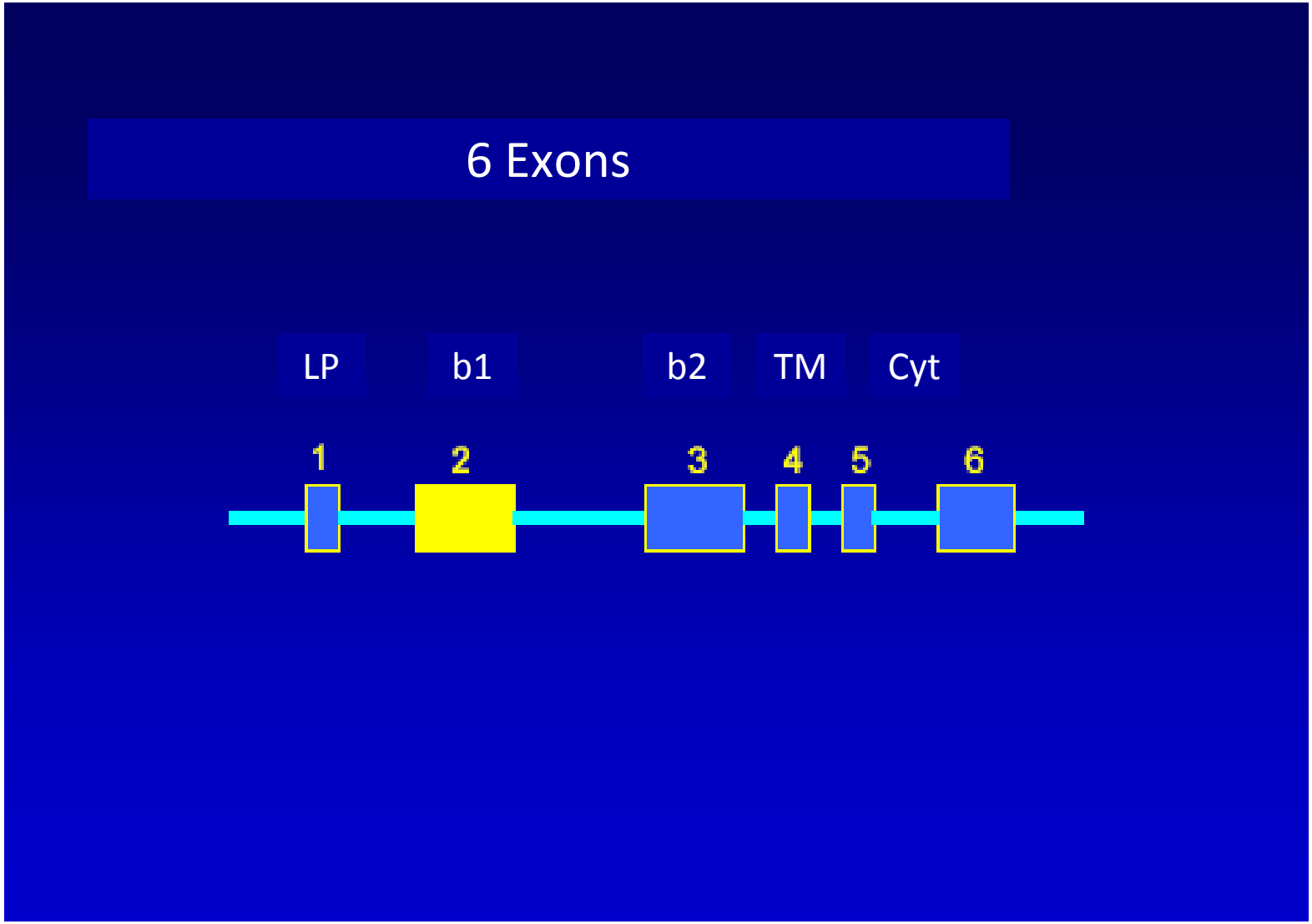




Struttura cristallografica HLA Classe II

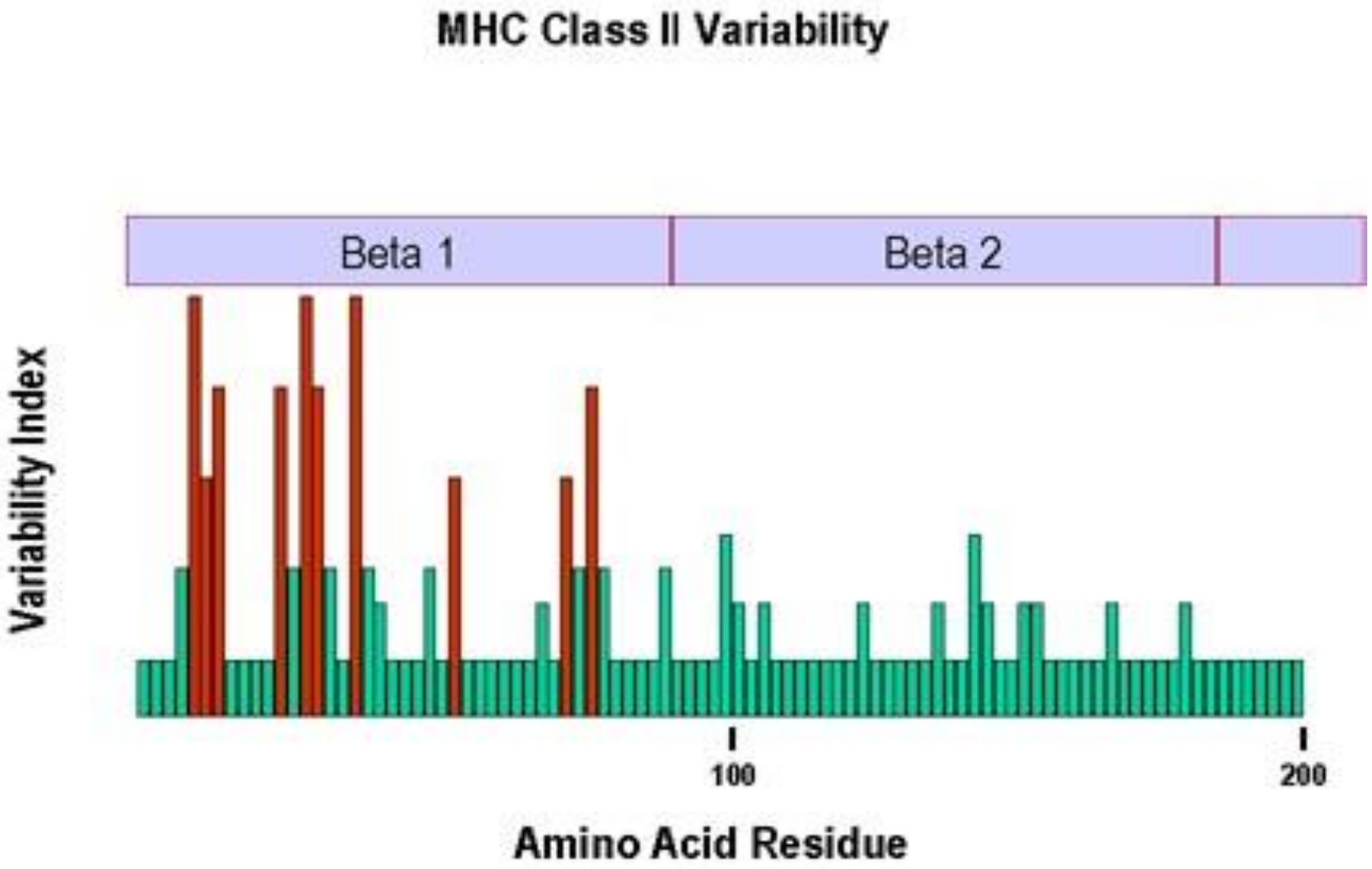


Gene Organization of HLA Class II

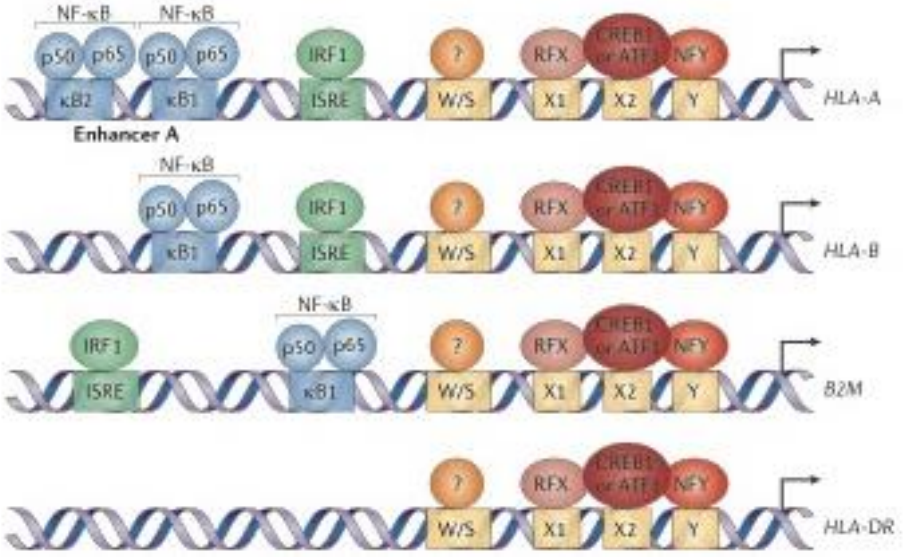


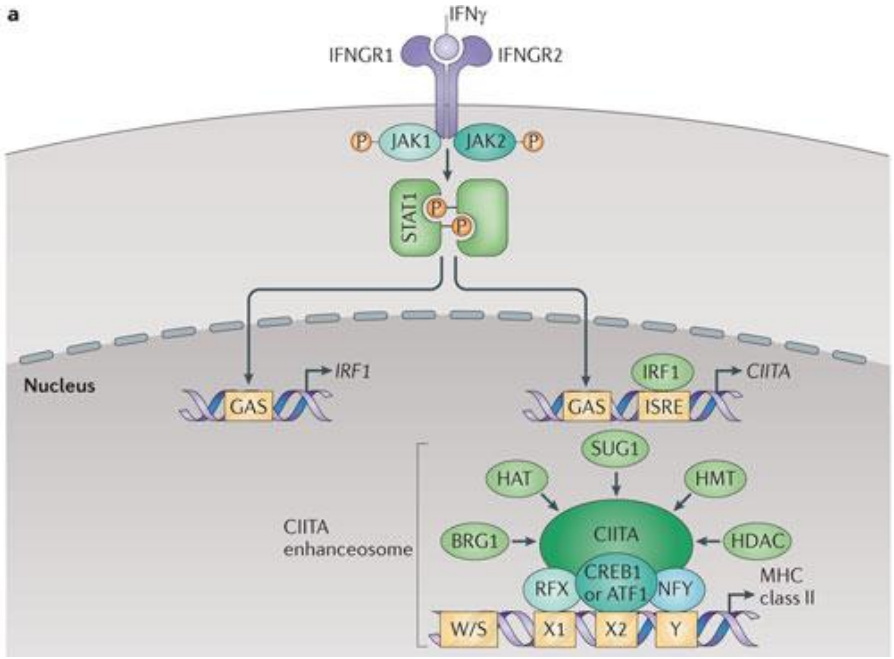
Cortesia del Prof. C. Caracassi

HLA Class II Polymorphism



HLA Class I Transcriptional Regulation

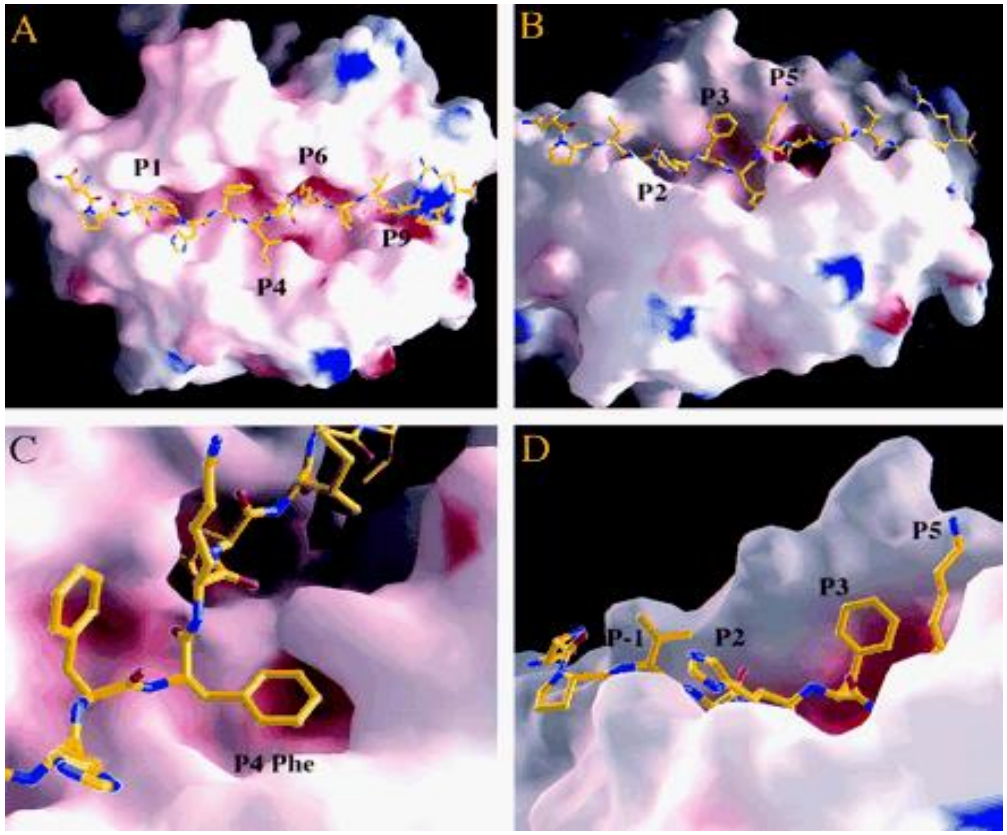




