Pre-Transplant Categorization of Immunological Risk: Tissue Typing, PRA, CXM

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4th GCC Organ Transplantation & Nephrology Congress Kuwait – 23rd January 2025

Outline

Risk Stratification

Risk weight of HLA antigens

HLA specification

AM Acceptable Mismatches

PM Permissive Mismatches

Eplet and Epitope Matching

c-PRA vs TS

KDRI / KDPI & EPTS

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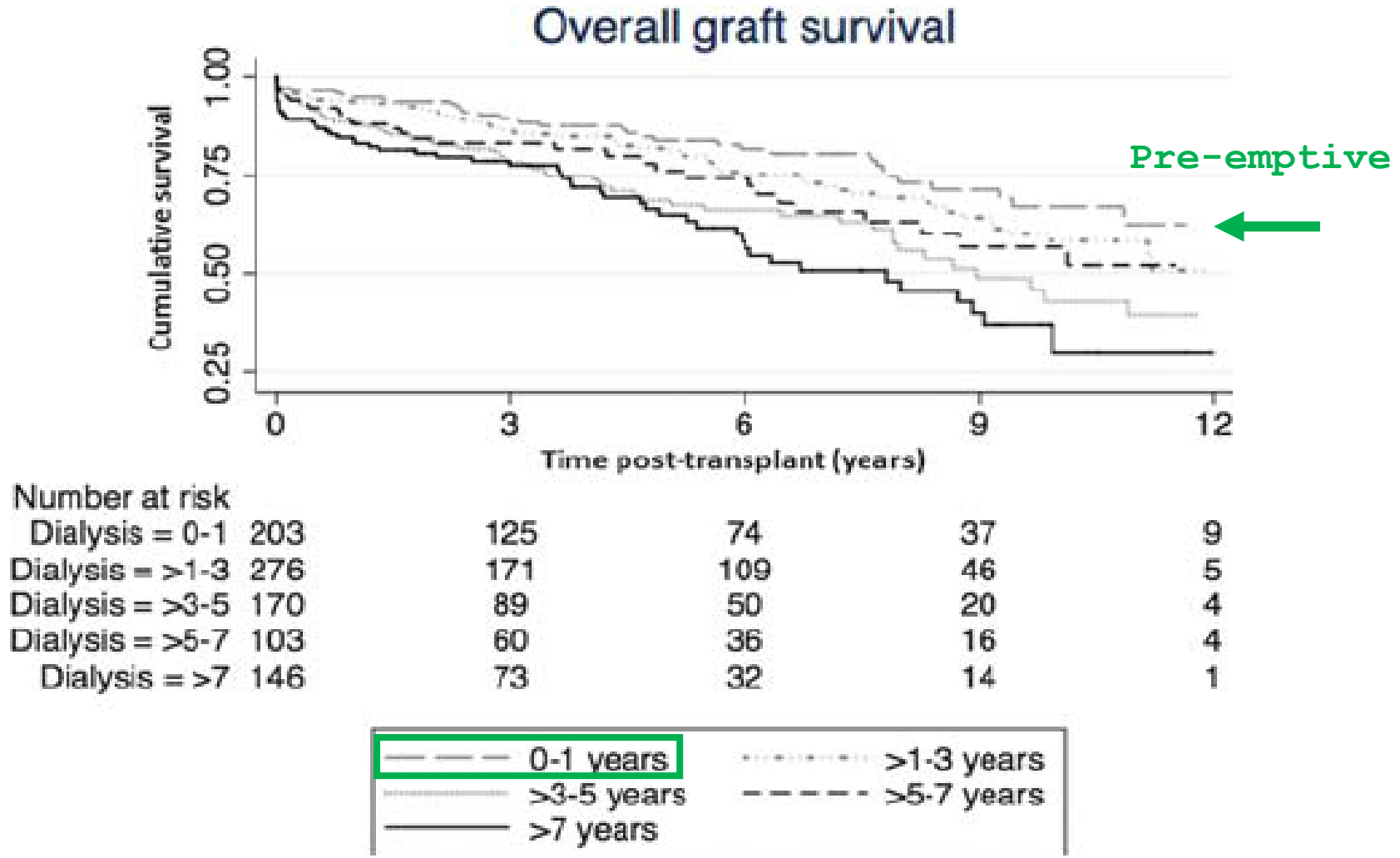
FIGURE 4. Cumulative adjusted patient survival curves of recipients who h
received second transplants stratified by waiting time (log- rank P value



