Challenges of Tissue Typing and Allocation for Kidney Transplantation

Towards Better Matching For Better Outcome of Kidney Transplantation

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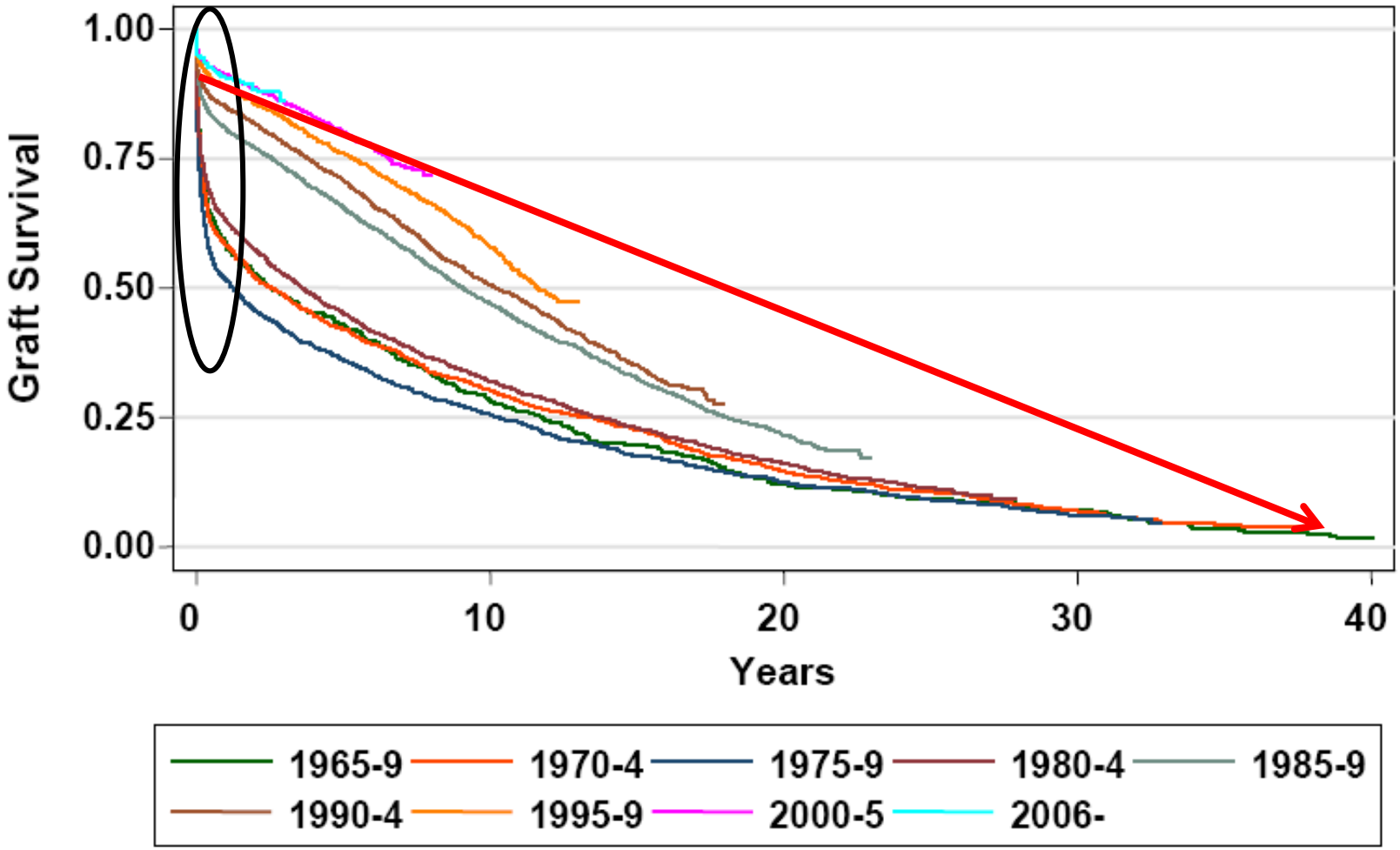
Cairo University

Junior Master Classes - ESPNT 6th Oct 2023

Longer-term outcomes remain a challenge in kidney transplantation

Primary graft survival of deceased donors by year of transplant to 31/12/07: Australia and NZ



ANZDATA 31st annual report 2008 (http://www.anzdata.org.au/anzdata/AnzdataReport/31stReport/Ch08Txpart2.pdf)

Outline

Risk Stratification

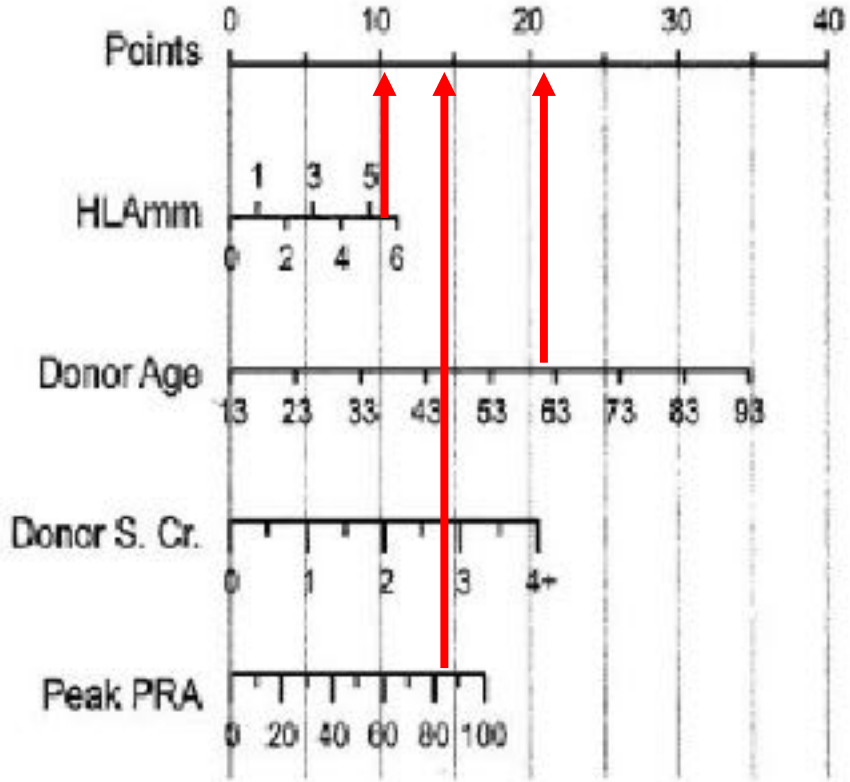
HLA specification

Eplet and Epitope Matching

AM Acceptable Mismatches & PM Permissive Mismatches

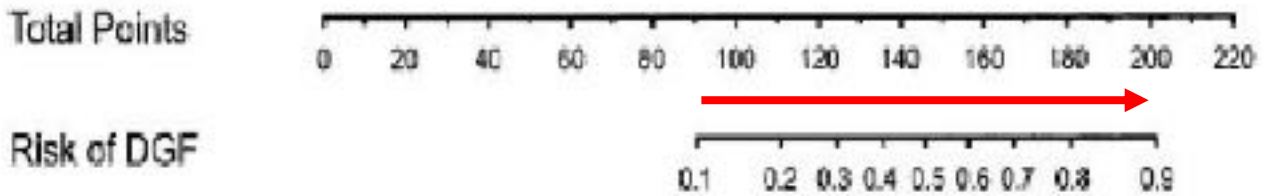
Detection of sensitization c-PRA vs TS

KDPI & EPTS

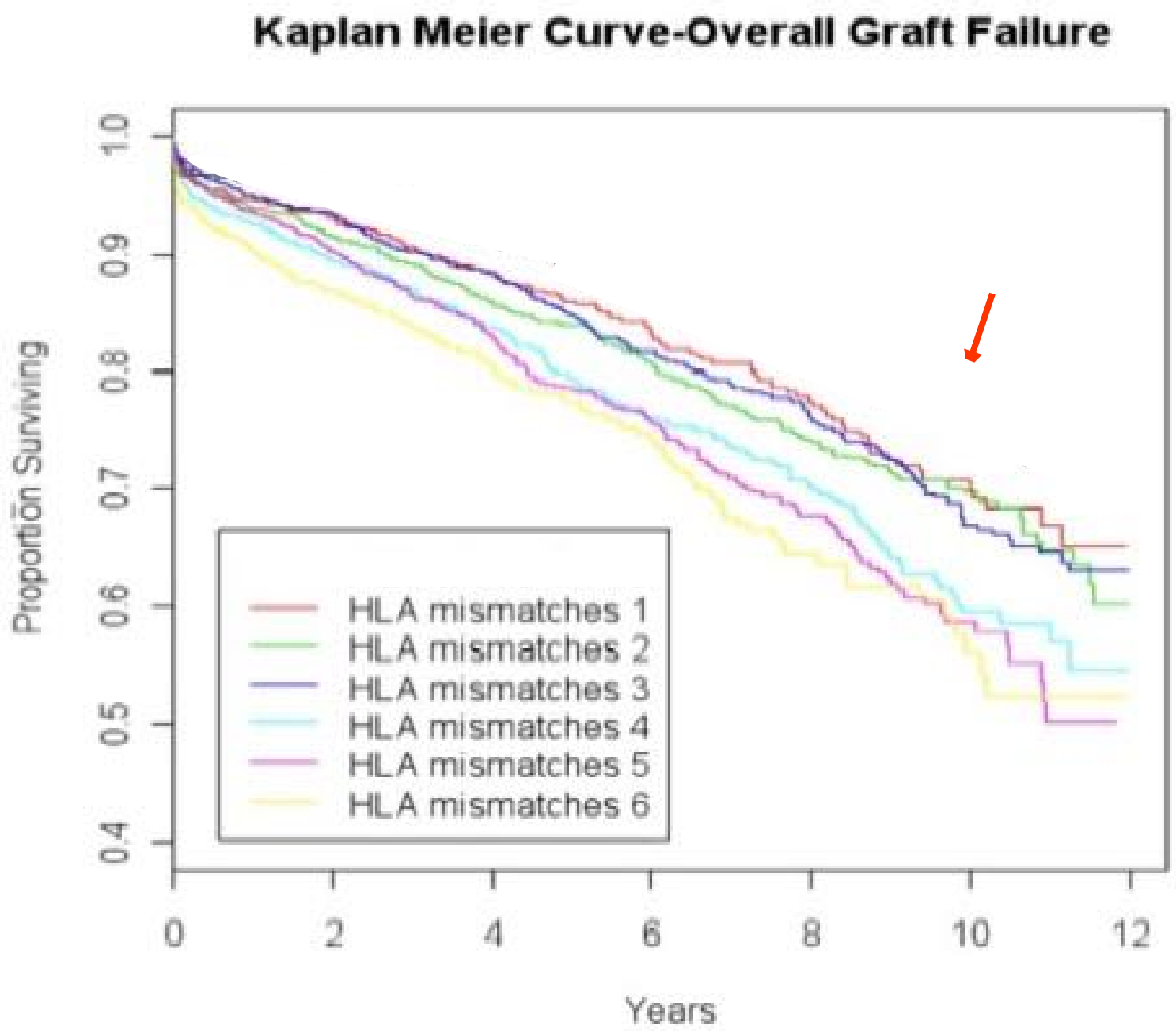


CIT Add 1 point for each hour

If		Add
RR = Black	& PID = Yes	46
RR = Black	& PID = No	44
RR = Non-Black	& PID = Yes	38
RR = Non-Black	& PID = No	18
SOT = Yes	& PID = Yes	40
SOT = No	& PID = Yes	20
Non-Heart Beating Donor		29
Recipient of Previous Transplant(s)		10
Donor Had Hypertension		7
Male Recipient		7
Diabetic Recipient		5
DCOD = Anoxia		5
DCOD = Cerebrovascular/Stroke		4
Pre-Transplant Transfusion		4