Struttura cristallografica HLA

Superficiale

Tasca P4

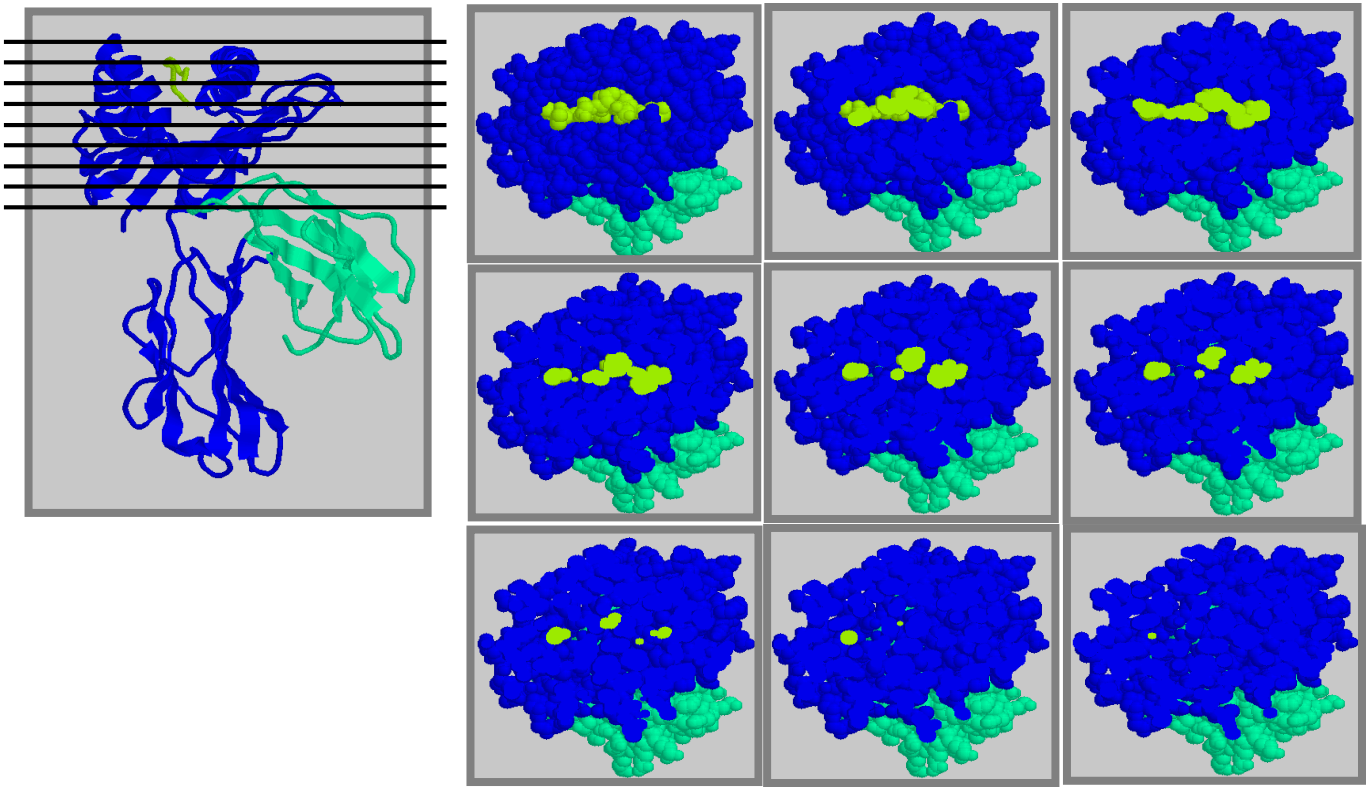


Laterale

Interazioni AA-TCR

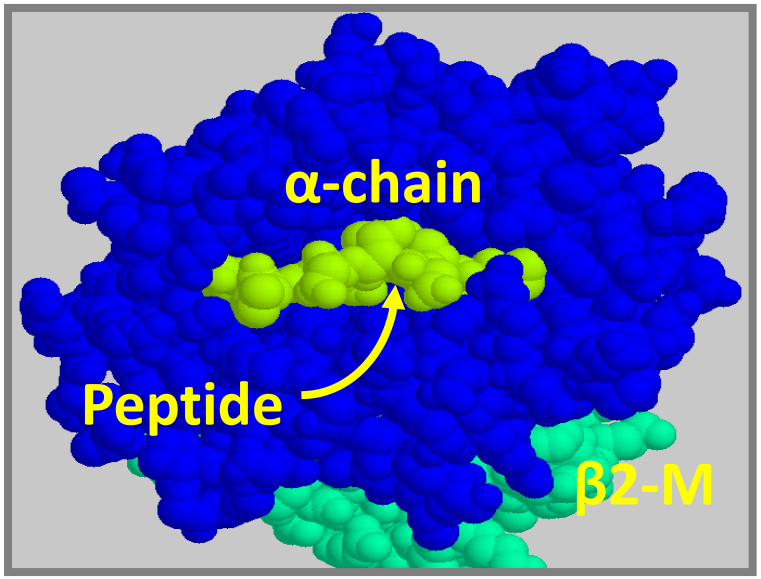
HLA-DR2 + MBP peptide

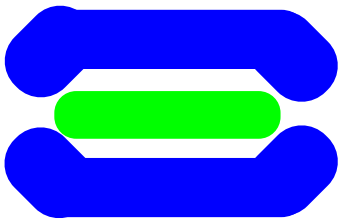
Interazione fra peptide e tasca



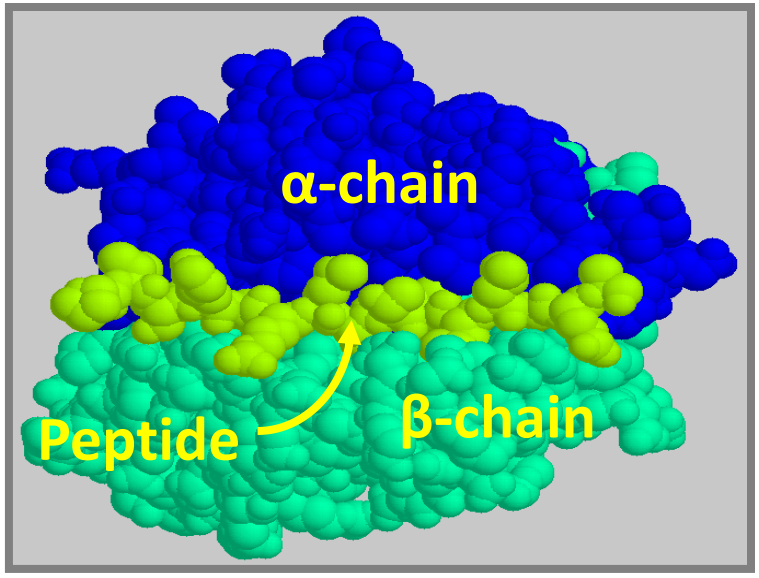
Tagli orizzontali: tasche conservate accomodano le catene aminoacidiche del peptide