Wong G, et al, Transplantation, Oct 2015

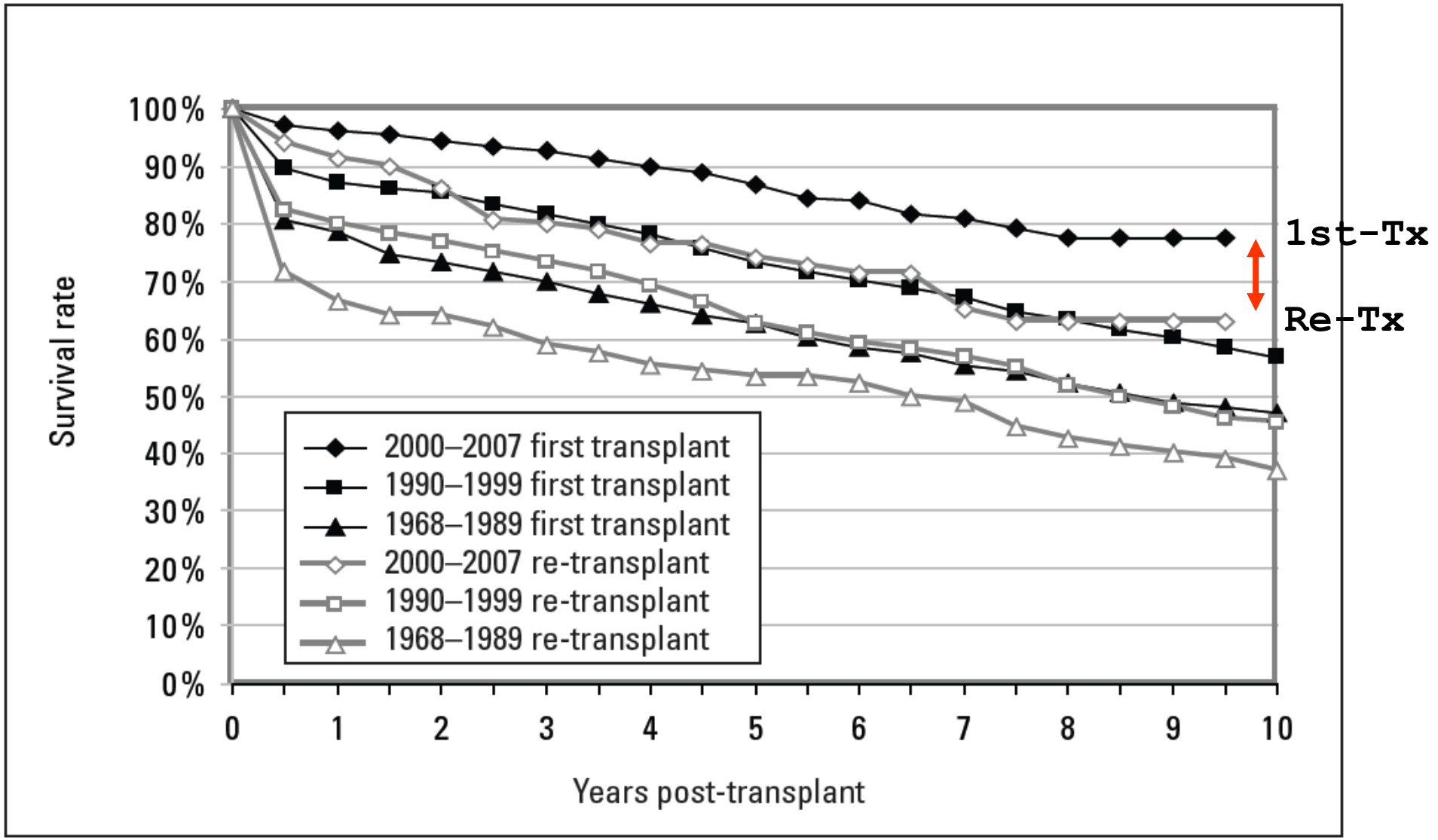
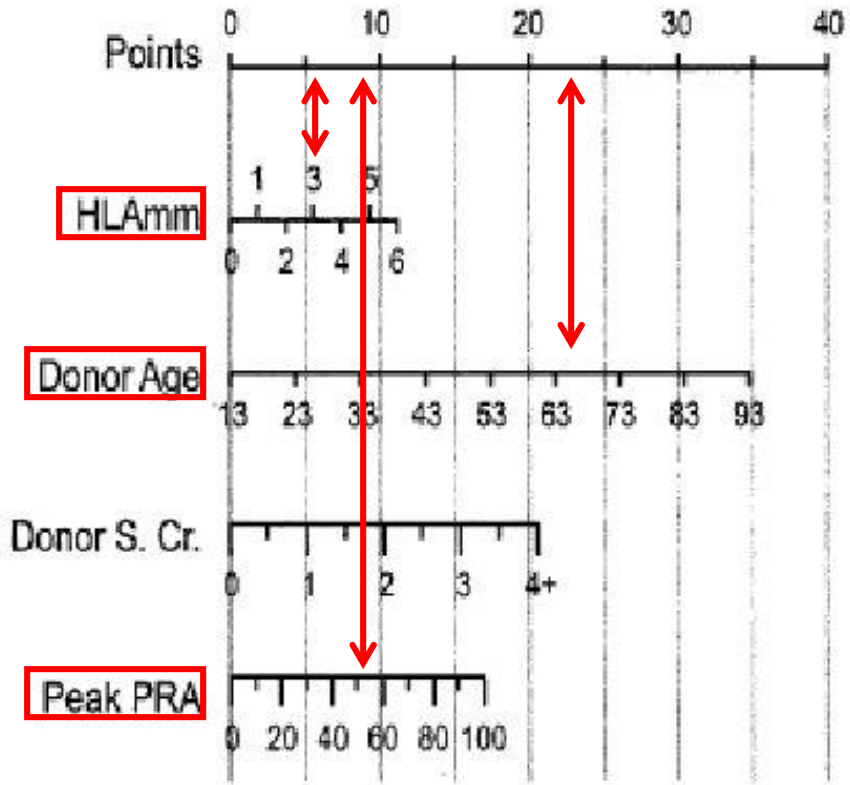