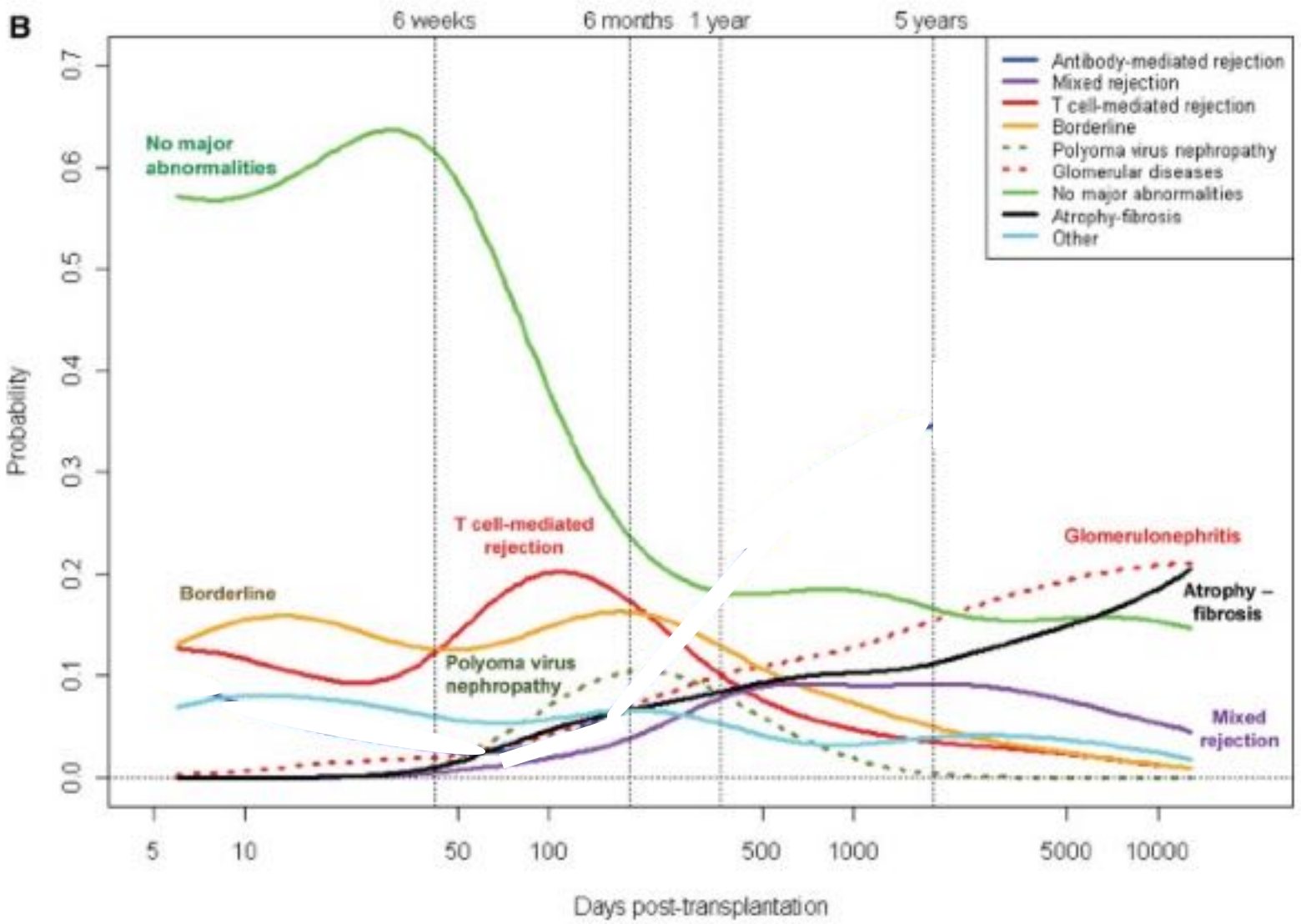


Abbreviations: DCOD = Donor Cause of Death PID = Pre-Transplant Dialysis
RR = Recipient Race SOT = Single Organ Transplant


Kaplan Meier Curve-Overall Graft Failure
Proportion Surviving
1.0
0.9
0.8
0.7
0.6
0.5
0.4
HLA mismatches 1
HLA mismatches 2
HLA mismatches 3
HLA mismatches 4
HLA mismatches 5
HLA mismatches 6
0
2
4
6
8
10
12
Years

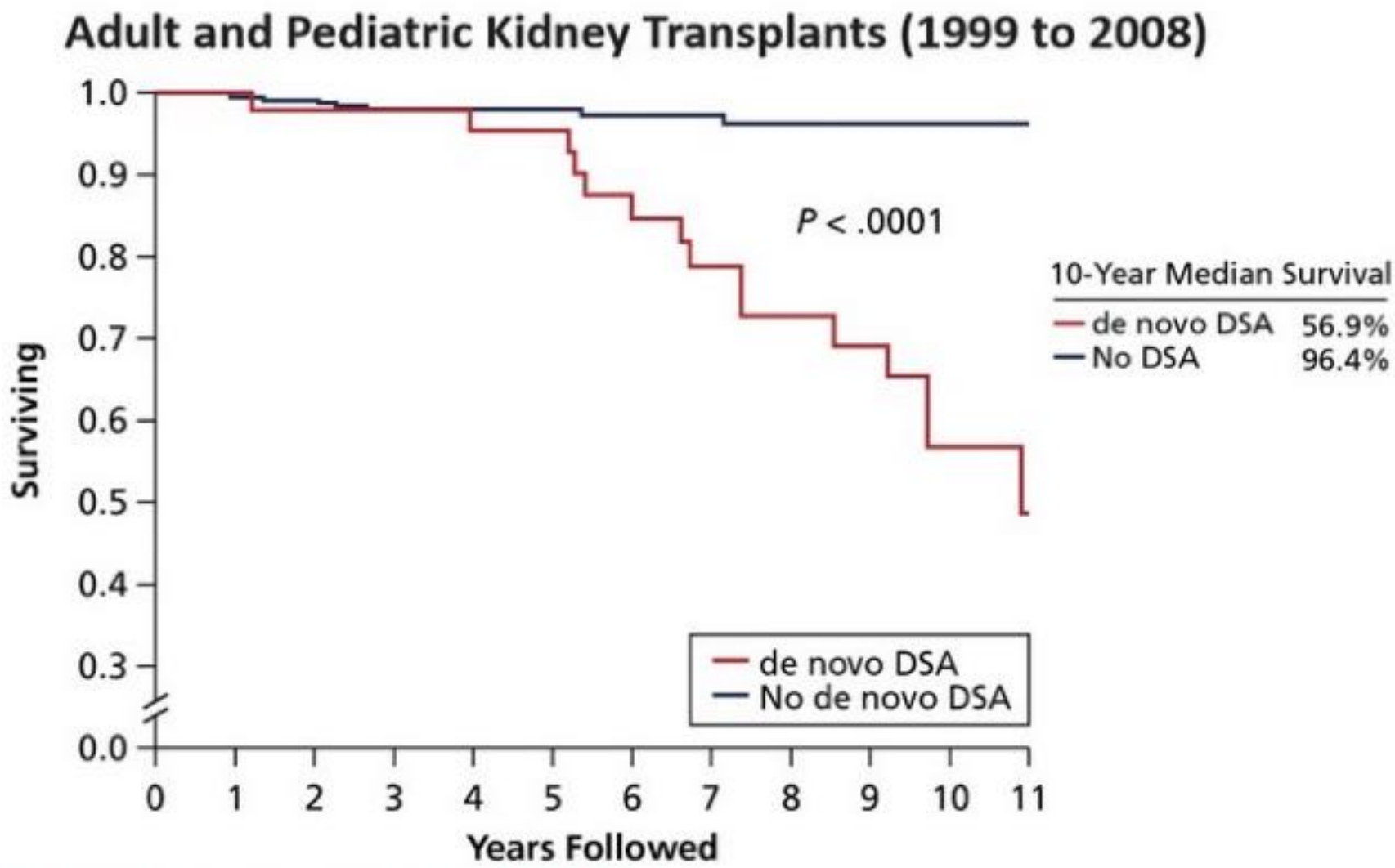
	Number of patients				Total
Non-adherent[e]	0	1	0	25	26
Adherent or Unknown[f]	59	39	30	161	289

Sellares J et al. AJT 2011



Understanding the Causes of Kidney Transplant Failure: The Dominant Role of Antibody-Mediated Rejection and Nonadherence

De Novo DSA and Graft Survival[1]



1. Wiebe C et al. *Am J Transplant*. 2012;12:1157-1167.

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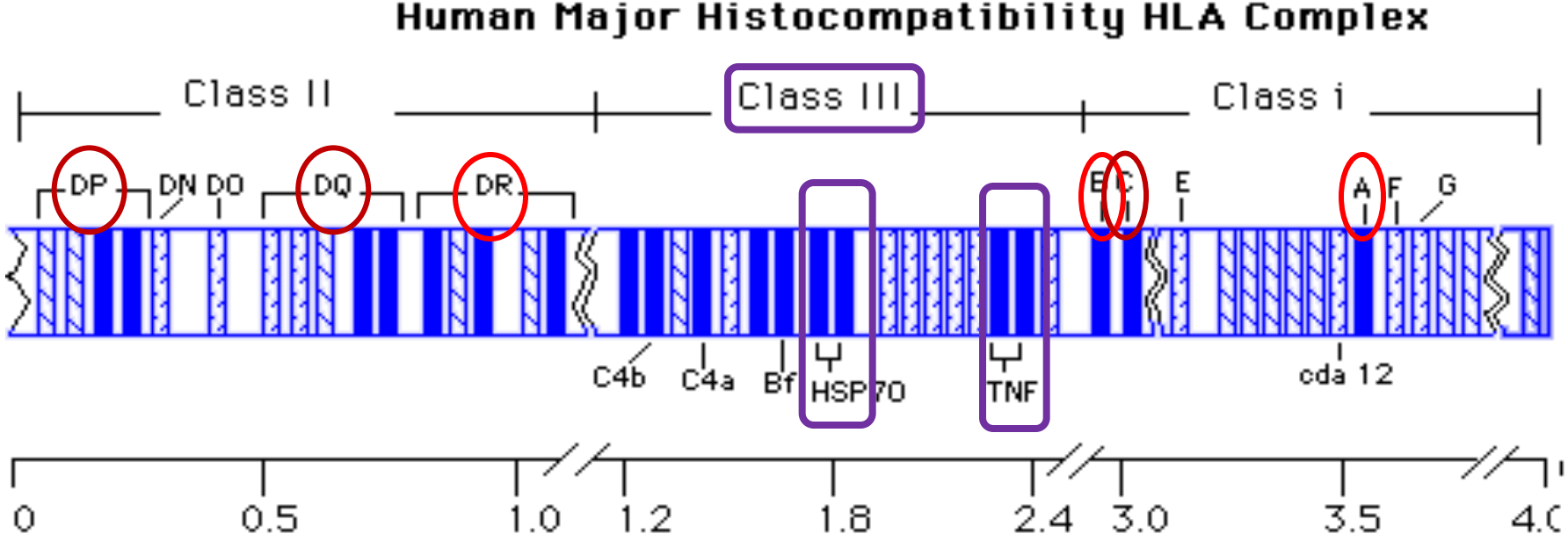