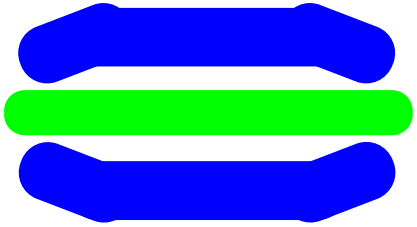
Interazione fra peptide e tasca





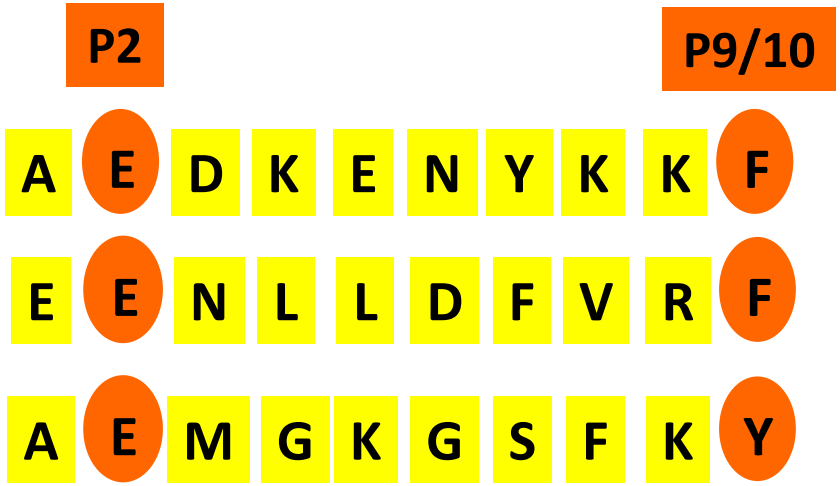
Classe I: 8-10 aa



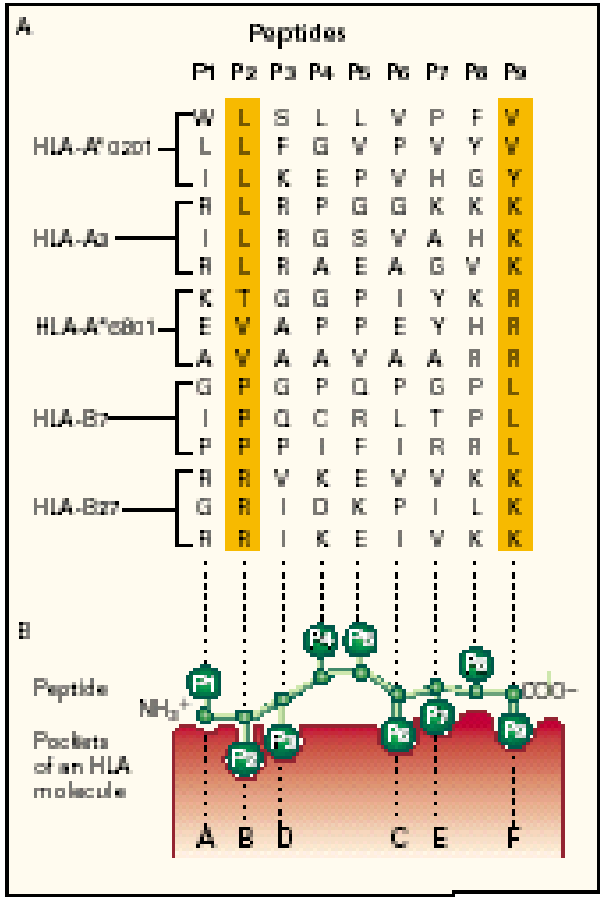


Classe II: >13 aa

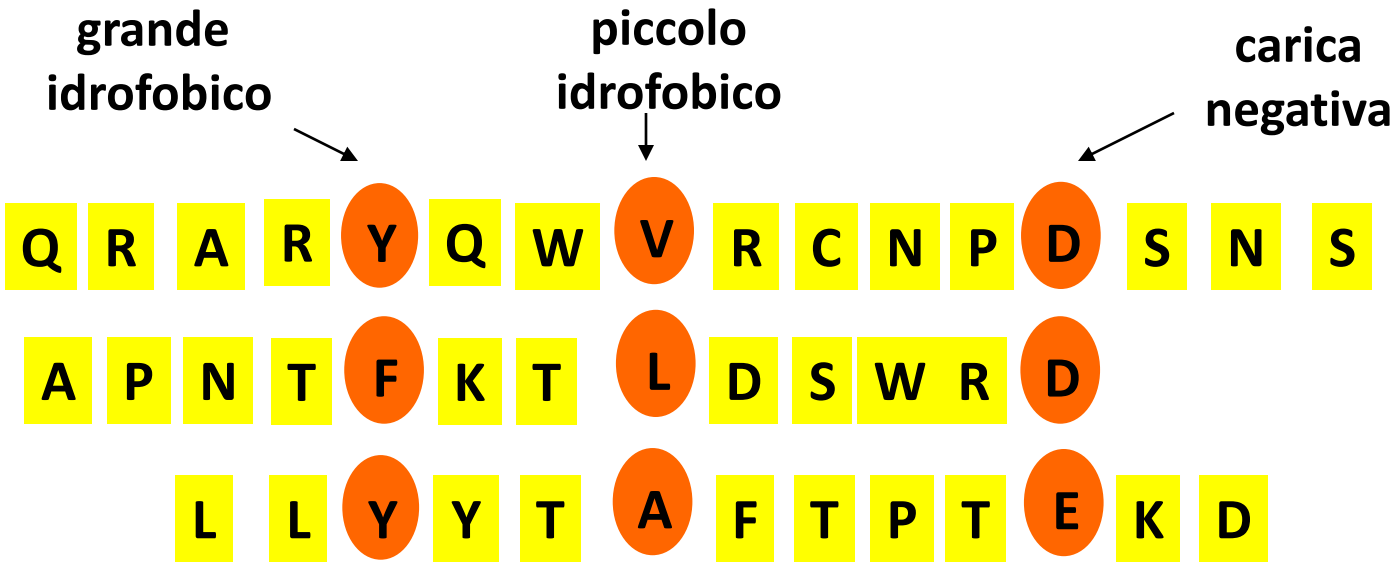
Il peptide binding motif: classe I



- **Allele-specificità**
- **Ancore ben definite**

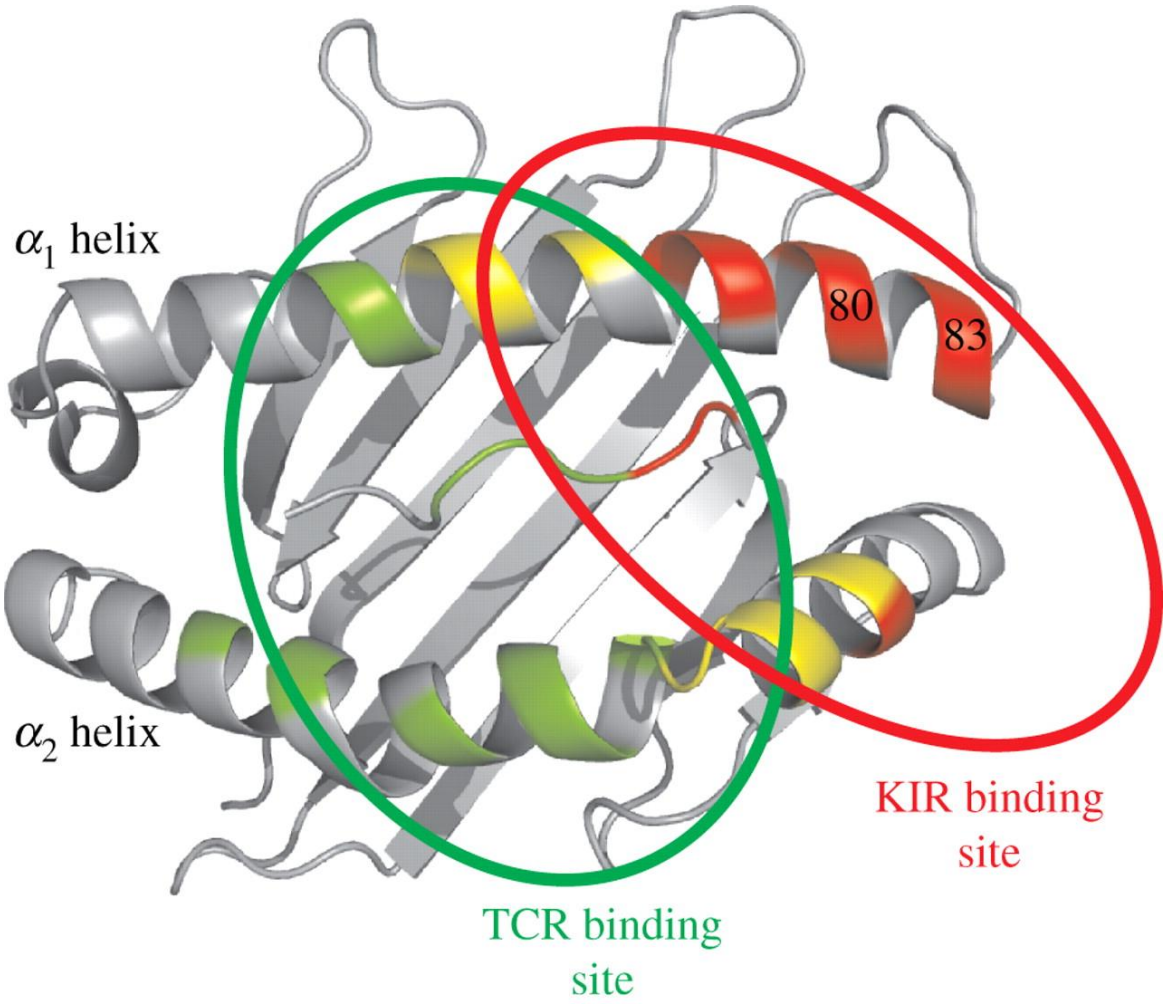


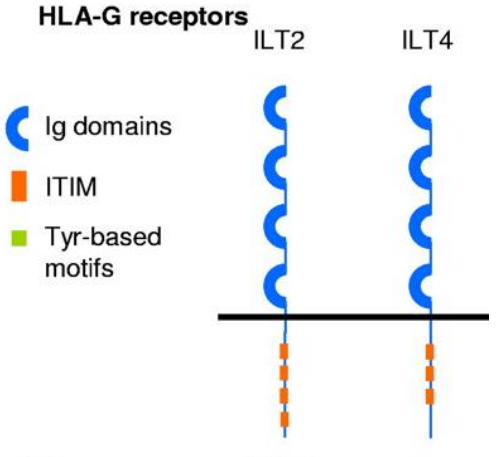
Il peptide binding motif: classe II

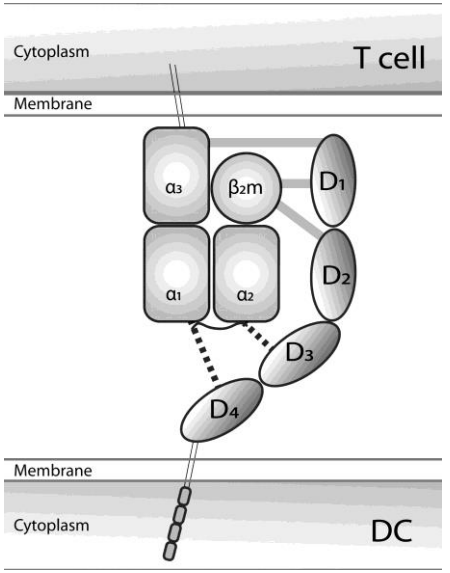


- **Promiscuità**

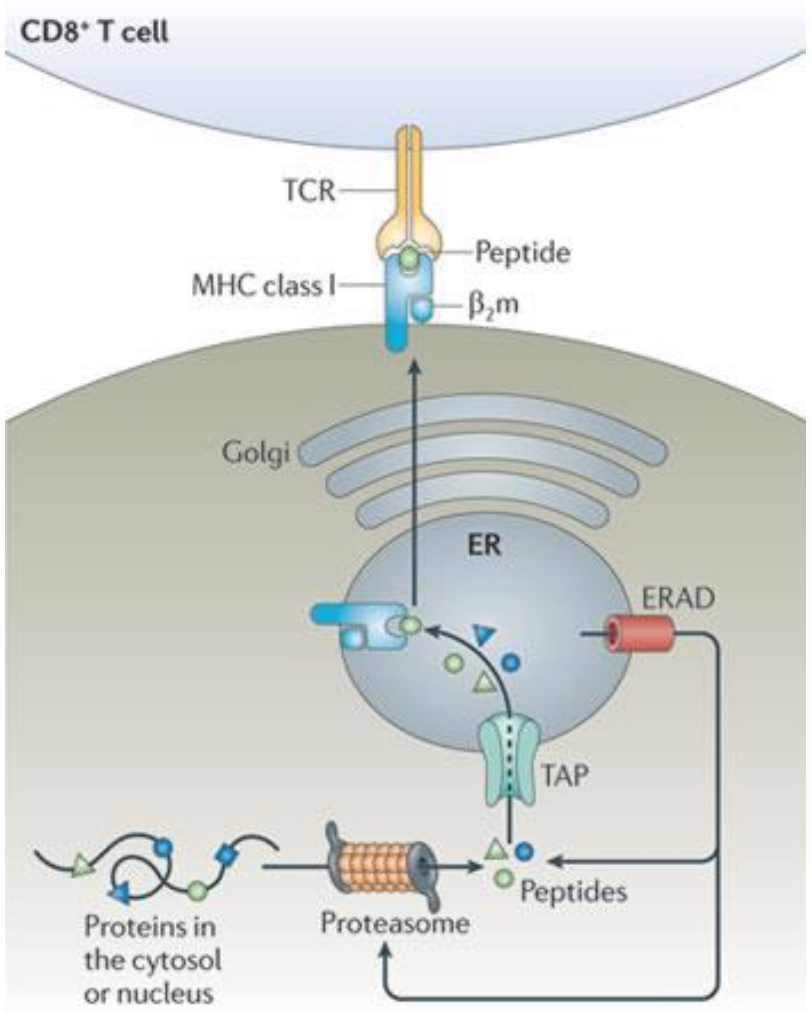
Interazione tra HLA e Recettori

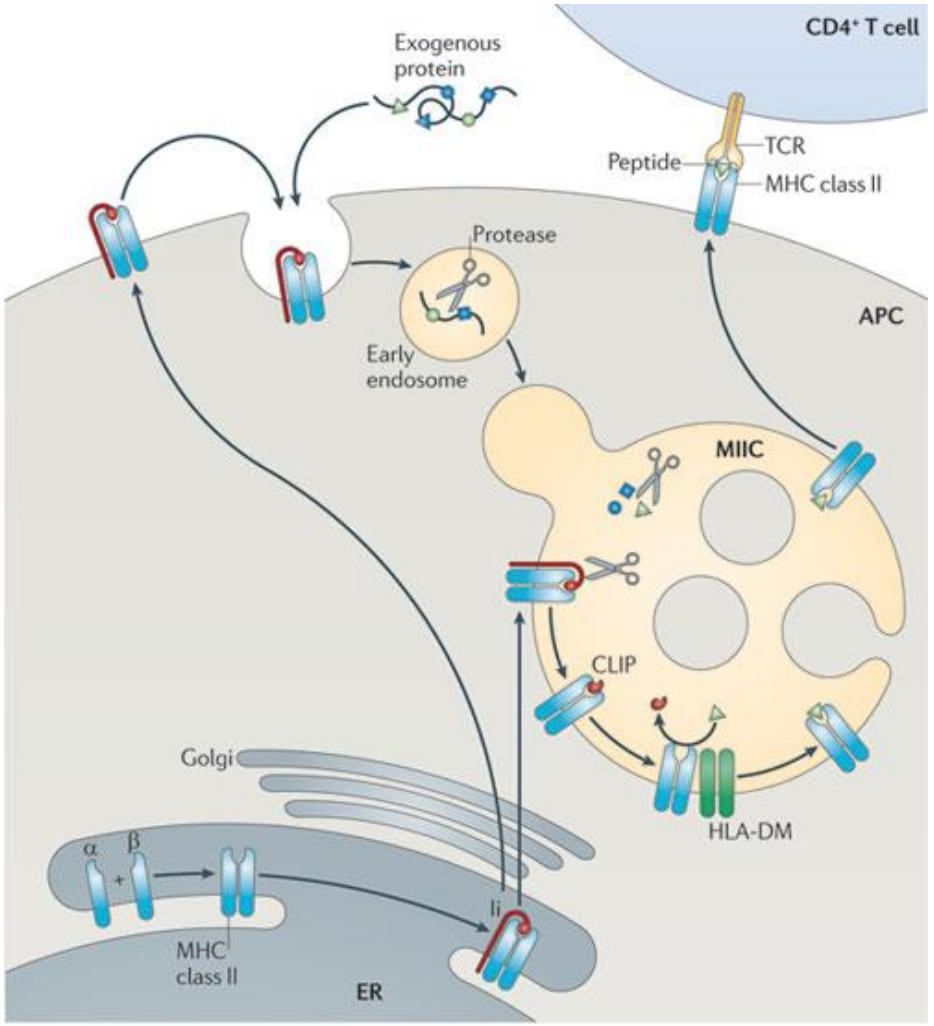






Presentazione HLA Classe I e Classe II



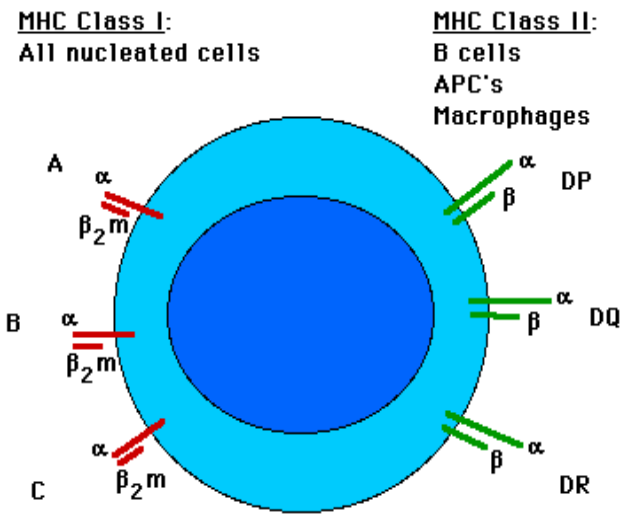


Neefjes et al. Nat Rev Immunol, 2011

Function of HLA Molecules

HLA Class I (ubiquituous)