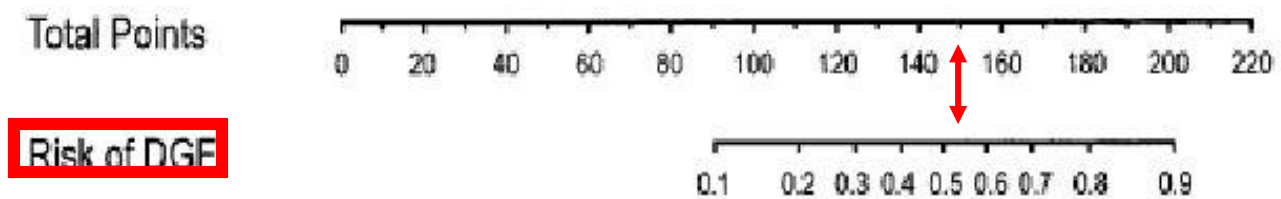
Figure 1. Graft survival for kidney transplants by graft number and year of transplant, 1968–2007.

Source: BC Transplant

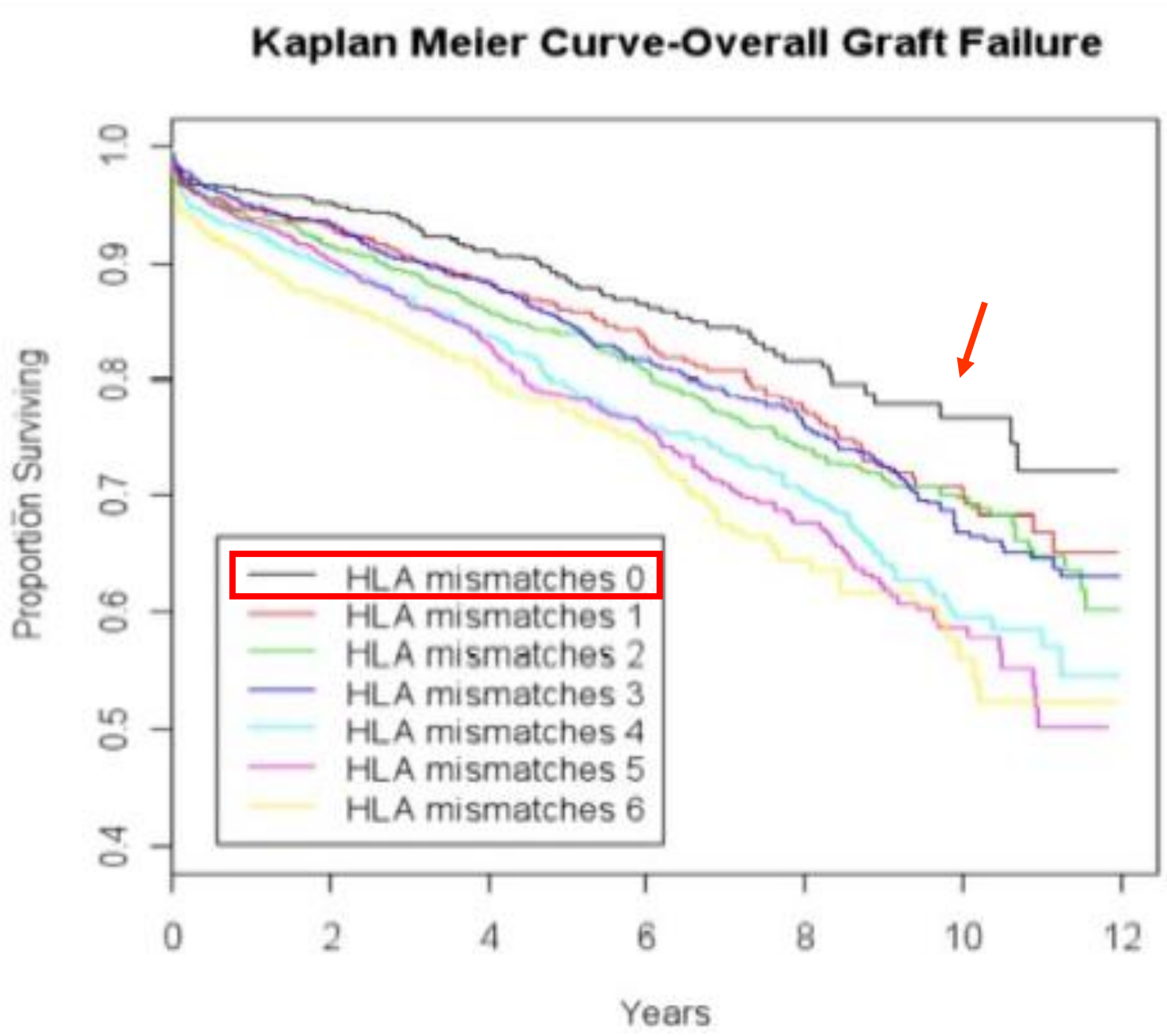


CIT Add 1 point for each hour

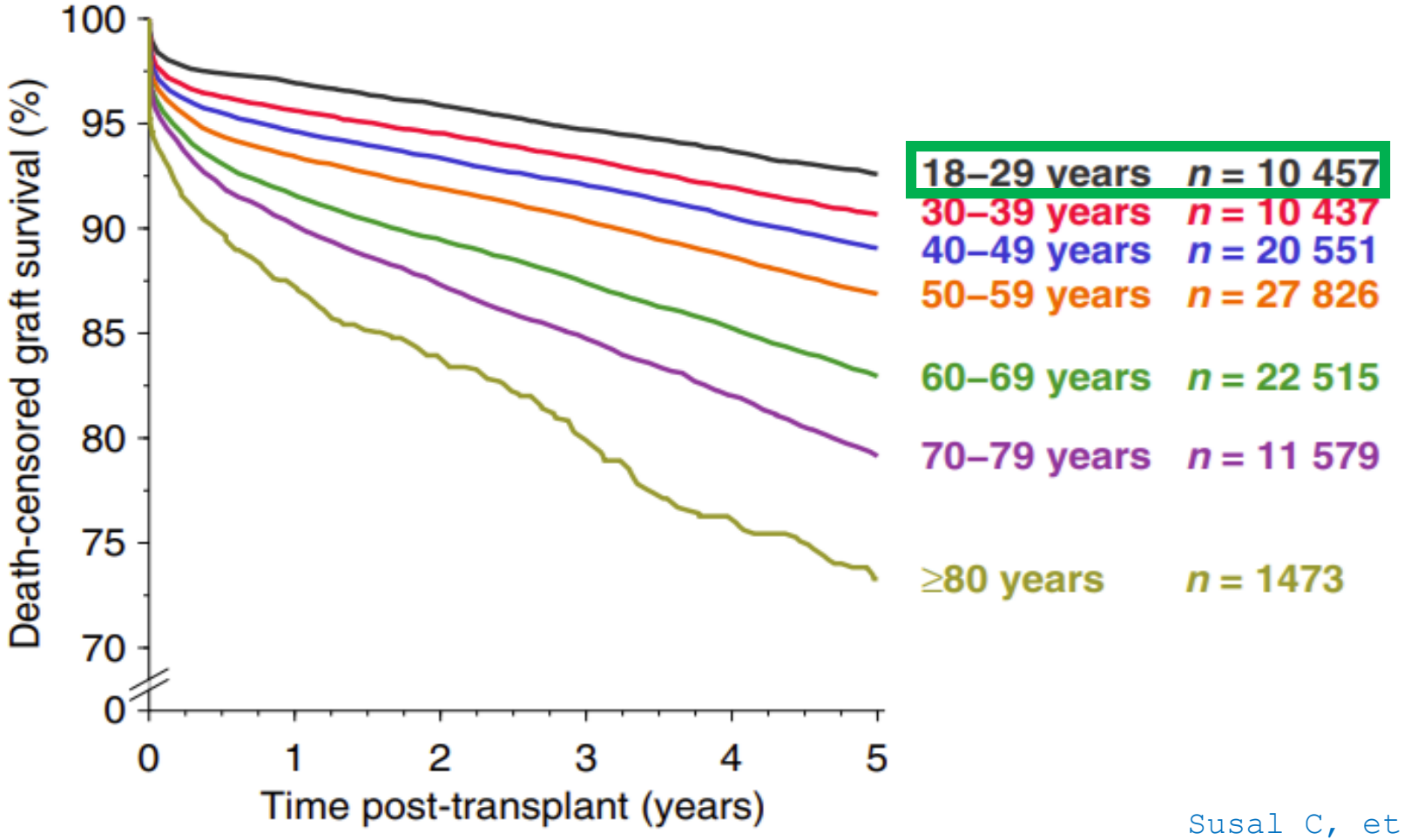
If		Add
RR = Black	& PID = Yes	46
RR = Black	& PID = No	44
RR = Non-Black	& PID = Yes	38
RR = Non-Black	& PID = No	18
SOT = Yes	& PID = Yes	40
SOT = No	& PID = Yes	20
Non-Heart Beating Donor		29
Recipient of Previous Transplant(s)		10
Donor Had Hypertension		7
Male Recipient		7
Diabetic Recipient		5
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 0
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

Impact of donor age on death-censored graft survival



Susal C, et al. Transplant International · April 2020
DOI: 10.1111/tri.13628

Outcomes in Living Donor Kidney Transplantation: *The Role of Donor's Kidney Function*

Study	Donor-recipient pairs	Donor's GFR	Method	Correlation between donor's and recipient's kidney function	Notes
Norden et al. [24]	344	Measured	^{51}Crom-EDTA	Yes	Donor's mGFR <80 mL/min/1.73 m^2 increase the risk of graft loss
Poggio [52]	119	Measured	^{125}I-iothalamate urinary clearance	Yes	Transplanted kidney GFR >55 mL/min/1.73 m^2 associated with better recipient's kidney function at 2 years
Issa [53]	248	Measured	^{125}I-iothalamate urinary clearance	Yes	Donor's iGFR >110 mL/min was associated with a better recipient's renal function at 2 years
Chang [54]	83	Estimated	MDRD equation	No	
Young et al. [26]	2,057	Estimated	CKD-EPI equation	No	No differences in graft survival between donor's with eGFR > or <80 mL/min/1.73 m^2
Godinho et al. [25]	48	Estimated	CKD-EPI equation	Yes	

Studies assessing the effect of donor's kidney function on recipient's graft functi