Expressed genes

Unknown Status

Pseduogenes genes (which are homologous to expressed genes occurring elsewhere in the genome)

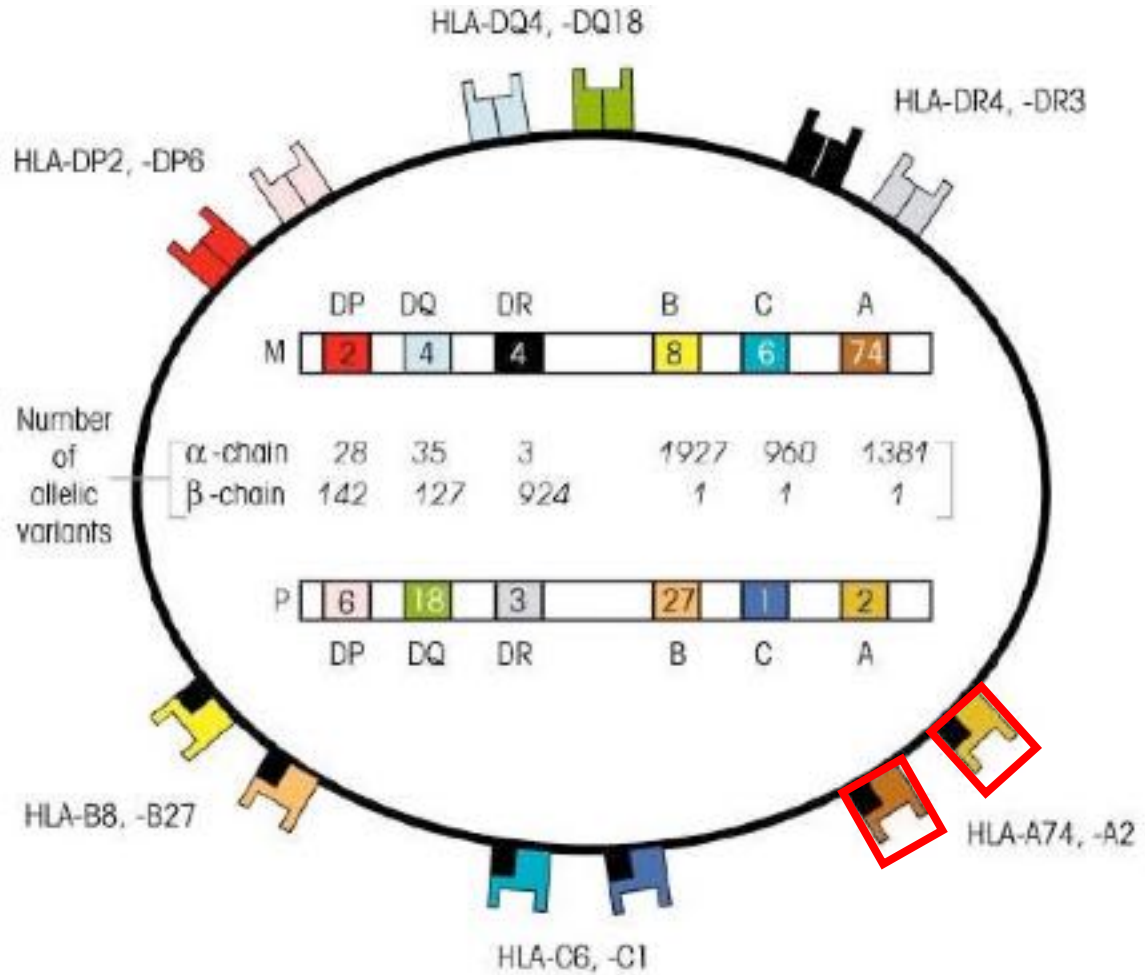
Class 1 antigens
Glycoproteins (40 ro 45kD) see figure 7.7

Class II antigens

Short limb of

Chromosome 6

Polymorphic HLA specificities and their inheritance



Delves *et al. Roitt's Essential Immunology, 12th ed.*

Name	الطفل/ احمد هشام سيد فاروق
Date Of Birth	09-05-2015
Ref. By Prof. Dr.	فتينه فاضل

Mismatch 2 / 6 & precisely 1-1-0 = 110

Name	الاستاذه/ ريهام رافت عبد قاسم
	Female

Molecular Biology
HLA Class I - A

Serologic Type: A24(9) A24(9)

Generic Type :HLA-A*24 & *24

Molecular Biology
HLA Class I - A

Serologic Type: A1 A24(9)

Generic Type :HLA-A*01 & *24

Molecular Biology
HLA Class I - B

Serologic Type: B38(16) ; B51(5)

Generic Type :HLA-B*38 & *51

Molecular Biology
HLA Class I - B

Serologic Type: B63(15) B51(5)

Generic Type :HLA-B*15 & *51

Molecular Biology
HLA Class II DR

Serologic Type: DR13(6) ; DR14(6)

Generic Type :HLA-DRB1*13 & *14

Molecular Biology
HLA Class II DR

Serologic Type: DR13(6) DR14(6)

Generic Type :HLA-DRB1*13 & *14

Name	الطفل/ احمد هشام سيد فاروق	Req
Date Of Birth	09-05-2015	
Ref. By Prof. Dr.	فتينه فاضل	

Mismatch 1 / 6 & precisely 0-1-0 = 010

Name	الاستاذ/ هشام سيد فاروق عبد المتجلي	Re
Sex	Male	Pri
Ref. By Prof. Dr.		

Molecular Biology
HLA Class I - A

Serologic Type: A24(9) ; A24(9)

Generic Type :HLA-A*24 & *24

Molecular Biology
HLA Class I - A

Serologic Type: A?4(9) ; A24(9)

Generic Type :HLA-A*24 & *24

Molecular Biology
HLA Class I - B

Serologic Type: B38(16) ; B51(5)

Generic Type :HLA-B*38 & *51

Molecular Biology
HLA Class I - B

Serologic Type: B35 ; B38(16)

Generic Type :HLA-B*35 & *38

Molecular Biology
HLA Class II DR

Serologic Type: DR13(6) ; DR14(6)

Generic Type :HLA-DRB1*13 & *14

Molecular Biology
HLA Class II DR

Serologic Type: DR?3(6) ; DR13(6)

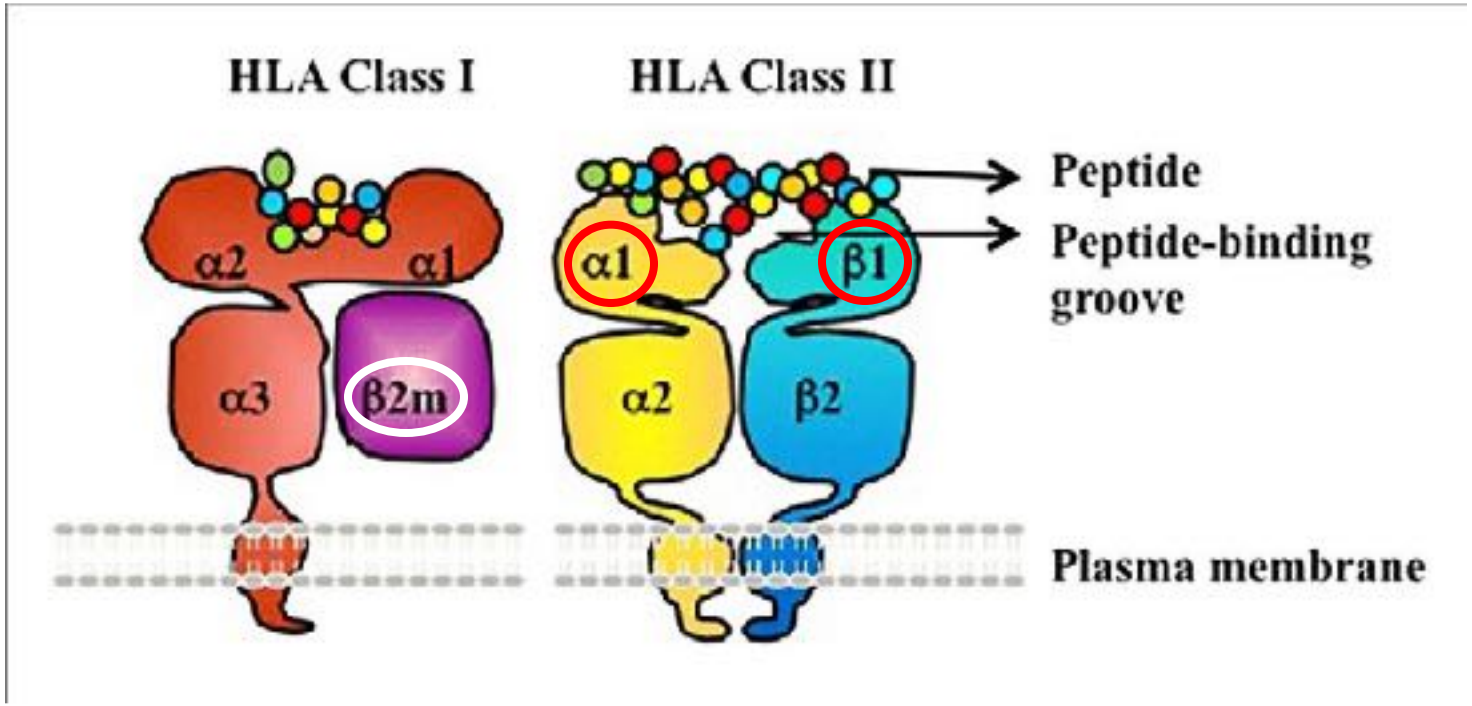
Generic Type :HLA-DRB1*13 & *13

HLA Mismatch Level

Introduction

The national deceased donor matching scheme used in the UK takes into account the HLA mismatch between donor and potential recipient when assigning points to determine the matching sequence. Rather than using a crude number of antigen mismatches, it takes into account the differing immunological effect of mismatches at different loci and assigns a mismatch level from the A:B:DR mismatch, as in the table below:

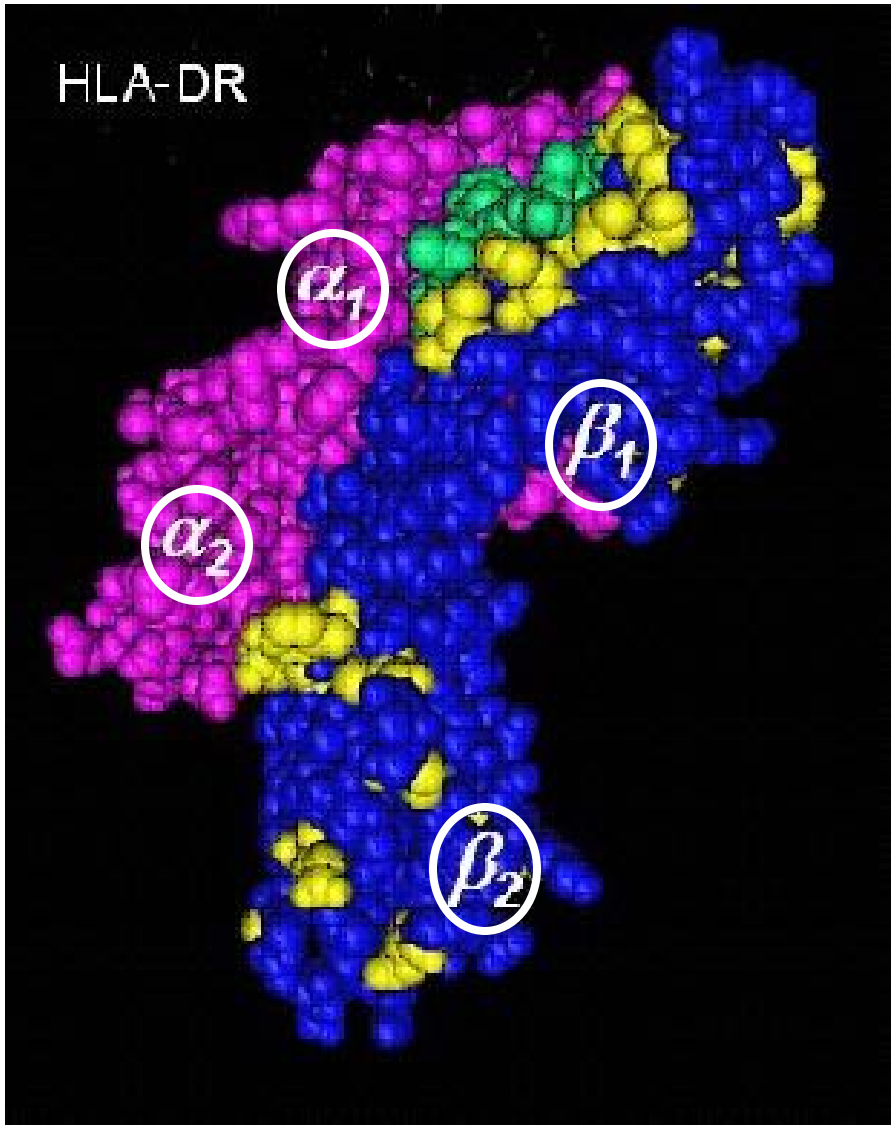
Level	HLA mismatch summary	HLA mismatch combinations
1	000	000
2	[0 DR and 0/1 B]	100, 010, 110, 200, 210
3	[0 DR and 2 B] or [1 DR and 0/1 B]	020, 120, 220, 001, 101, 201, 011, 111, 211
4	[1 DR and 2 B] or [2 DR]	021, 121, 221, 002, 102, 202, 012, 112, 212, 022, 122, 222



The Class I molecule is composed of one polypeptide chain and a B2 microglobulin chain.e Alpha1 and alpha2 domains form the peptide binding site for class Ifor class I;

class II molecule has 2 polypeptide chains. alpha1 and beta1 domains form the peptide binding site for class II.alpha1 and beta1 domains form the peptide binding site for class II

Hickey MJ, Valenzuela NM and Reed EF (2016) Alloantibody Generation and Effector Function Following Sensitization to Human Leukocyte Antigen. Front. Immunol. 7:30. doi: 10.3389/fimmu.2016.00030



Rene Duquesnoy 2020

Topographic Three-dimensional modeling of HLA DR polymorphic residues

Allo-typing

- CDC Serologic: Terasaki's antihuman leukocyte antigen (HLA) antibodies from sera of sensitized patients

Serologic / cellular CDC [Low resolution typing]

Smith LK, Methods Mol Biol. 2012;882:67-86

TABLE 3.3 Cross-reactive antigen groups

On serologic basis

A LOCUS													
1.	A1	A3	A11	A36									
2.	A9	A23	A24										
3.	A10	A25	A26	A34	A66	A43							
4.	A19	A29	A30	A31	A32	A33	A74						
5.	A2	A28	A68	A69									
B LOCUS													
1.	B5	B18	B35	B51	B52	B53	B70	B71	B72				
2.	B12	B21	B44	B45	B49	B50							
3.	B14	B64	B65										
4.	B8	B59											
5.	B15	B17	B46	B57	B58	B62	B63	B70	B71	B72	B75	B76	B77
6.	B16	B38	B39	B67									
7.	B37												
8.	B7	B27	B42	B73									
9.	B7	B22	B54	B55	B56	B67							
10.	B7	B40	B41	B48	B60	B61							
11.	B13	B47											

Broad and Split

Outline

Risk Stratification

HLA specification … [Intermediate/ High Resolution]

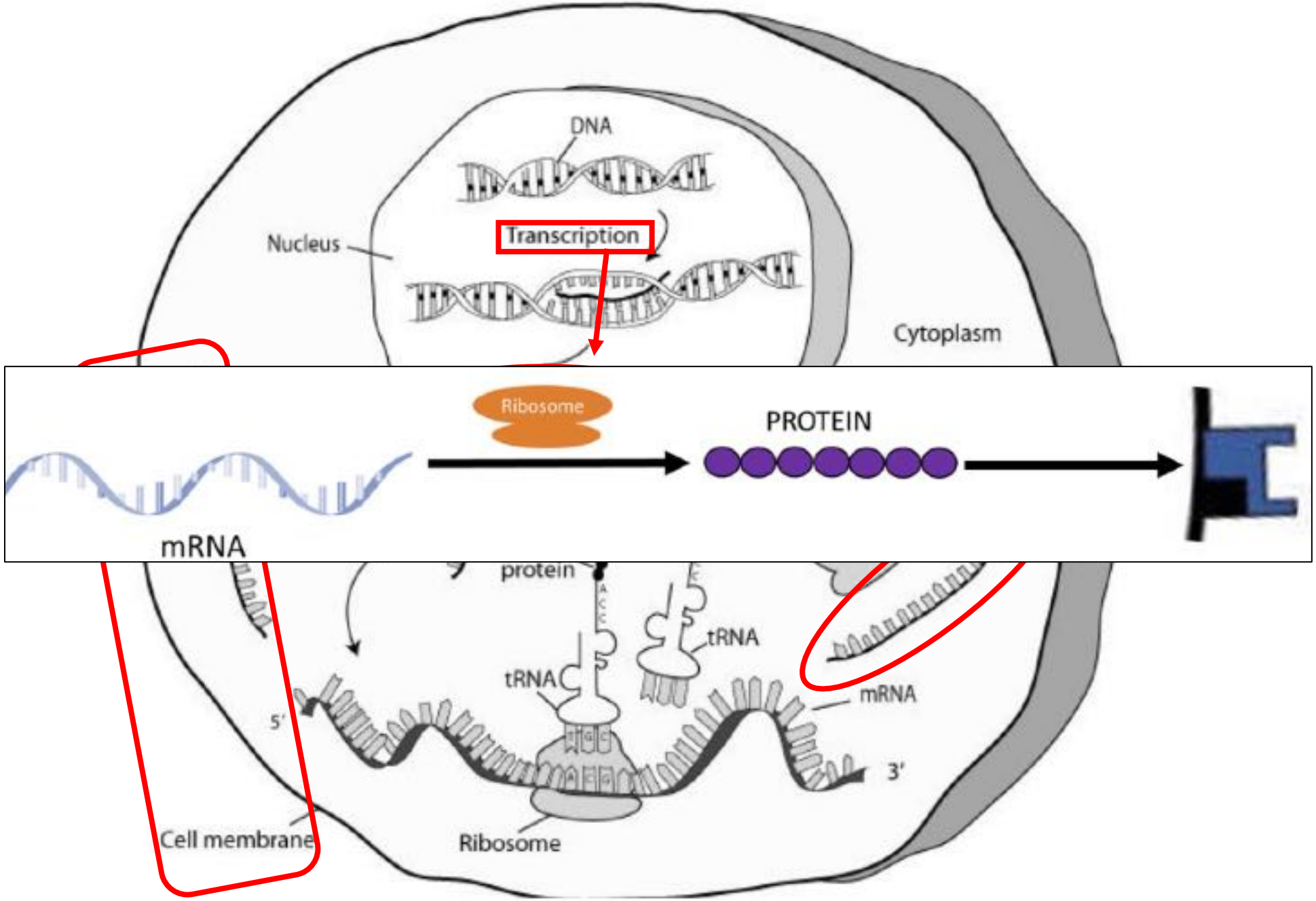
Eplet and Epitope Matching

AM Acceptable Mismatches & PM Permissive Mismatches

Detection of sensitization c-PRA vs TS

KDPI & EPTS

Katharina Petsche, (2016) askabiologist.asu.edu/making-cells-dna-protein



Allo-typing

- Oligotyping

R. J. Duquesnoy & M. Marrari, Tissue Antigens ISSN 0001-2815

Oligonucleotide Probes Used for Identification of DR1, DR2, DR3, DR4, DR8, DR11, and DR13 Subtypes

Oligonucleotide	Sequence (5′ to 3′)	Amino Acids
E71	GGCCCGCTCGTCTTCCAGG	68–73
K71	CGGCCCGCTTGTCTTCCAG	68–73
R71	GGCCCGCCTGTCTTCCAGG	68–73
F67	CTTCCAGGAAGTCCTTCTG	64–69
I67	CTTCCAGGATGTCCTTCTG	64–69
G86	GAAGCTCTCACCAACCCCG	85–89
V86	GAAGCTCTCCACAACCCCG	85–89
N37	AGCGCACGTTCTCCTCCTG	34–39
S57	GTACTCGGCGCTAGGCCGC	55–60
QK71	CGGCCCGCTTCTGCTCCAG	68–73
QR71	CCGCGGCCCGCCTCTGCTC	69–74
E74	GTGTCCACCTCGGCCCGCC	71–77
S37	AGCGCACGGACTCCTCTTG	34–39
QR71/2	CGGCCCGCCTCTGCTCCAG	68–73
AV86	GCTCACCACAGCCCCGTAG	83–88
D37	GAAGCGCAAGTCCTCCTCT	35–40
R71/2	CCGCGGCGCGCCTGTCTTC	69–74
R11	CTCAGACTTACGCAGCTCC	9–14
E28	GGAAGTATCTCTCCAGGAAC	26–31
H30	GGAAGTGTCTCTCCAGGAAC	26–31

Tiercy JM, et al. Am. J. Hum. Genet. 51:592-608, 1992

Luminex-Based Methods in High-Resolution HLA Typing

Abstract

Luminex-based technology has been applied to discriminate between the different Human Leukocyte Antigens (HLA) alleles. The typing method consists in **a reverse-SSO assay: Target DNA is PCR-amplified** using biotinylated group-specific primers. A single PCR reaction is used for each HLA locus. The biotinylated PCR product is **chemically denatured using a pH change and allowed to rehybridize to complementary DNA probes conjugated to microspheres**. **These beads are characterized by two internal fluorescent dyes that create a unique combination of color, make them identifiable**. Washes are performed to eliminate any additional PCR product that does not exactly match the sequence detected by the probe. The biotinylated PCR product bound to the microsphere is labelled with streptavidin conjugated with R-phycoerythrin (SAPE). A flow analyzer identifies the fluorescent intensity SAPE on each microsphere. Software is used to assign positive or negative reactions based on the strength of the fluorescent signal. The assignment of the HLA typing is based on positive and negative probe reactions compared with published HLA gene sequences. Recently kits characterized by an extensive number of probes/beads designed to potentially reduce the number of ambiguities or to directly lead to an allele level typing, have been made available.