- Antigen Presentation to cytotoxic T cells (Adaptive Immunity)
- Immune control from Infection, Tumors
- Self-Nonself Discrimination (Alloreactivity)
- Inhibiton of Natural Killer Cell Cytotoxicity



HLA Class II (Immune-related cells)

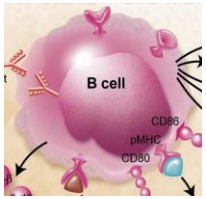
- Antigen Presentation to helper T cells (Adaptive Immunity)
- Immune control from Infection, Tumors
- Self-Nonself Discrimination (Alloreactivity)

Alloreactivity

Reactivity to Non-Self (Allos=Other)

Humoral

B Cell

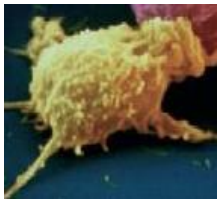


Alloantibodies

- Solid Organ Transplantion
- HLA-incompatible SCT

Cellular

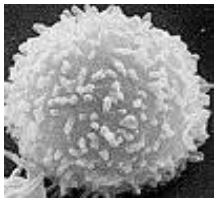
NK Cell



Lysis; Cytokines

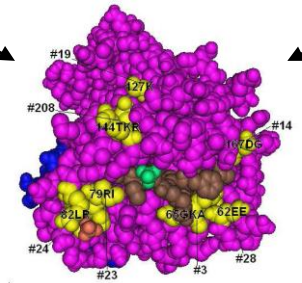
- HLA incompatible SCT
- Solid Organ Transplantion?

T Cell

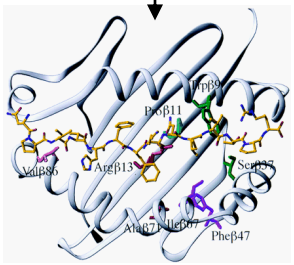


Lysis; Cytokines

- Allogeneic SCT
- Solid Organ Transplantion



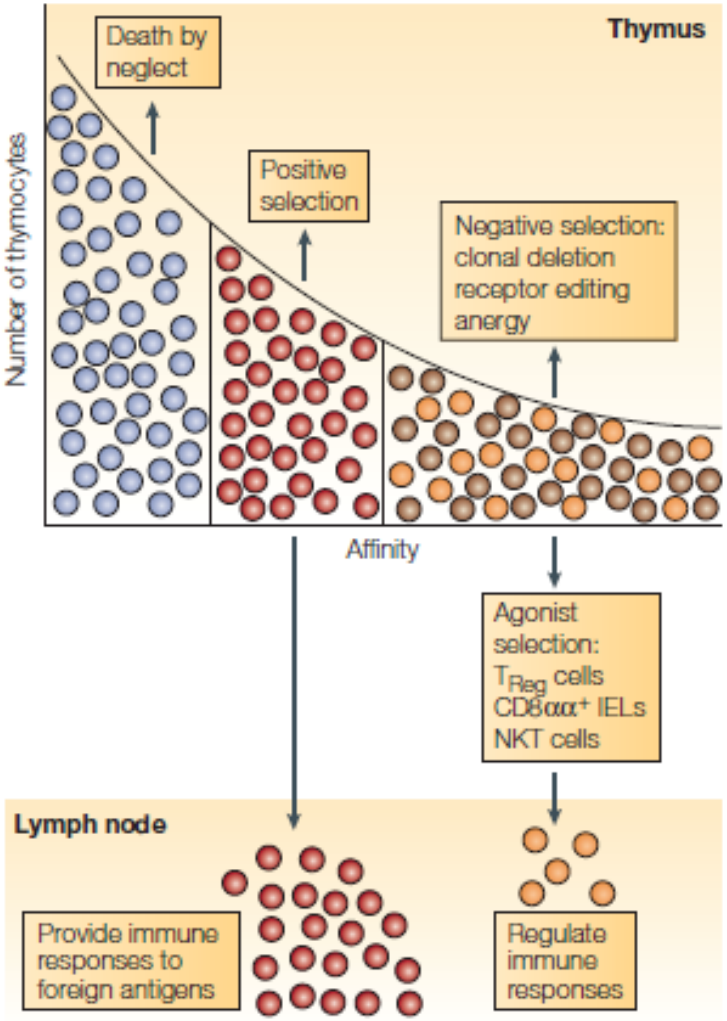
Structural Epitopes (Eplets)



Peptide-dependent T Cell Epitopes (TCE)

T Cell Alloreactivity

Central Tolerance – Clonal Deletion



- Positive Selection for T cells with
  - ➢ low affinity for self-HLA
- Negative Selection against T cells with
  - ➢ no affinity for self-HLA
  - ➢ too high affinity for self-HLA

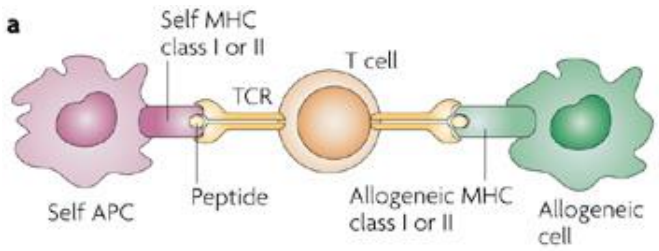
↓

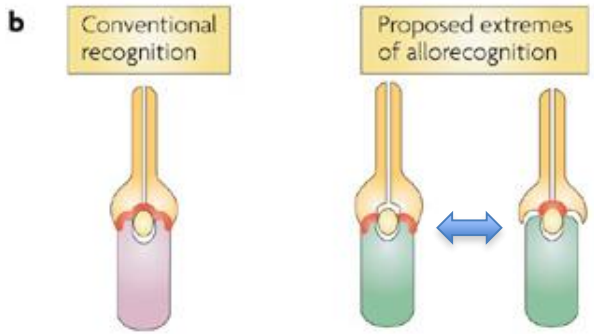
Deletion of T cells reactive to dominant TCE!

Hogquist, Nat Rev Immunol 2005

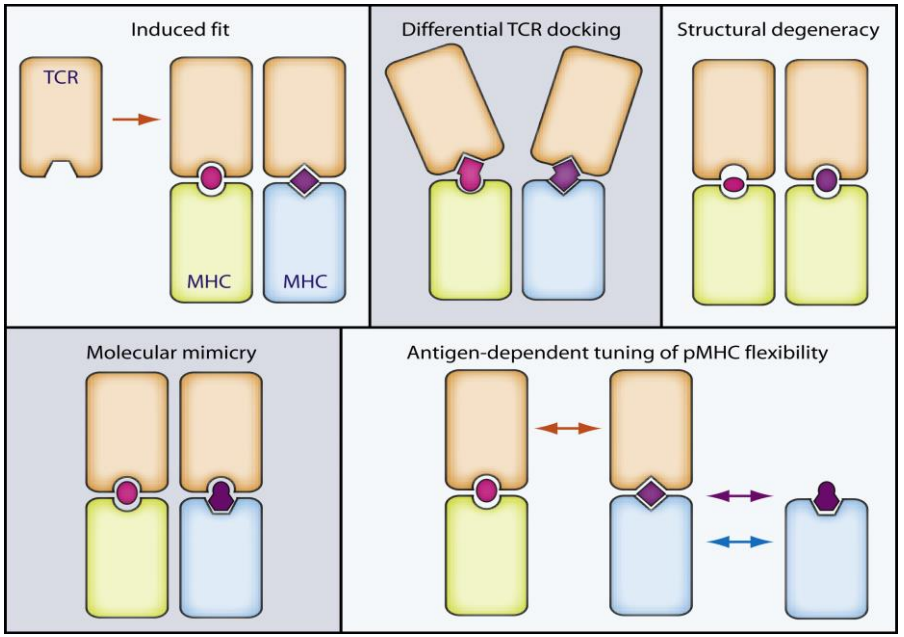
Mechanism of T cell allorecognition

Indirect Direct



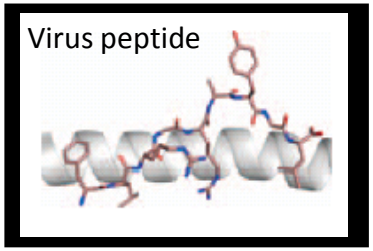


5 different models of cross-recognition

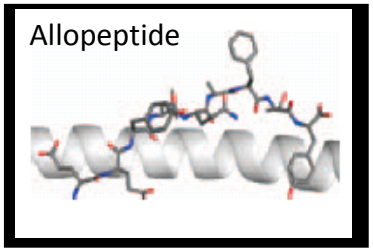


Molecular mimicry

HLA-B8 (Self)



HLA-B44 (Allo)



Unrelated HSCT Rejection

Serologically HLA-identical MUD

BONE MARROW–ALLOGRAFT REJECTION BY T LYMPHOCYTES RECOGNIZING A SINGLE AMINO ACID DIFFERENCE IN HLA-B44

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NANCY A. KERNAN, M.D.,
RICHARD J. O'REILLY, M.D.,
BO DUPONT, M.D., D.SC.,
AND SOO YOUNG YANG, PH.D.

Fleischhauer et al., New Engl J Med, 1990

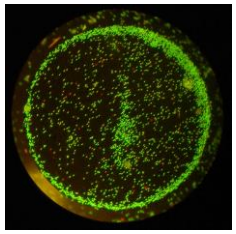
Unrelated HSCT Rejection

Serologically HLA-identical MUD

Patient

Serologically HLA-identical (B44)

Donor

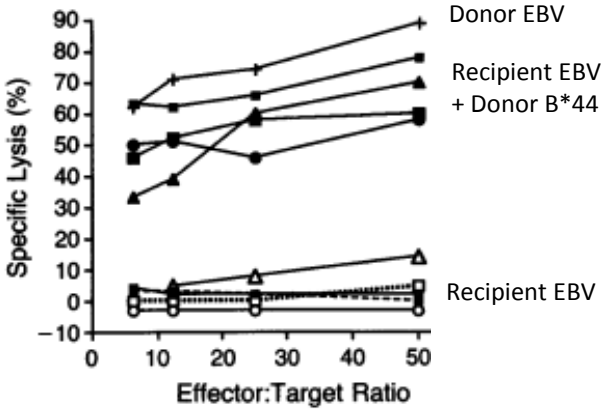


Acute HSCT-Rejection

Test Patient T Cell Alloreactivity

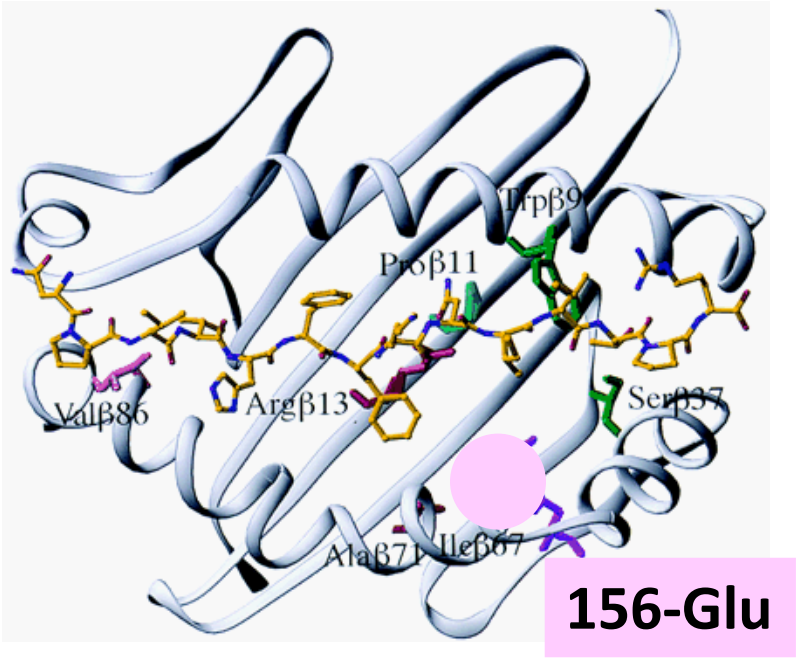
Specific Cytotoxicity against Donor Cells