Torreggiani M, et al, Kidney Blood Press Res **2021**; 46: 84–94

DOI: 10.1159/000512177

graft function of the recipients 3 months after transplantation

Plage H, et al, Therapeutics and Clinical Risk Management
2020,16: 787–793
doi: 10.2147/TCRM.S256962

A multinational cohort study uncovered sex differences in excess mortality after kidney transplant.

kidney INTERNATIONAL

ISN INTERNATIONAL SOCIETY OF NEPHROLOGY

Methods & Cohort

SRTR CTS ANZDATA

IPD Meta-Analysis of Deceased Donor Kidney Transplant Recipients

Relative Excess Risk of Mortality (Above Baseline Risk)

Female vs Male Recipients, Accounting for Donor Sex and Recipient Age

1988-2019

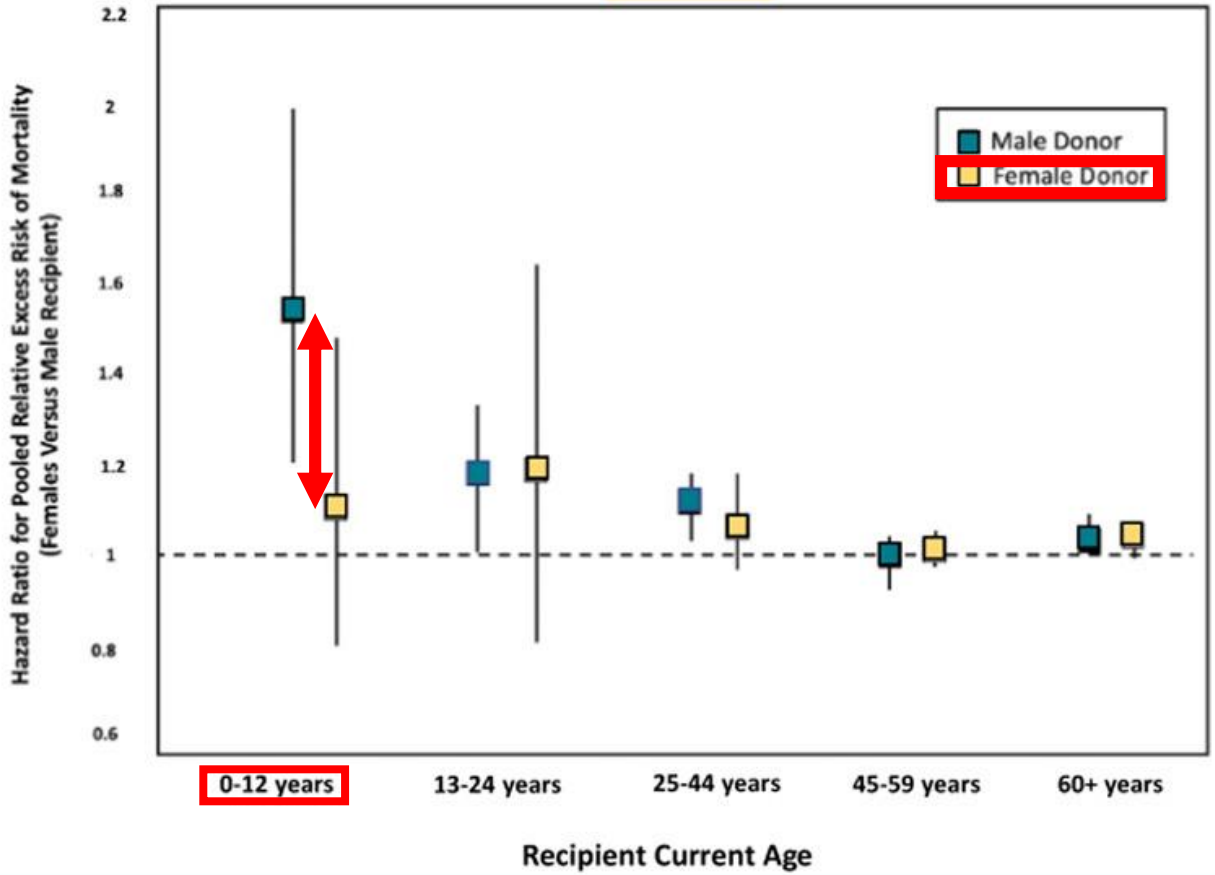
SRTR
Scientific Registry of Transplant Recipients **(USA)**
N=243,371