Testi M, Andreani M. Methods Mol Biol. 2015;1310:231-45
doi: 10.1007/978-1-4939-2690-9_19

Multiple PCR-based typing methods

hybridization to an oligonucleotide probe (reverse sequence specific oligonucleotide-rSSO),

digestion with restriction enzymes

chain termination sequencing reactions (Sequence-Based Typing-SBT)

mobility pattern using gel electrophoresis (sequence-specific primer-SSP)

Muluhngwi P, and Tumer G. Human Leukocyte Antigens and Transplantation **(2023),** DOI: 10.5772/intechopen.1001276

The asterisk "*" sign indicates that typing is performed by a "*molecular method*"

HLA-A*02:101:01:02N

HLA-A*02:101:01:02N

Surface Antigen detected serologically

HLA-A*02:101:01:02N

Cytoplasmic Molecular Protein (Missense) detected by PCR / NGS / Amino Acid sequencing

HLA-A*02:101:01:02N

DNA (coding exons) detected by PCR / NGS

HLA-A*02:101:01:02N

DNA (noncoding region) detected
by PCR / NGS

In cases of apparent homozygosity, the typing should be entered in both boxes, or an error will be reported

n

(Petersdorf et al. Blood **(2020)** 136:362-9)

HLA allele[a]	Pre 2010 designation	HLA specificity
A*24:54	A*2454	—
A*24:55	A*2455	—
A*24:56	A*2456	—
A*24:57	A*2457	—
A*24:58	A*2458	A24(9)
A*24:59	A*2459	—
A*24:60N	A*2460N	Null
A*24:61	A*2461	—
A*24:62	A*2462	—
A*24:63	A*2463	—
A*24:64	A*2464	—
A*24:65	A*2465	—
A*24:66	A*2466	—
A*24:67	A*2467	—
A*24:68	A*2468	—
A*24:69	A*2469	—
A*24:70	A*2470	—
A*24:71	A*2471	—
A*24:72	A*2472	—
A*24:73	A*2473	—
A*24:74	A*2474	—
A*24:75	A*2475	—
A*24:76	A*2476	A24(9)
A*24:77	A*2477	—
A*24:78	A*2478	A24(9)
A*24:79	A*2479	—
A*24:80	A*2480	—
A*24:81	A*2481	—

Marsh SGE, et al. WHO Nomenclature for factors of the HLA system.
Tissue Antigens **2010**, 75: 291–455
doi: 10.1111/j.1399-0039.2010.01466.x

LABORATORIES

HLA MatchMaker report

www.ansarylabs.com

Name: Ahmed Hesham Sayed Farouk **Reporting date:** 26/9/2023

Serologic Mismatch 2 / 6 & precisely 1-1-0 = 110

Molecular Mismatch = 211

Age	8 Years	30 Years
Sex	Male	Female
Relative	Son	Mother
HLA Class I		
HLA-A	A*24:01 ; A*24:03 ?	A*24:02 ; A*01:01
HLA-B	B*38:01 ; B*51:01	B*15:17 ; B*51:01
HLA Class II		
HLA- DR	DR*13:01 ; DR*14:01	DR*13:03 ; DR*14:01
Total		

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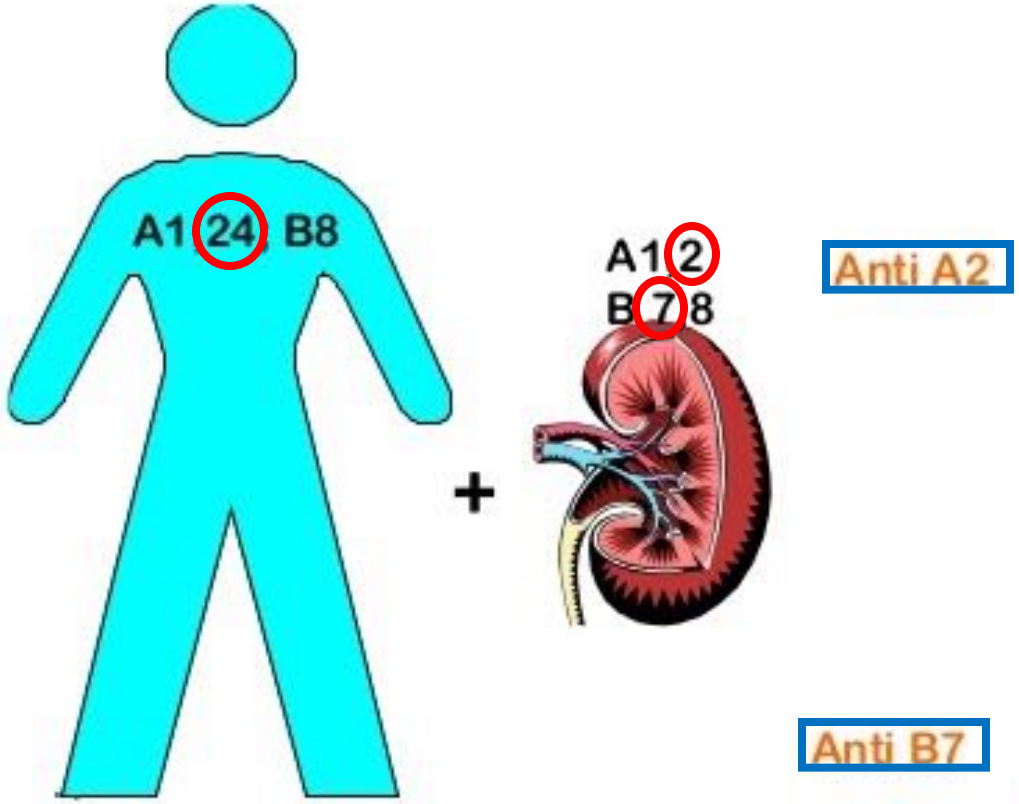
Generation of DSA

DSA are rarely generated alone and generally antibodies to HLA molecules related to the donor HLA are also often found:



Wiebe C et al. Am J Transplant. 2012; 12: 1157-1167


24

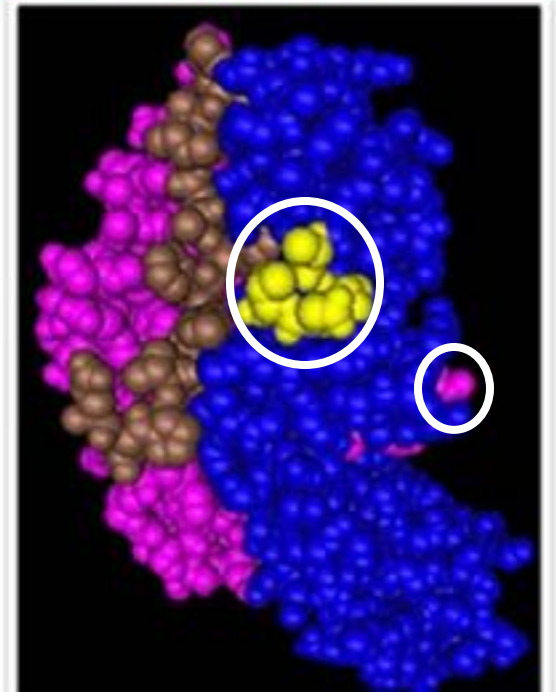

24

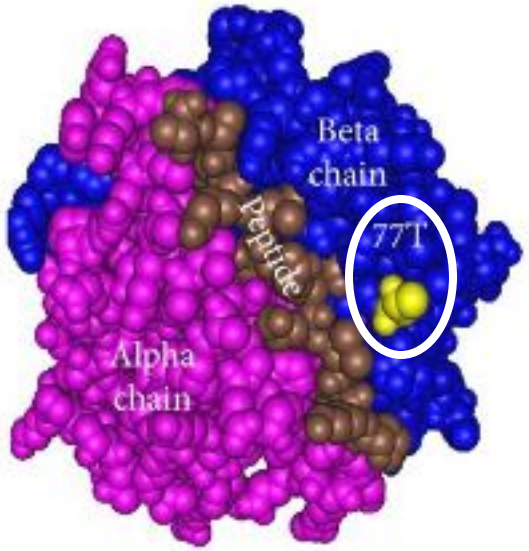

www.hisupplier.com

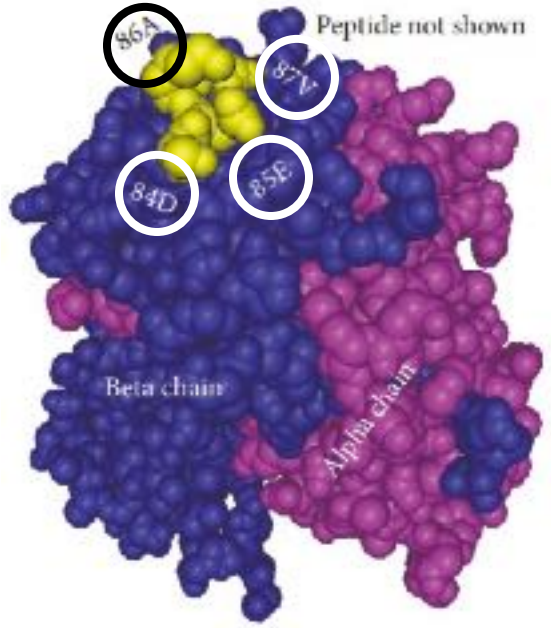
Allo-typing

- HLA antigens have multiple epitopes that are determined by amino acid residues in polymorphic positions
- HLA epitopes recognized, especially by mouse monoclonal antibodies (mAbs)

R. J. Duquesnoy & M. Marrari, Tissue Antigens ISSN 0001-2815







Duquesnoy RJ. Tissue Antigens. 2011; 524 - 534

El-Awar N, et al. Journal of Immunology Research 2017, Article ID 3406230, doi.org/10.1155/2017/3406230