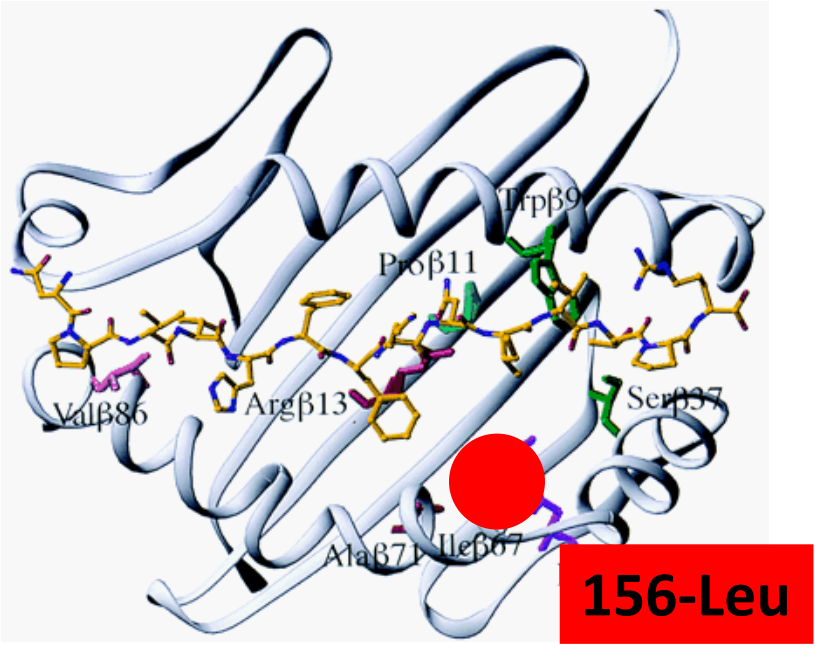
Cytotoxicity Assay



Fleischhauer et al., New Engl J Med, 1990

Alloreattività T può discriminare un singolo aa





Paziente: HLA-B*44:02

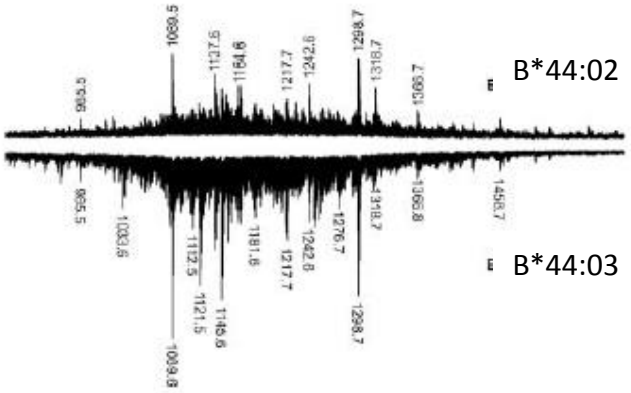
Donatore: HLA-B*44:03

Fleischhauer et al., New Engl J Med, 1990

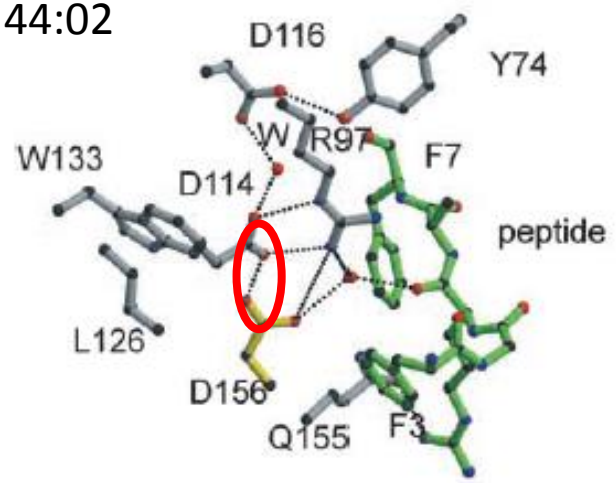
HLA-B*4402 vs B*4403: Structural differences...

HLA-B*4402	Position 1	2	3	4	5	6	7	8	9
Anchor residue		E							Y F W
Preferred residue	E		D M	P					

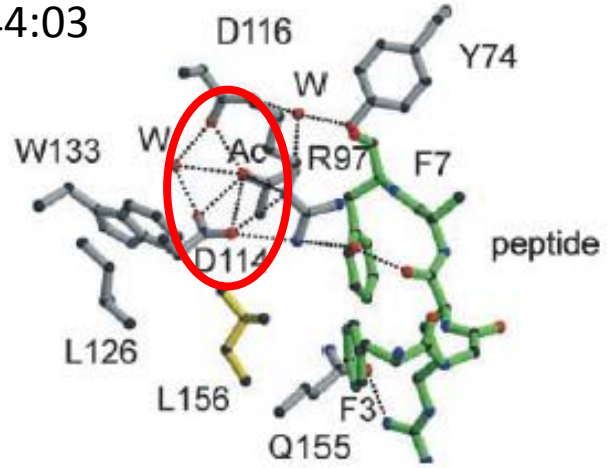
HLA-B*4403	Position 1	2	3	4	5	6	7	8	9
Anchor residue		E							Y F
Preferred residue	E		D M V	P					



B*44:02



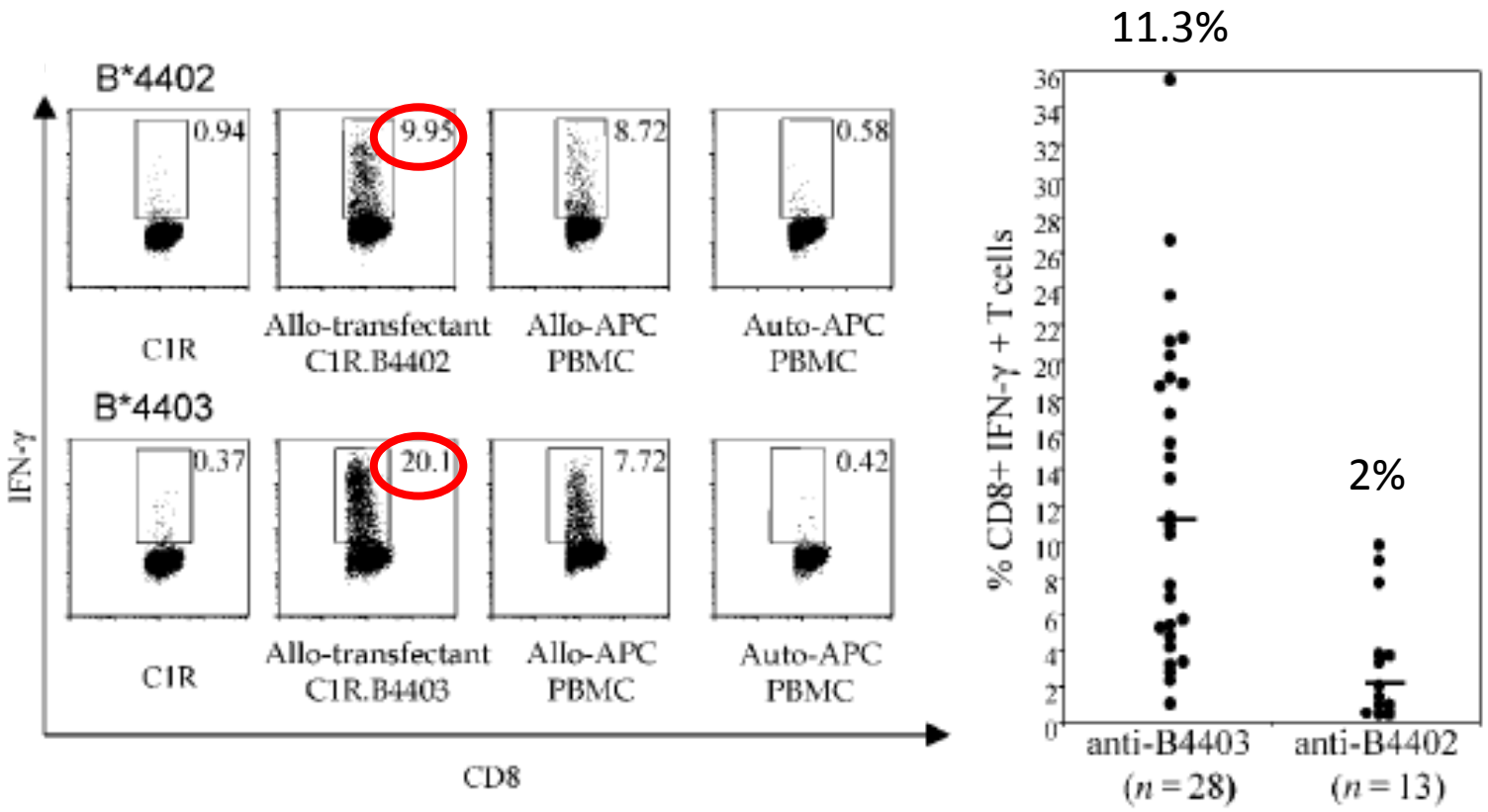
B*44:03



- Similar peptide motifs and 5% unique peptides
- 80% of these were presented only by B*4403 (higher promiscuity)