EPITOPE Function

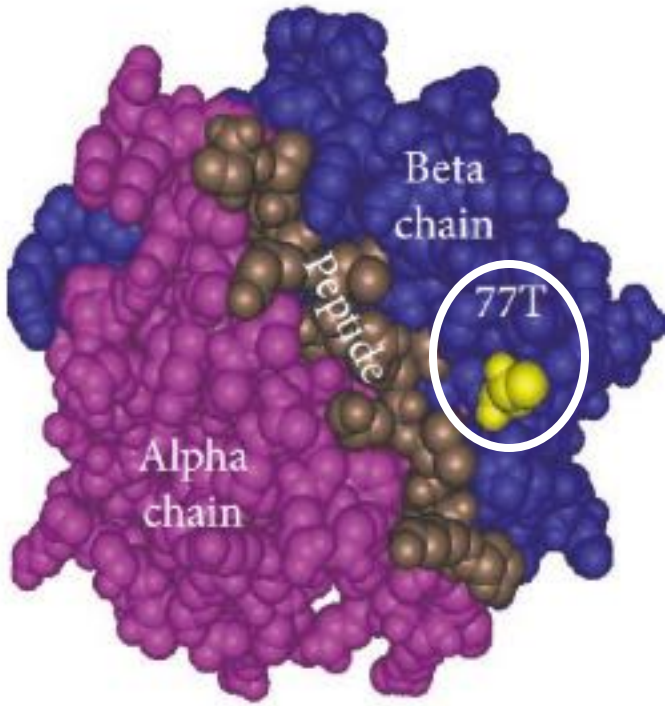
An epitope has two characteristics

- immunogenicity, inducing Ab response
- antigenicity, determining Ab reactivity
- mismatch permissibility # reactivity

Epitope 1028 shared by class II DR antigens DR1, DR4, DR7, DR9, DR10, DR11, DR12, DR13, DR14, DR15, DR16, DR51, DR53, and DR103 and defined by threonine (T) at position 77

HLA class II DR epitope

Antigen	aa pos. 77
DR1	T
DR103	T
DR10	T
DR11	T
DR12	T
DR15(2)	T
DR16(2)	T
DR4	T
DR4	T
DR13(6)	T
DR14(6)	T
DR7	T
DR8	T
DR9	T



El-Awar N, et al. Journal of Immunology Research 2017, Article ID 3406230, doi.org/10.1155/2017/3406230

An antibody paratope consists of three light-chain and three heavy-chain complementarily determining regions (CDR-L1, -L2, -L3, - H1, -H2, and -H3).

A "Structural" antigen epitope, consisting of 15–22 amino acids, constitutes the whole binding site for antibodies

A "Functional" epitope of 2–5-amino-acid-long determines the specificity of antibody

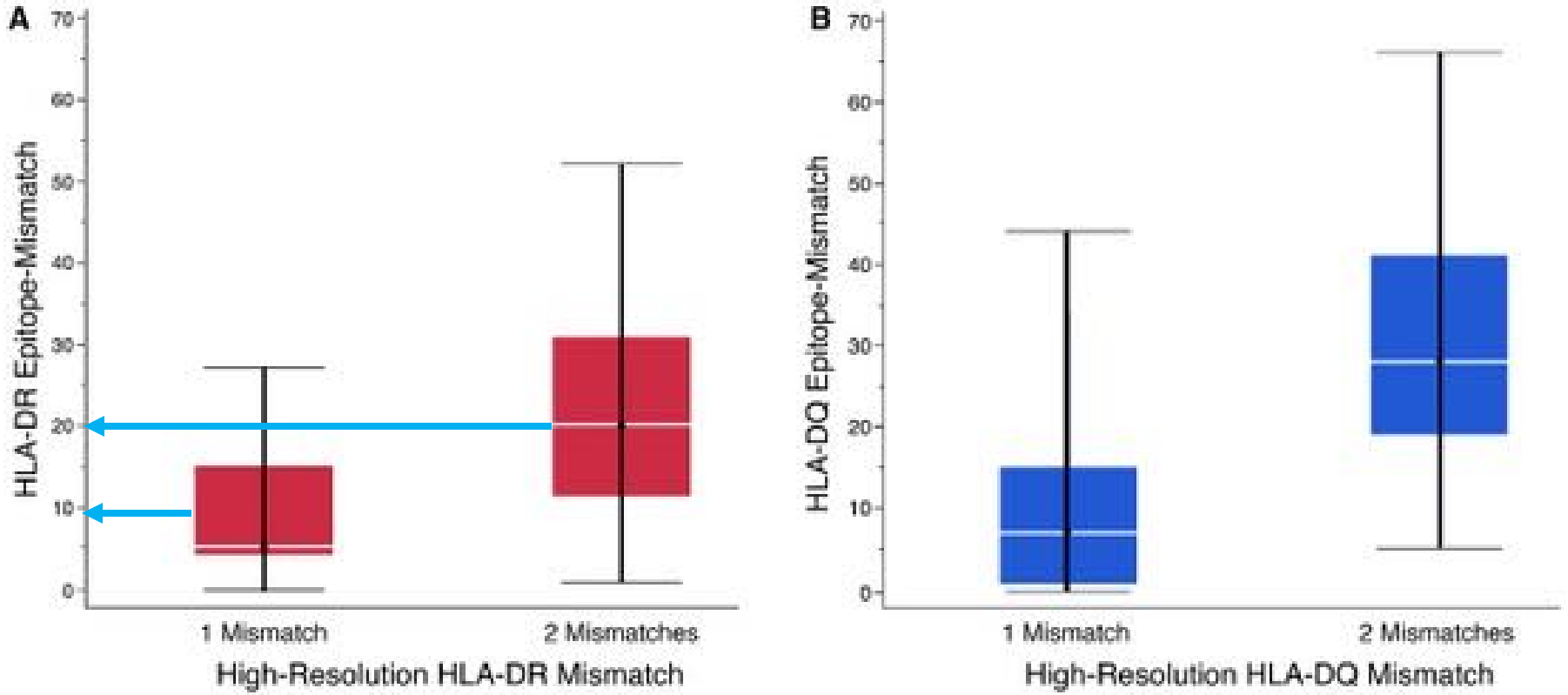
The highly variable and specific CDR-H3 regions of the HLA antibody recognize this small core structure

Binding of the antibody to the remaining parts of the structural epitope helps to increase the stability of the antigen-antibody complex.

antibodies cannot be induced against an eplet if it is present on a self-HLA antigen

Duquesnoy RJ. Hum Immunol 2006;67:847–62. doi:10.1016/j.humimm.2006.08.001.

Sahin GK, Unterrainer C, and Süsal C. Transplantation Reviews(2020), doi.org/10.1016/j.trre.2020.100533



Wiebe et al *American Journal of Transplantation* 2015; 15: 2197–2202

Amino Acid / Epitope and High Resolution Typing

DNA Sequence based typing

Sequence-based typing (is considered the gold standard for HLA allele identification, High resolution typing)

Sanger sequencing

Next generation sequencing (NGS), millions of DNA fragments are simultaneously sequenced per run hence it is known as massive parallel sequencing. The first step in NGS usually involves amplifying the HLA gene region by PCR using primers, monitoring pH changes or capturing fluorescent signals to identify bases (A, C, G, T)

Muluhngwi P, and Tumer G. Human Leukocyte Antigens and Transplantation **(2023),** DOI: 10.5772/intechopen.1001276

The Accuracy of Sequence-Specific Oligonucleotide and Real-Time Polymerase Chain Reaction HLA Typing in Determining the Presence of Pre-Transplant Donor-Specific Anti-HLA Antibodies and Total [Eplet] Mismatches for Deceased Donor Kidney Transplantation

High resolution human leukocyte antigen (HLA) typing is important in establishing eplet compatibility and the specificity of donor-specific anti-HLA antibodies (DSA). In deceased donor kidney transplantation, high resolution donor HLA typing may not be immediately available, leading to inaccuracies during the organ allocation process. We aimed to determine the concordance and agreement of HLA-Class I and II eplet mismatches calculated using population frequency based allelic haplotype association (linkage disequilibrium, LD) from sequence-specific oligonucleotide (SSO) and real-time polymerase chain reaction (rtPCR) donor HLA typing (available at time of donor kidney allocation) compared to high-resolution Next Generation Sequencing (NGS) donor typing. NGS high resolution HLA typing were available for all recipients prior to donor kidney allocation. A cohort of 94 deceased donor-recipient pairs from a single Western Australian center were included (77 individual donors typed, 55 local and 22 interstate). The number of class I (HLA-A+B+C) and class II (HLA-DRB1+DRB3/4/5+DQB1+DQA1+DPB1+DPA1) eplet mismatches were calculated using HLAMatchmaker, comparing LD- and NGS-HLA typing. The accuracy in assigning pre-transplant DSA was compared between methods. The concordance correlation coefficient (95%CI) for HLA-class I and II eplet mismatches were 0.994 (0.992 to 0.996) and 0.991 (0.986 to 0.993), respectively. The 95% limits of agreement for class I were -1.3 (-1.6 to -1.1) to 1.4 (1.2 to 1.7) and -4.8 (-5.7 to -3.9) to 5.0 (4.1 to 5.9) for Class II. Disagreement between the two methods were present for 11 and 37 of the Class I and II donor/recipient pairs. Of which, 5 had a difference of ≥5 class II eplet mismatches. There were 34 (36%) recipients with potential pre-transplant DSA, of which 8 (24% of recipients with DSA) had indeterminate and ultimately false positive DSA assigned by donor LD-typing. While the concordance between NGS- and LD-typing was high, the limits of agreement suggest meaningful differences between these two techniques. The inaccurate assignment of DSA from donor LD-typing may result in associated HLA being considered unacceptable mismatches, inappropriately precluding candidates' access to transplantation. Accurate imputation of two-field HLA alleles based on LD from SSO and rtPCR HLA typing remains a substantial challenge in clinical practice in-lieu of widely available, rapid, high-resolution methods.