MacDonald, J Exp Med, 2003

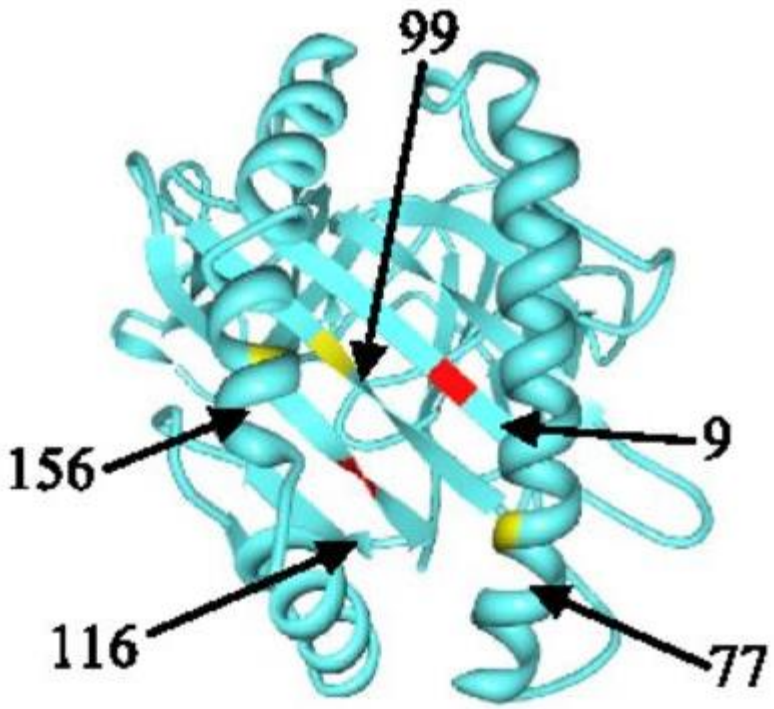
...and functional implications

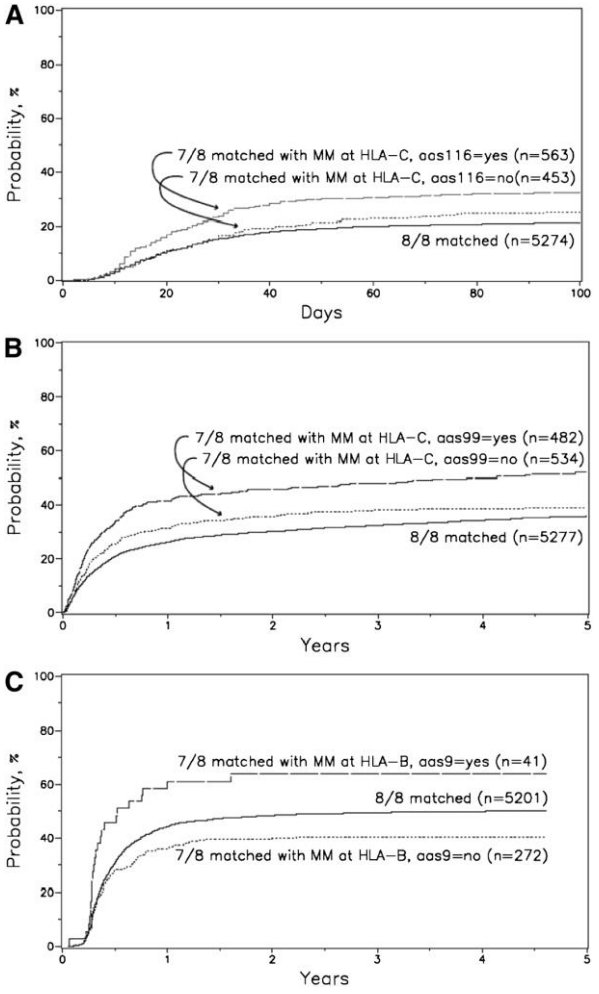


- Stronger Immunogencity of HLA-B*44:03 vs B*44:02

MacDonald, J Exp Med, 2003

HLA-C: Mismatches nella PBG e incidenza di GvHD





Pidala et al., Blood, 2013

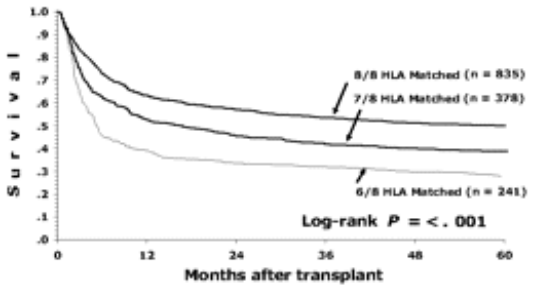
Structural Matching

8/8 Allelic Identity Improves Survival after VUD-HSCT

- 3857 VUD Tx from unrelated adult volunteer donors *(Lee, Blood 2007)*

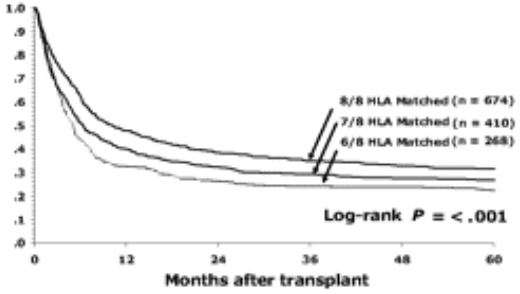
Early Stage

P<0.001



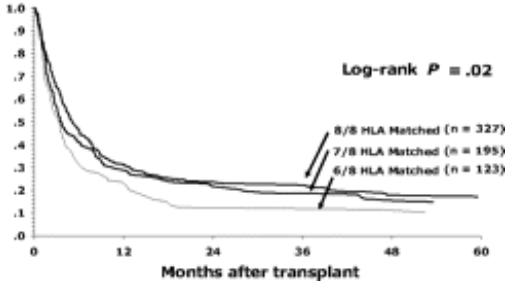
Intermediate Stage

P<0.001



Late Stage

P=0.02



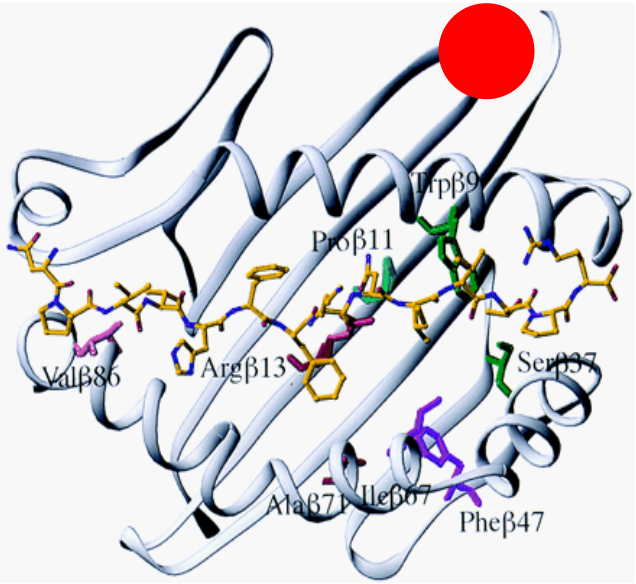
MAINLY IN PATIENTS WITH EARLY DISEASE

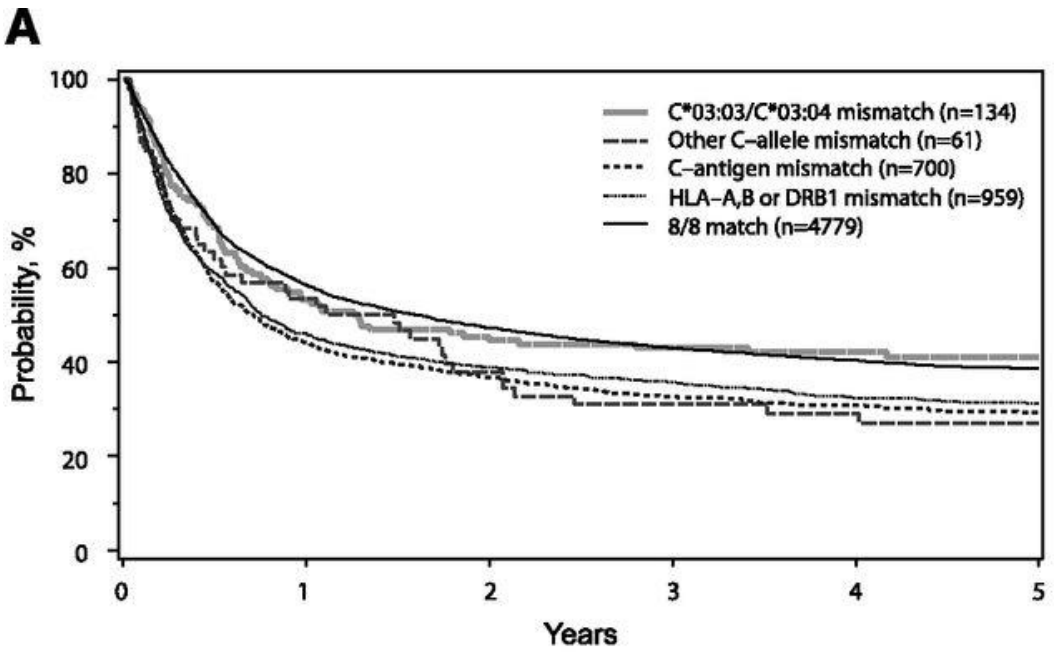
Lee et al., Blood, 2007

E’ sempre vero?

Line	Allele	Population	% of individuals that have the allele	Allele Frequency (in_decimals)	Sample Size	IMGT/HLA[1] Database	Distribution[2]	Haplotype[3] Association	Notes[a]
1	C*03:03	Germany pop 8		0.0511	39,689	See			
2	C*03:04	Germany pop 8		0.0741	39,689	See			

pos 91





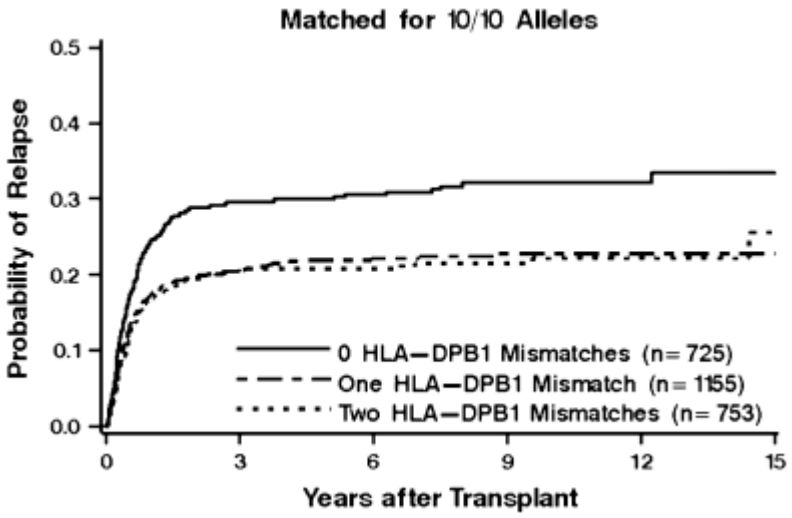
Fernandez Vina et al., Blood, 2014

Relevance of HLA-DP mismatches in HSCT

- 80% of MUD-HSCT across HLA-DPB1 mismatches
- Protection from Relapse, Higher GvHD, No net effect on Survival

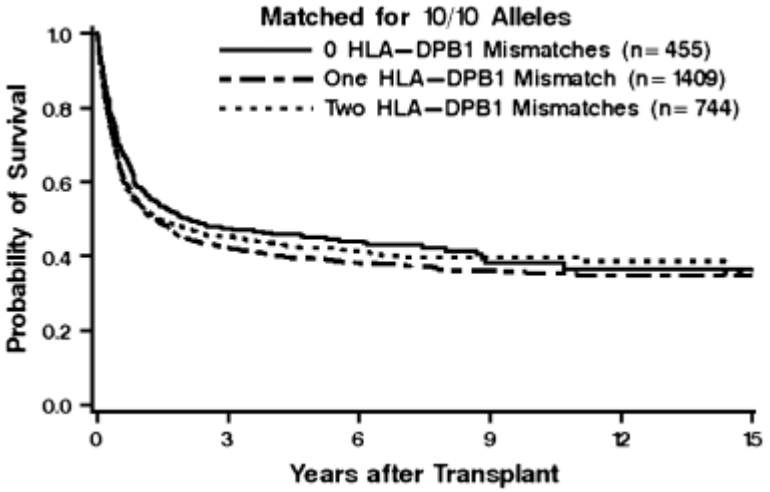
Relapse

HR 0.68; p=0.0001



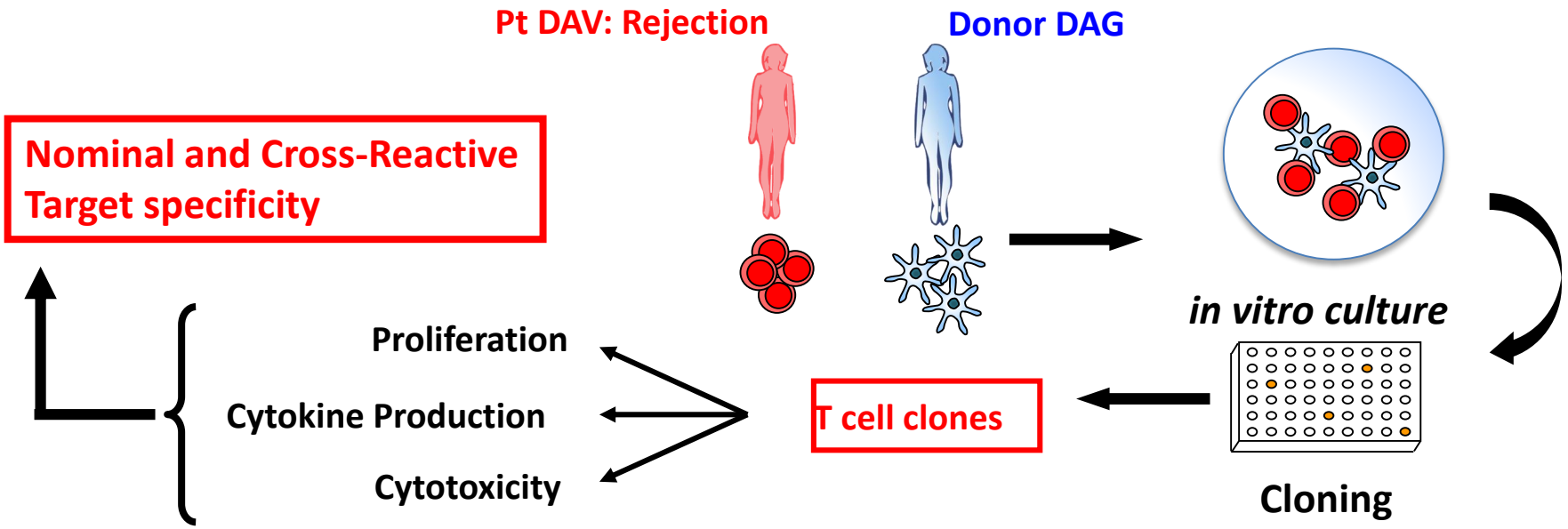
Survival

HR 1.08; p=0.1



Shaw et al., Blood, 2007

HLA-DP mismatch target of Rejection



Functional Characterization of alloreactive T cells from a patient with rejection of a 10/10 matched HSCT allograft

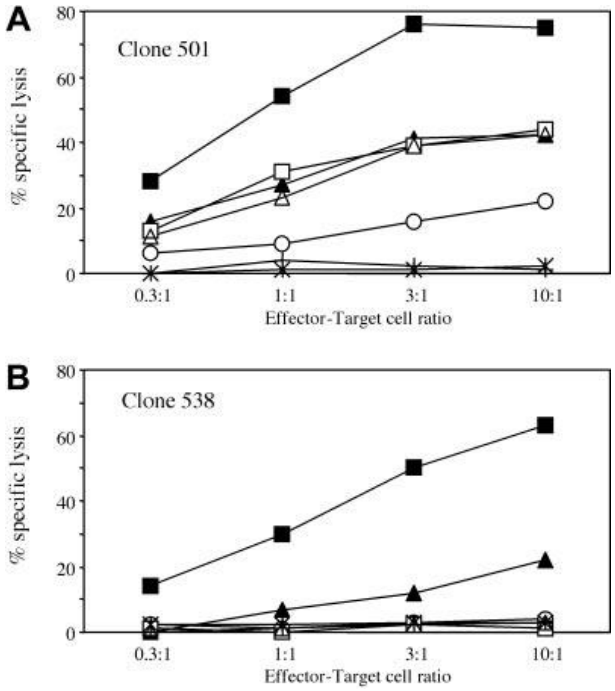
Target alloantigen on donor cells was HLA-DP9

Fleischhauer et al., Blood, 2001

Cross-Reactive anti-DPB1 Specificities

- Cross-reactivity of T cell clones from Patient DAV with rejection
- Patient DAV: DPB1*0201,*0401; Donor DAG: DPB1*0201,*0901

Nominal Ag: DPB1*0901



DPB1*1001

DPB1*1701

DPB1*0301

DPB1*1401

DPB1*4501

DPB1*1001

DPB1*1701

Group 1: DPB1*0901,1001,1701

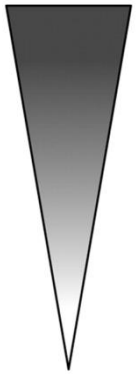
Group 2: DPB1*0301,1401,4501

Group 3: Others

Zino et al., Blood, 2004

Summarizing DPB1 Immunogenicity Groups

Immunogenicity Hierarchy

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

← TCE3 Grouping

↓

60% permissive
40% non-perm.