Larkins NG, et al. Front. Immunol. **2022**, 13: 844438. doi: 10.3389/fimmu.2022.844438

LABORATORIES

HLA MatchMaker report

www.ansarylabs.com

Name: Ahmed Hesham Sayed Farouk **Reporting date:** 26/9/2023

	Patient	Donor	MisMatch	Cause of MisMatch
	Ahmed Hesham Sayed	**Reham Rafaat Eid**		
Age	8 Years	30 Years		
Sex	Male	Female		
Relative	Son	Mother		
HLA Class I				
HLA-A	A*24:01 ; A*24:03	A*24:02 ; A*01:01	**14**Eplets MisMatch	**Due to A*01:01 allele as following (1,1E,12,15,16,238 ,5041C,5044PC, 5045PC,5053PC 5059E**
HLA-B	B*38:01 ; B*51:01	B*15:17 ; B*51:01	**0** Eplets MisMatch	**-**
HLA Class II				
HLA- DR	DR*13:01 ; DR*14:01	DR*13:03 ; DR*14:01	**3** Eplets MisMatch	**Due to DRB1*13:03 allele As following (1016,1021,1409)**
Total			**17**	

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	Ahmed Hesham Sayed	**Hesham Sayed Farouk**		
Age	8 Years	32 Years		
Sex	Male	Male		
Relative	Son	Father		
HLA Class I				
HLA-A	A*24:01 ; A*24:03	A*24:02 ; A*24:03	**0** Eplets MisMatch	-
HLA-B	B*38:01 ; B*51:01	B*38:01 ; B*35:02	**0** Eplets MisMatch	-
HLA Class II				
HLA- DR	DR*13:01 ; DR*14:01	DR*13:01 ; DR*13:03	**3** Eplets MisMatch	**Due to DRB1*13:03 allele As following (1016,1021,1409)**
Total			**3**	

Transplant outcome

Epitope Matching Outperforms Traditional Antigen Matching as a Predictor of De Novo Donor Specific Antibody Development after Renal Transplantation

C. Wiebe, D. Pochinco, T. Blydt-Hansen, I. Gibson, D. Rush, P. Nickerson

Medicine, University of Manitoba, Winnipeg, Canada
Immunology, University of Manitoba, Winnipeg, Canada
Diagnostic Services of Manitoba, University of Manitoba, Winnipeg, Canada
Pediatric and Child Health, University of Manitoba, Winnipeg, MB, Canada
Pathology, University of Manitoba, Winnipeg, Canada

Meeting: 2013 American Transplant Congress

Abstract number: 408

Outline

Risk Stratification

HLA specification

Eplet and Epitope Matching

AM Acceptable Mismatches & PM Permissive Mismatches

Detection of sensitization c-PRA vs TS

KDPI & EPTS

AM patients had a significantly superior 10-year graft survival compared to highly sensitized patients transplanted on the basis of avoidance of unacceptable mismatches.

Before the current allocation system is shifted to epitope matching, it has to be demonstrated that this method is significantly superior to the conventional HLA matching

Sahin GK, Unterrainer C, and Süsal C. Transplantation Reviews(2020), doi.org/10.1016/j.trre.2020.100533

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Acceptable Mismatch

Definition of permissible and immunogenic HLA antigens based on epitope analysis of the HLA specific antibodies produced in sensitized patients

It has been suggested that shared HLA **specific epitopes might result in down-regulation or clonal deletion of T cells directed to donor-derived HLA peptides**, which contribute to indirect allorecognition in chronic rejection (Suciu-Foca et al., 1998; Colovai et al., 2000). These donor derived HLA peptides, which have shared epitopes and which might be activating or suppressor peptides, are presented by the antigen-presenting cells (APCs) of the recipients, and their immunogenicity is affected by the HLA phenotype of the recipient (Maruya et al., 1993; Doxiadis et al., 1996; Fuller & Fuller, 1999; Papassavas et al., 2002)

distinct anergic phenotypes can be induced in the responding T cells upon subsequent interaction with professional APC presenting the same peptide. These can range from the absence of Tcell anergy (i.e. T-cell activation), to an anergic phenotype, to a **suppressive anergic phenotype** that can be persistently present (Taams & Wauben, 2000).

different peptide–MHC complexes have the ability to **trigger Tcell receptor (TCR) responses via receptor antagonism**

Papassavas AC, et al. European Journal of Immunogenetics 2002, 29, 401–407

HLA-DQ Mismatching and Kidney Transplant Outcomes

Conclusions: HLA-DQ mismatching is associated with lower graft survival independent of HLA-ABDR in living donor kidney transplants and deceased donor kidney transplants with cold ischemia time #17 hours, and a higher 1-year risk of acute rejection in living and deceased donor kidney transplants

Leeaphorn N, et al. Clin J Am Soc Nephrol 13: 763–771, 2018.
doi.org/10.2215/CJN.10860917

The MHC class I MICA gene is a histocompatibility antigen in kidney transplantation

The identity of histocompatibility loci, besides human leukocyte antigen (HLA), remains elusive. The major histocompatibility complex (MHC) class I MICA gene is a candidate histocompatibility locus. Here, we investigate its role in a French multicenter cohort of 1,356 kidney transplants. MICA mismatches were associated with decreased graft survival (hazard ratio (HR), 2.12; 95% confidence interval (CI): 1.45–3.11; P < 0.001). Both before and after transplantation **anti-MICA donor-specific antibodies (DSA) were strongly associated with increased antibody-mediated rejection (ABMR) (HR, 3.79**; 95% CI: 1.94–7.39; P < 0.001; HR, 9.92; 95% CI: 7.43–13.20; P < 0.001; HR, 9.92; 95% CI: 7.43–13.20; P < 0.001, respectively). This effect was synergetic with that of anti-HLA DSA before and after transplantation (HR, 25.68; 95% CI: 3.31–199.41; P= 0.002; HR, 82.67; 95% CI: 33.67–202.97; P < 0.001, respectively). **De novo-developed anti-MICA DSA were the most harmful because they were also associated with reduced graft survival (HR, 1.29**; 95% CI: 1.05–1.58; P= 0.014). Finally, the damaging effect of anti-MICA DSA on graft survival was confirmed in an independent cohort of 168 patients with ABMR (HR, 1.71; 95% CI: 1.02–2.86; P= 0.041). In conclusion, assessment of MICA matching and immunization for the identification of patients at high risk for transplant rejection and loss is warranted.

Carapito R, et al. Nature Medicine 2022, 28: 989–998
doi.org/10.1038/s41591-022-01725-2

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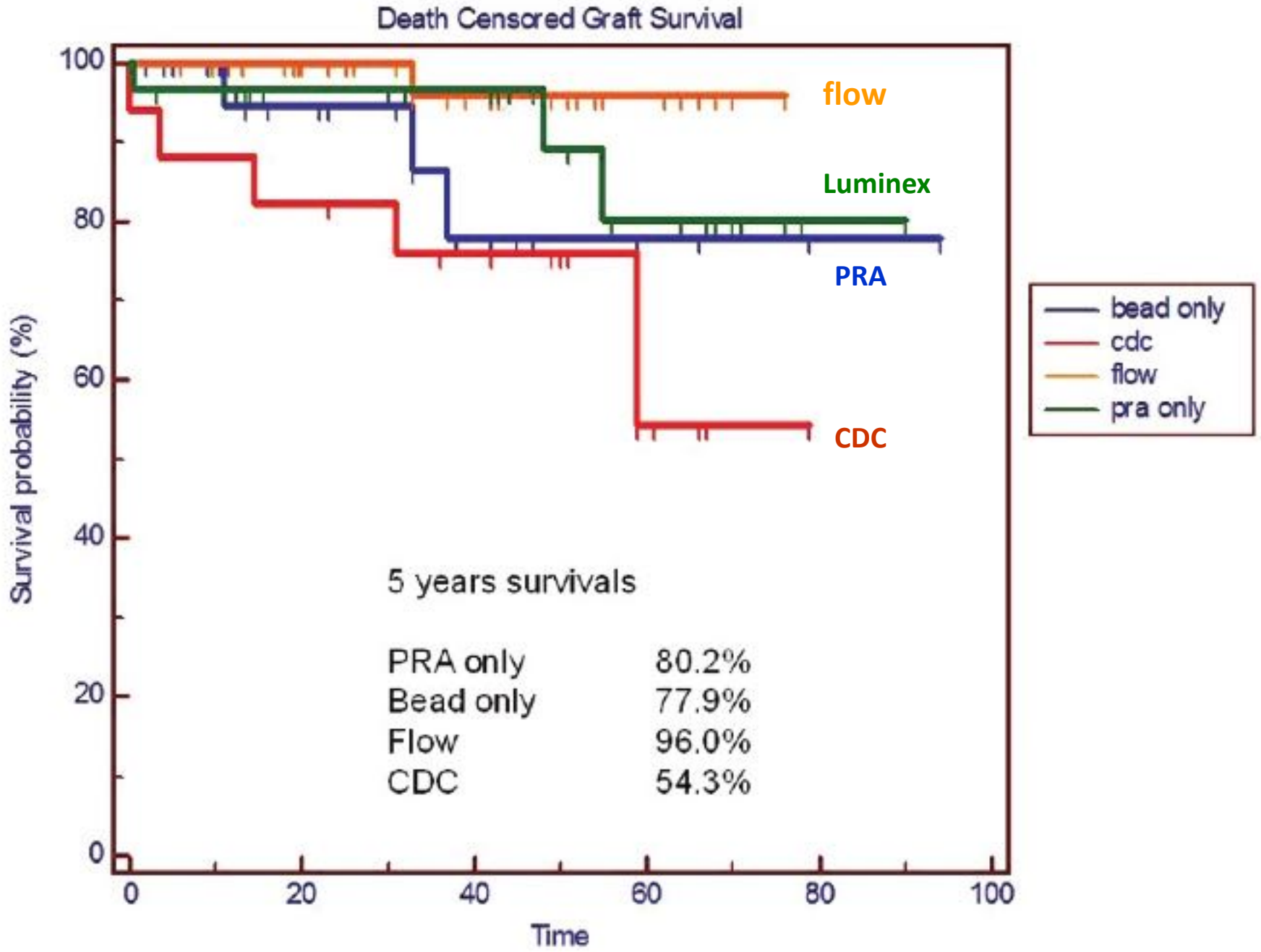
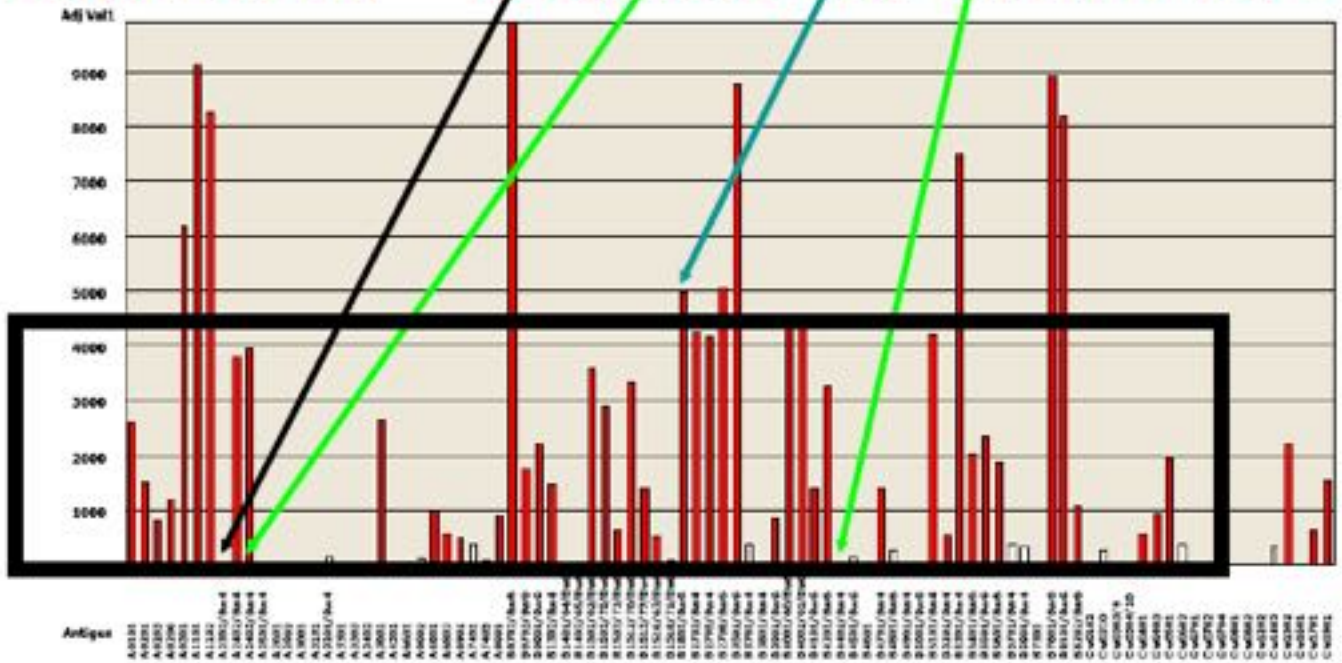