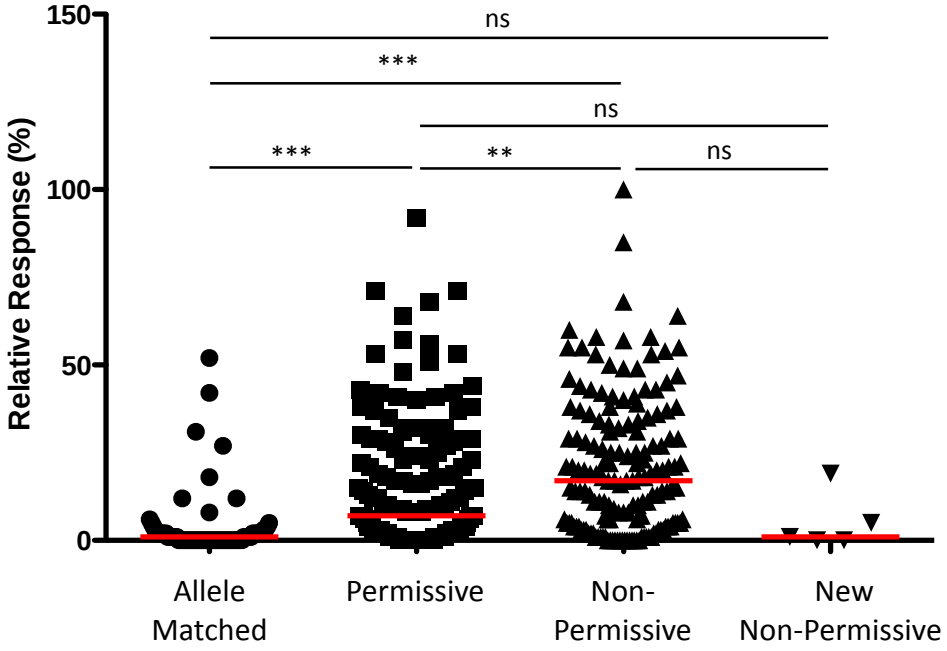
↓

Donor \ Recipient	1/1	1/2	1/3	2/2	2/3	3/3
1/1	permissive			non-permissive (HvG)		
1/2						
1/3						
2/2	non-permissive (GvH)			permissive		
2/3						
3/3						permissive

Zino et al., Blood, 2004

MLC Responses: Non-permissive vs Permissive

N=419 MLC



N	75	179	161	5
Median	1	7	17	1

P<0.001

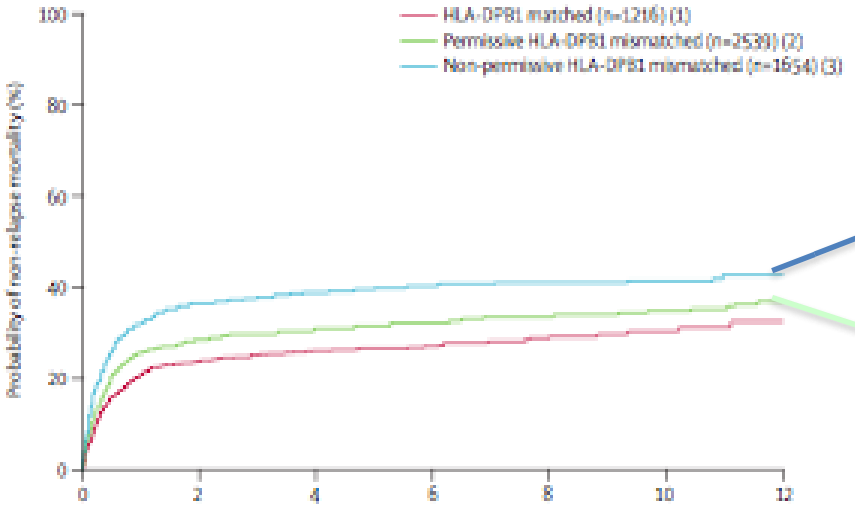
Sizzano et al., Blood, 2010

From Allele- to T cell epitope- (TCE-) matching for DPB1

Effect of T-cell-epitope matching at HLA-DPB1 in recipients of unrelated-donor haemopoietic-cell transplantation: a retrospective study

Katharina Fleischhauer, Bronwen E Shaw*, Theodore Gooley, Mari Malkki, Peter Bardy, Jean-Denis Bignon, Valérie Dubois, Mary M Horowitz, J Alejandro Madrigal, Yasuo Morishima, Machteld Oudshoorn, Olle Ringden, Stephen Spellman, Andrea Velardi, Elisabetta Zino, Effie W Petersdorf, on behalf of the International Histocompatibility Working Group in Hematopoietic Cell Transplantation*

Non-Relapse Mortality



Non-permissive *TCE Mismatch*

Permissive *TCE Mismatch*

DPB1 Allele Mismatched

Fleischhauer et al., Lancet Oncol, 2012

From Allele- to T cell epitope- (TCE-) matching for DPB1

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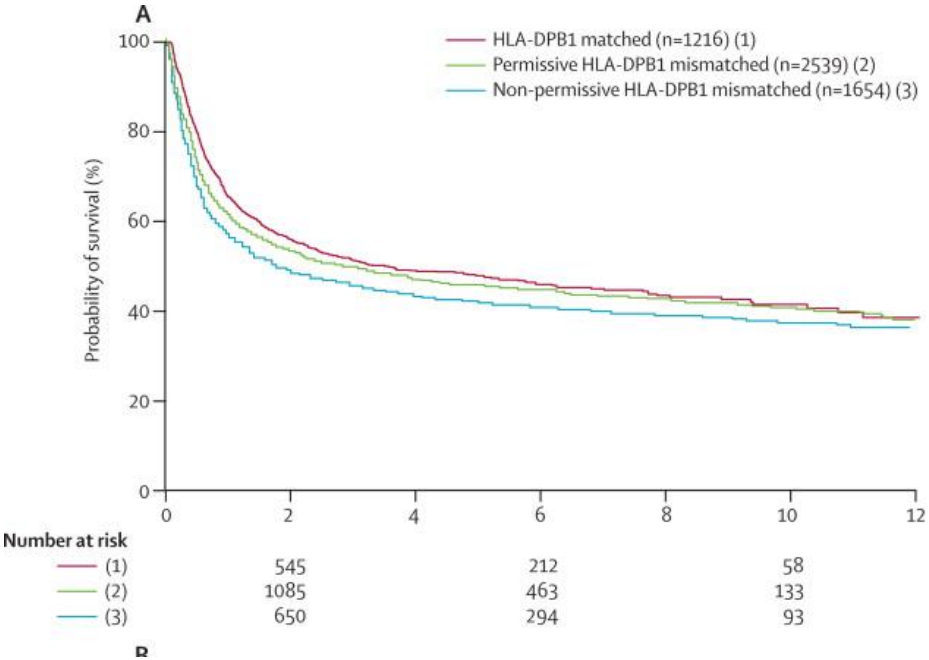
Overall Survival



Permissive *TCE Mismatch*

Non-permissive *TCE Mismatch*

DPB1 Allele Mismatched

Fleischhauer et al., Lancet Oncol, 2012

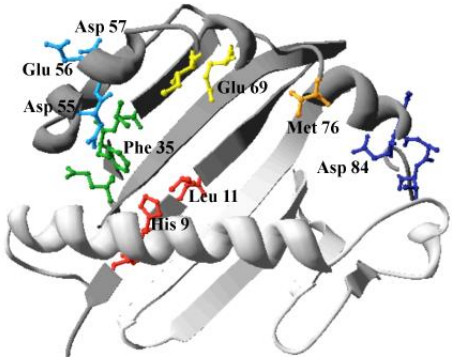
Amino acid Alignment of TCE-Group 1-3 DPB1 alleles

		A			B			C			D		E	F			
TCE Group	DPB1*	8	9	11	33	35	36	55	56	57	65	69	76	84	85	86	87
1	0901	V	H	L	E	F	V	D	E	D	I	E	V	D	E	A	V
	1001	-	-	-	-	-	-	-	-	E	-	-	-	-	-	-	-
	1701	-	-	-	-	-	-	-	-	-	-	-	M	-	-	-	-
2	0301	-	Y	-	-	-	-	-	-	-	L	K	-	-	-	-	-
	1401	-	-	-	-	-	-	-	-	-	L	K	-	-	-	-	-
	4501	-	-	-	-	-	-	-	-	E	L	K	-	-	-	-	-
	8601	-	-	-	-	-	-	-	-	-	-	-	M	G	G	P	M
3	0101	-	Y	G	-	Y	A	A	A	E	-	K	-	-	-	-	-
	0201	L	F	G	-	-	-	-	-	E	-	-	M	G	G	P	M
	0401	L	F	G	-	-	A	A	A	E	-	K	M	G	G	P	M
	0402	L	F	G	-	-	-	-	-	E	-	K	M	G	G	P	M

Polymorphic Key Amino Acids for TCE

Site Directed Mutagenesis of HLA-DPB1*09:01

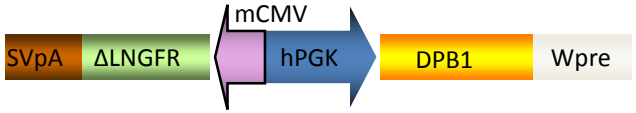
HLA-DP Homology Modeling



Site-Directed Mutagenesis of DPB1*09:01

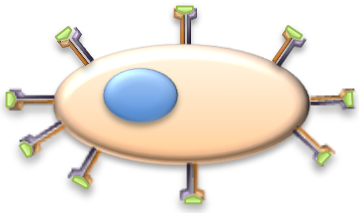
DPB1*09:01	9	35	55	56	57	69	76	84
	H	F	D	E	D	E	V	D
LVM1	Y							
LVM2	F							
LVM3		Y						
LVM4		L						
LVM5			A					
LVM6								G
LVM8				A				
LVM9							M	
DPB1*10:01					E			
DPB1*35:01						K		

Bi-directional Lentiviral Expression Platform



Amendola et al., Nat Biotechnol 2005

Read-Out BLCL MGAR

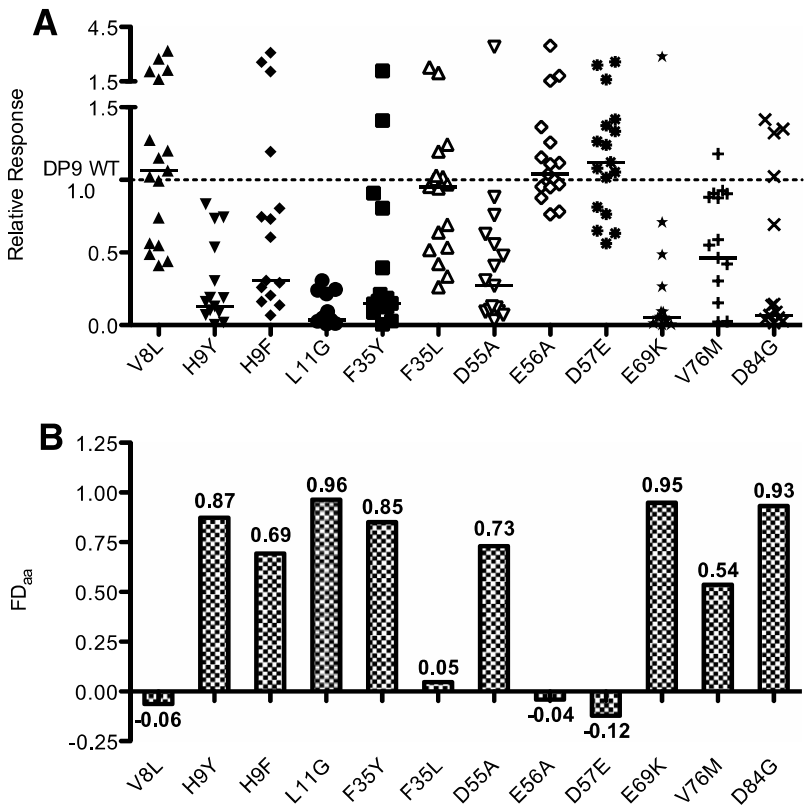


Endogenous DPB1*04:01
(TCE Group 3)