Death Censored Graft Survival
Survival probability (%)
100
80
60
40
20
0
0
20
40
60
80
100
Time
flow
Luminex
PRA
CDC
bead only
cdc
flow
pra only
5 years survivals
PRA only 80.2%
Bead only 77.9%
Flow 96.0%
CDC 54.3%

Patient 6092: HLA A*26,A*31,B*1402,B*49,DRB1*01,*11
Donor 6092D3; A*23,A*25, B*18,B*44, DRB1*13,*15



22/02/2007 PRA-CDC 56 % Anti- A3, B40, B51

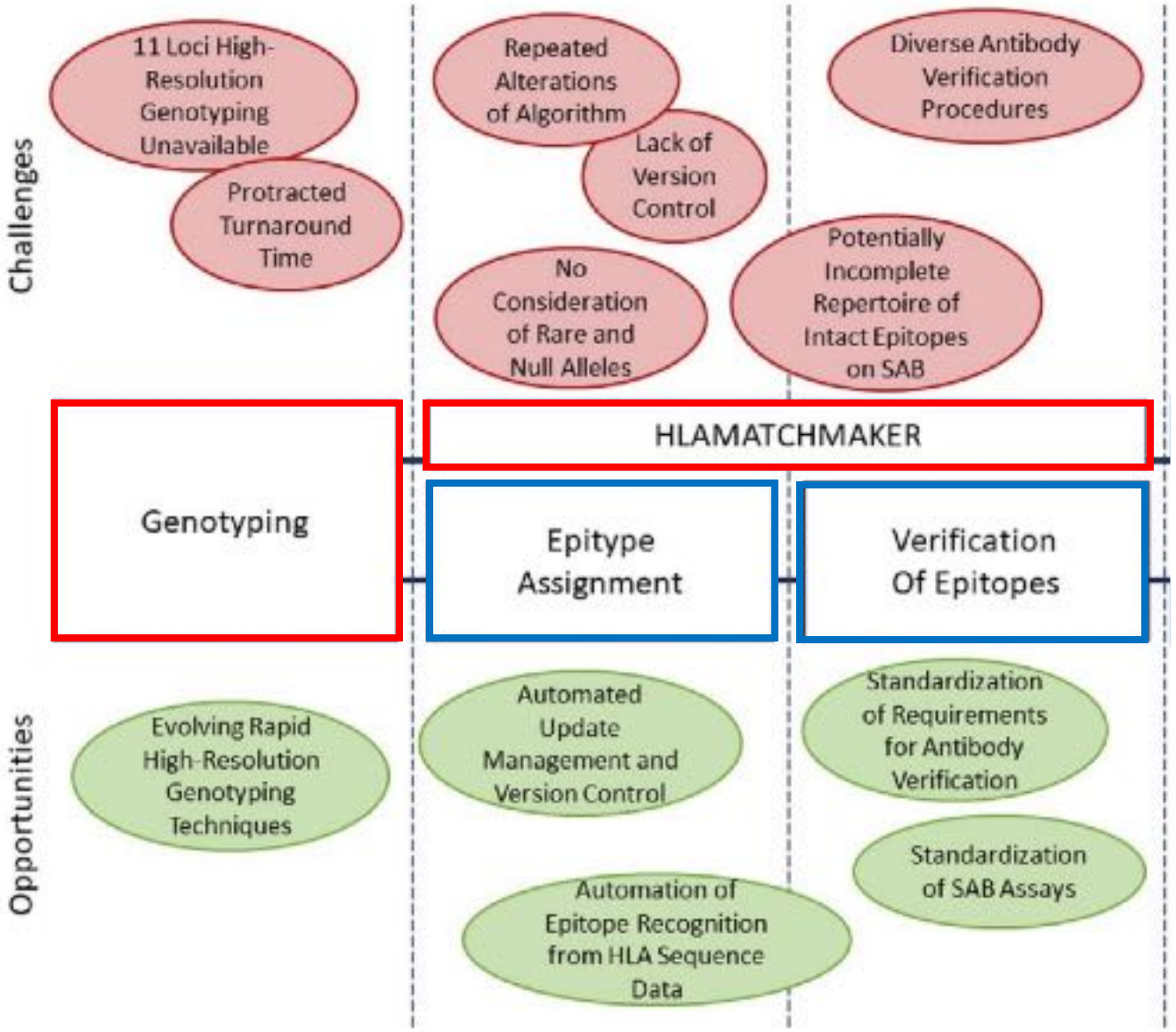
Crossmatch: XM-CDC Negative; XM-FCM Negative

Isolated Pre-existing HLA-DP Donor-Specific Antibodies are Associated With Poorer Outcomes in Renal Transplantation

Introduction: The importance of donor-specific antibodies (DSAs) in renal transplantation has long been recognized, but the significance of human leukocyte antigen (HLA)-DP antibodies remains less clear. We performed a retrospective single center study of renal transplants with pre-existing isolated HLA-DP-DSAs to assess clinical outcomes. Methods: Twenty-three patients with isolated HLA-DP-DSAs were compared with 3 control groups as follows: standard immunological risk (calculated reaction frequency [cRF] < 85%, no current or historical DSA, no repeat mismatched antigens with previous transplants, n ¼ 46), highly sensitized (cRF > 85%, n ¼ 27), and patients with HLA-DP antibodies that were not donor-specific (n ¼ 18). Univariate and multivariate analyses were performed comparing antibody-mediated rejection (ABMR)-free and graft survival. Factors in the final multivariable models included patient group, % cRF, B-cell flow crossmatch (BFXM) positivity and regrafts. Results: Over a median follow-up of 1197 days, **65% of HLA-DP-DSA patients had ABMR** on indication biopsies, and **30% of HLA-DP-DSA patients lost their graft.** Pre-existing HLA-DP DSAs remained the single factor associated with ABMR after multivariable analysis (hazard ratio [HR] ¼ 9.578, P ¼ 0.012). Patients with HLA-DP DSAs had increased microvascular scores (P ¼ 0.0346) and worse transplant glomerulopathy (P ¼ 0.015) on biopsy compared with the standard immunological risk group. Furthermore, **flow crossmatch (FXM) positivity did not help inform on the risk of graft failure or ABMR in patients with preformed DP-DSA**. Conclusion: Transplants with pre-existing HLA-DP-DSAs should be considered high risk. Routine laboratory tests are unable to further risk stratify these patients. Recipients should be considered for intensified immunosuppression and closely monitored.

Seitz A, et al. Kidney Int Rep **(2022)** 7: 2251–2263
doi.org/10.1016/j.ekir.2022.07.014

incorporating HLA eplet compatibility in immune risk assessment and organ allocation.



Lemieux W, et al. Int J Immunogenet. 2021;48:135–144
DOI: 10.1111/iji.12525

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Identification of antigenic epitopes recognized by tumor infiltrating lymphocytes in high grade serous ovarian cancer by multi-omics profiling of the auto-antigen repertoire

Patient ID	Somatic SNV	HLA-A		HLA-B		HLA-C		A*02:01 Predicted neo-epitopes
OV158	11	A*02:01	A*31:01	B*15:01	B*18:01	C*03:03	C*07:01	2
OV237	30	A*02:01	–	B*40:01	B*44:03	C*03:04	C*16:01	6
OV248	32	A*02:01	–	B*13:01	B*44:05	C*03:04	–	3
OV355	13	A*02:01	A*23:01	B*14:02	B*51:01	C*02:02	C*08:02	2
OV364	10	A*02:01	–	B*15:30	B*18:01	C*03:04	C*07:01	3
OV436	48	A*02:01	A*26:01	B*18:01	B*51:01	C*07:01	C*15:02	6
OV486	67	A*02:01	A*29:02	B*08:01	B*44:03	C*07:01	C*16:01	9
OV499	38	A*02:01	A*30:04	B*44:02	B*49:01	C*07:01	C*15:02	8
OV586	54	A*02:01	A*31:01	B*39:01	B*46:01	C*01:02	–	9

Neo-epitope prediction

Millar DG, et al. Cancer Immunology, Immunotherapy **(2023)** 72:2375–2392
doi.org/10.1007/s00262-023-03413-7

The shared susceptibility epitope of HLA-DR4 binds citrullinated self-antigens and the TCR

Individuals expressing HLA-DR4 bearing the shared susceptibility epitope (SE) have an increased risk of developing rheumatoid arthritis (RA). Posttranslational modification of self-proteins via citrullination leads to the formation of neoantigens that can be presented by HLA-DR4 SE allomorphs. However, in T cell–mediated autoimmunity, the interplay between the HLA molecule, posttranslationally modified epitope(s), and the responding T cell repertoire remains unclear. In HLA-DR4 transgenic mice, we show that immunization with a Fib□-74cit69-81 peptide led to a population of HLA-DR4Fib□-74cit69-81 tetramer+ T cells that exhibited biased T cell receptor (TCR) □ chain usage, which was attributable to selective clonal expansion from the preimmune repertoire. Crystal structures of pre- and postimmune TCRs showed that the SE of HLA-DR4 represented a main TCR contact zone. Immunization with a double citrullinated epitope (Fib□-72,74cit69-81) altered the responding HLA-DR4 tetramer+ T cell repertoire, which was due to the P2-citrulline residue interacting with the TCR itself. We show that the SE of HLA-DR4 has dual functionality, namely, presentation and a direct TCR recognition determinant. Analogous biased TCR □ chain usage toward the Fib□-74cit69-81 peptide was observed in healthy HLA-DR4+ individuals and patients with HLA-DR4+ RA, thereby suggesting a link to human RA.

Lim JJ, et al. Sci. Immunol. (2021) 6, eabe0896
DOI: 10.1126/sciimmunol.abe0896

certain posttranslational modifications (PTMs), such as the deamidation of glutamine (6), peptide trans-splicing (7), and citrullination (8), generate neoepitopes with improved binding to a given HLA molecule (5)

Lim JJ, et al. Sci. Immunol. **(2021)** 6, eabe0896
DOI: 10.1126/sciimmunol.abe0896

Take Home Message

Allosensitization is detrimental in kidney graft outcome

Serologic [Low Resolution] typing lacks specificity, accuracy and not inclusive

DSA to various HLA antigens are influential

High Resolution matching carries better specificity and spectrum than traditional serologic tests

HLA and Eplet / Epitope matching are complementary

Eplet mismatching with "recorded" epitopes is indicative, to be confirmed & "tested" on demand

Complexity of Matching and Allocation among c-PRA or TS requiring Match-Maker analysis