Crivello et al., Biol Blood Marrow Transpl, 2015

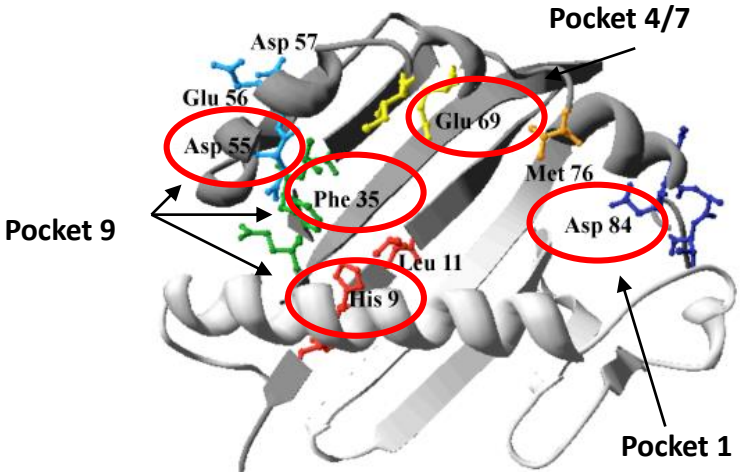
Polymorphic Key Amino Acids for TCE

17 Alloreactive T cell clones



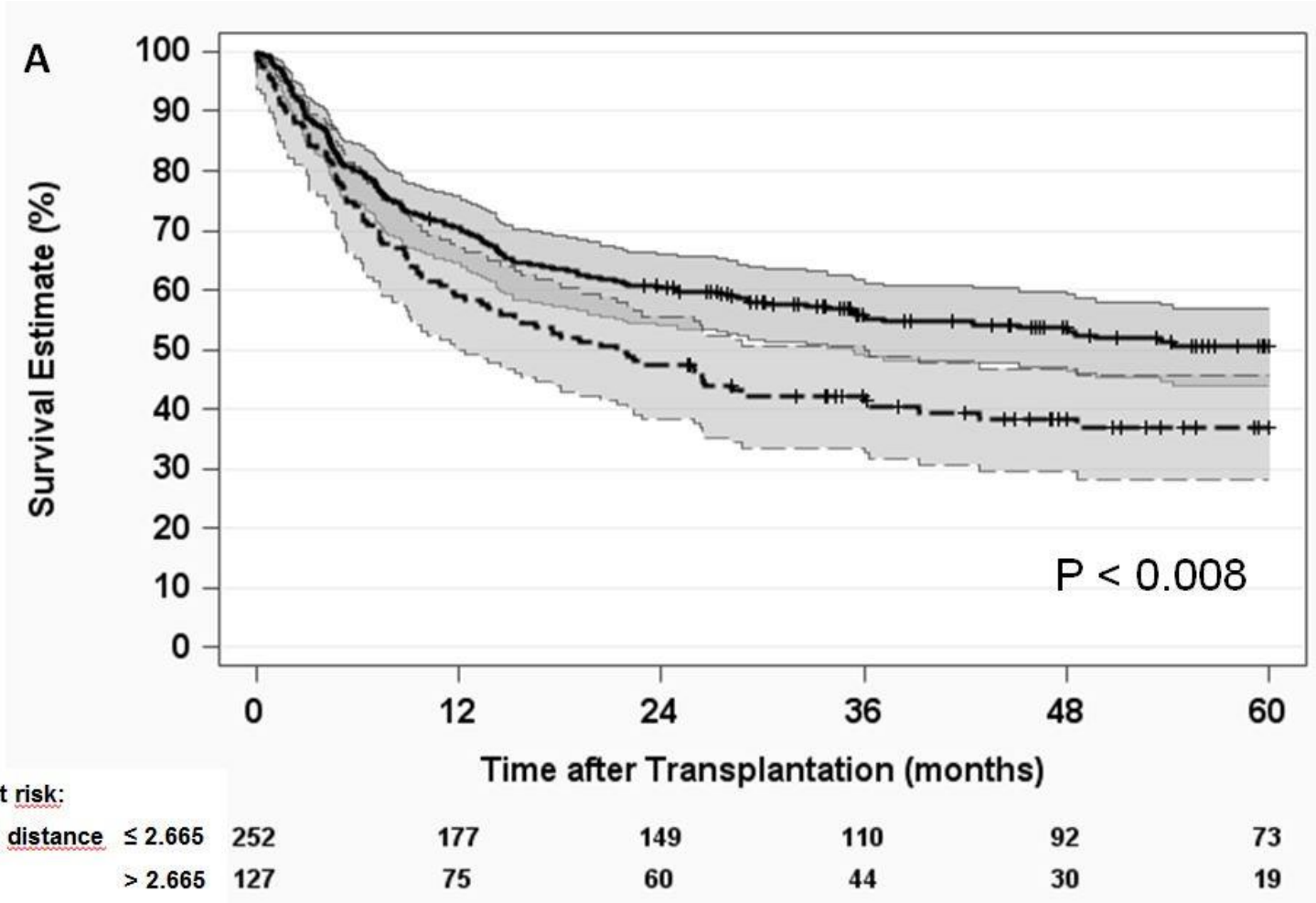
Relevant Amino Acid Residues

DPB1*09:01	9	35	55	56	57	69	76	84
	H	F	D	E	D	E	V	D
H9Y	Y							
H9F	F							
F35Y		Y						
F35L		L						
D55A			A					
D84G								G
E56A				A				
V76M							M	
D57E					E			
E69K						K		



Crivello et al., Biol Blood Marrow Transpl, 2015

TCE Functional Distance and Outcome



Crivello et al., Blood, 2016

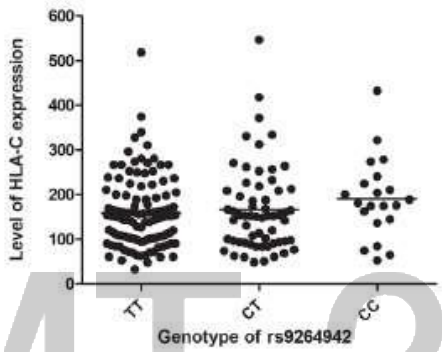
E’ Solo Una Questione di Struttura?

Livello di Espressione di HLA-C e Outcome

A

HLA-C allele	rs9264942 allele	Haplotype frequency	D'	p value
04:01	T	0.176	0.95	<0.0001
07:01	T	0.091	0.06	NS
16:01	T	0.086	0.85	<0.0001
06:02	C	0.085	0.94	<0.0001
17	T	0.071	1.00	<0.0001
07:02	T	0.061	0.99	<0.0001
03:04	T	0.055	0.90	<0.0001
18	T	0.044	0.93	<0.0001
08:02	C	0.033	0.83	<0.0001
05:01	C	0.028	0.97	<0.0001
02:02	C	0.025	0.31	<0.0001
02:10	T	0.021	0.26	NS
03:02	C	0.021	0.92	<0.0001
12:03	C	0.019	0.95	<0.0001
14:02	C	0.019	0.95	<0.0001
15:05	T	0.014	0.54	NS
08:04	C	0.013	0.97	<0.0001
03:03	T	0.010	0.97	0.03

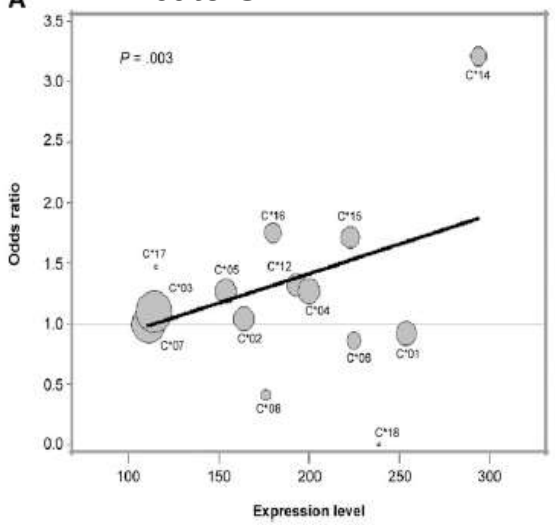
B



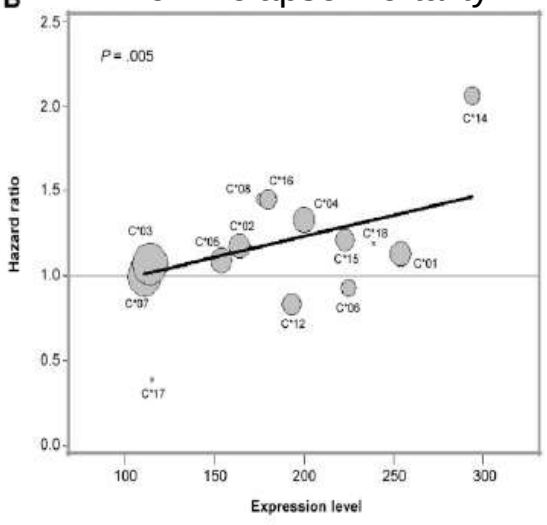
Apps 2013

EBMT 2016

A Acute GvHD III-IV



B Non-Relapse Mortality



Petersdorf 2014

E’ Solo Una Questione di Struttura?

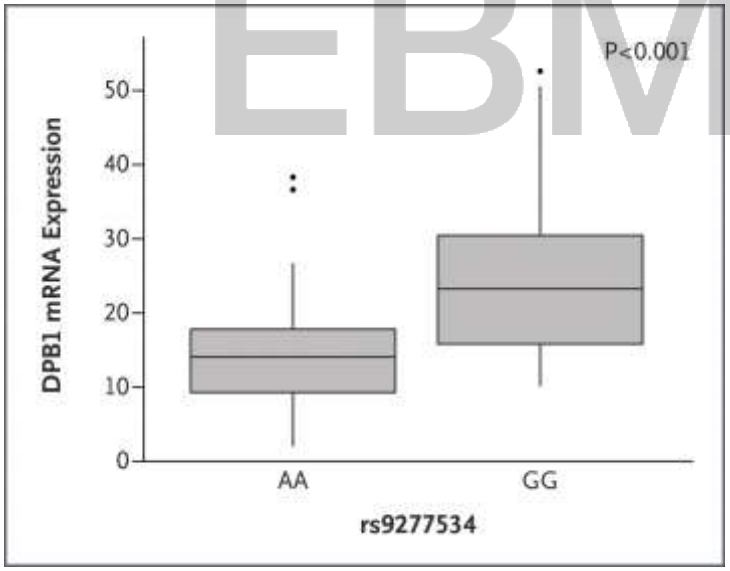
Livello di Espressione di HLA-C e Outcome

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

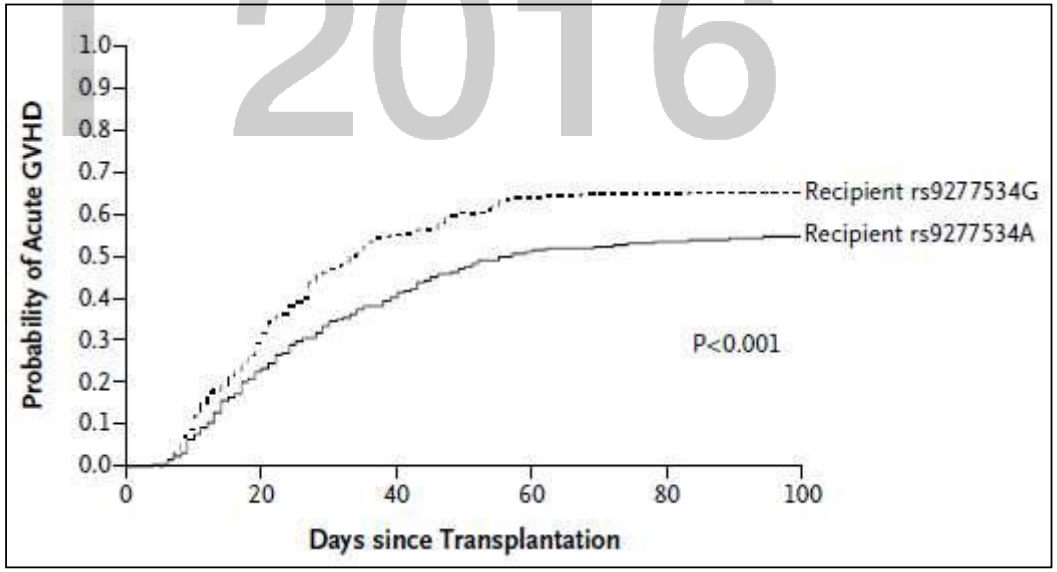
Effie W. Petersdorf, M.D., Mari Malkki, Ph.D., Colm O'hUigin, Ph.D., Mary Carrington, Ph.D., Ted Gooley, Ph.D., Michael D. Haagenson, M.S., Mary M. Horowitz, M.D., Stephen R. Spellman, M.B.S., Tao Wang, Ph.D., and Philip Stevenson, M.S.

N Engl J Med 373:599-609 (2015)

SNP rs9277534-G high expression



Kaplan Meier Probability of acute GvHD II-IV



EBMT 2016

Take Home Messages

- Ora che le tecnologie di tipizzazione ci permettono di ottenere un elevatissimo dettaglio nella tipizzazione HLA, diviene essenziale caratterizzare funzionalmente quali differenze strutturali/incompatibilita' siano funzionalmente rilevanti

- E' ancora largamente da esplorare l'impatto dei livelli di espressione dei singoli alleli HLA sui fenomeni di alloreattività