CTS
The Collaborative Transplant Study **(Europe/International)**
N=209,340

ANZDATA
Australia and New Zealand Dialysis and Transplant Registry **(Australia/New Zealand)**
N=14,181

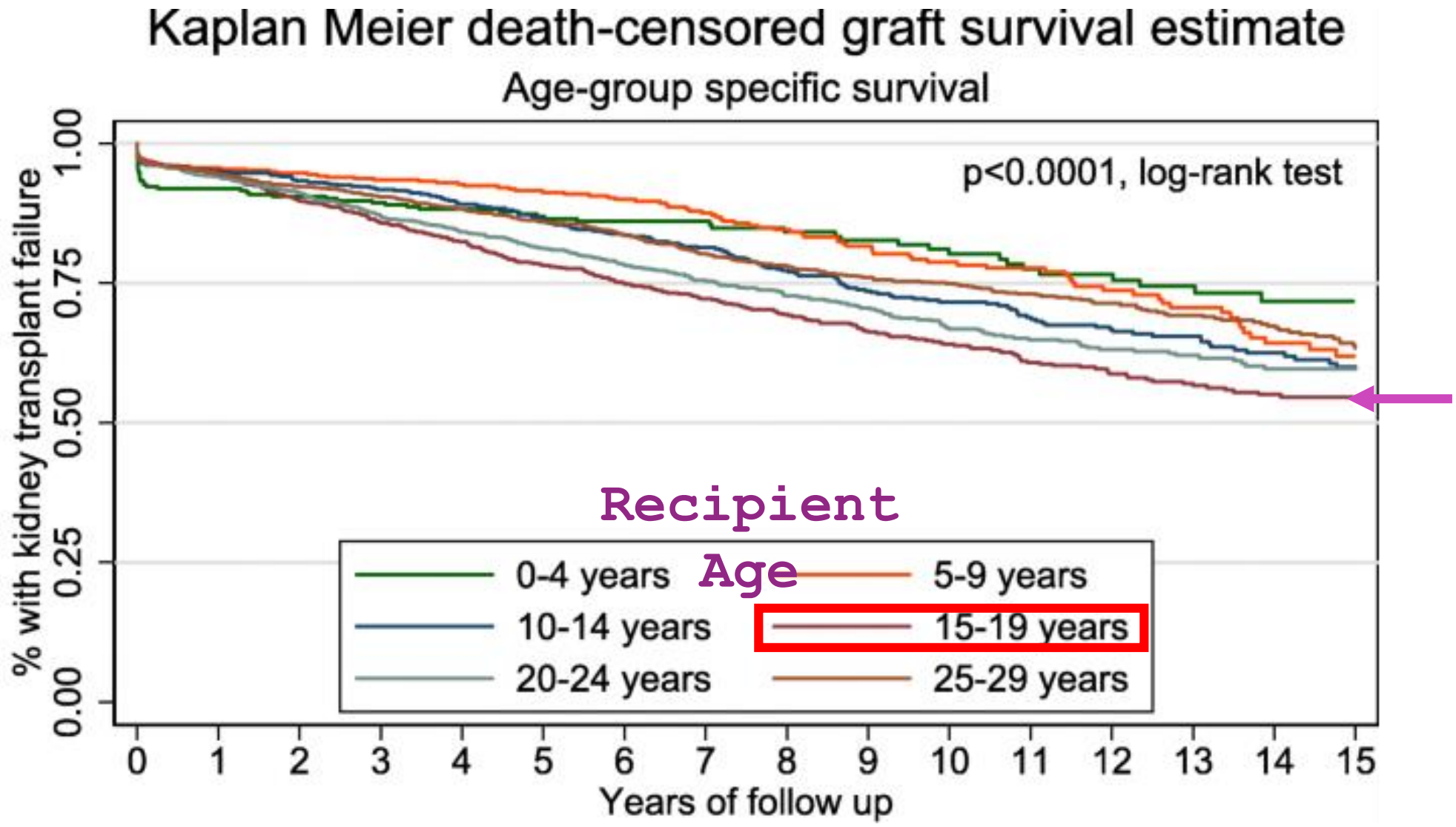
N=466,892 first deceased donor kidney transplant recipients

Results



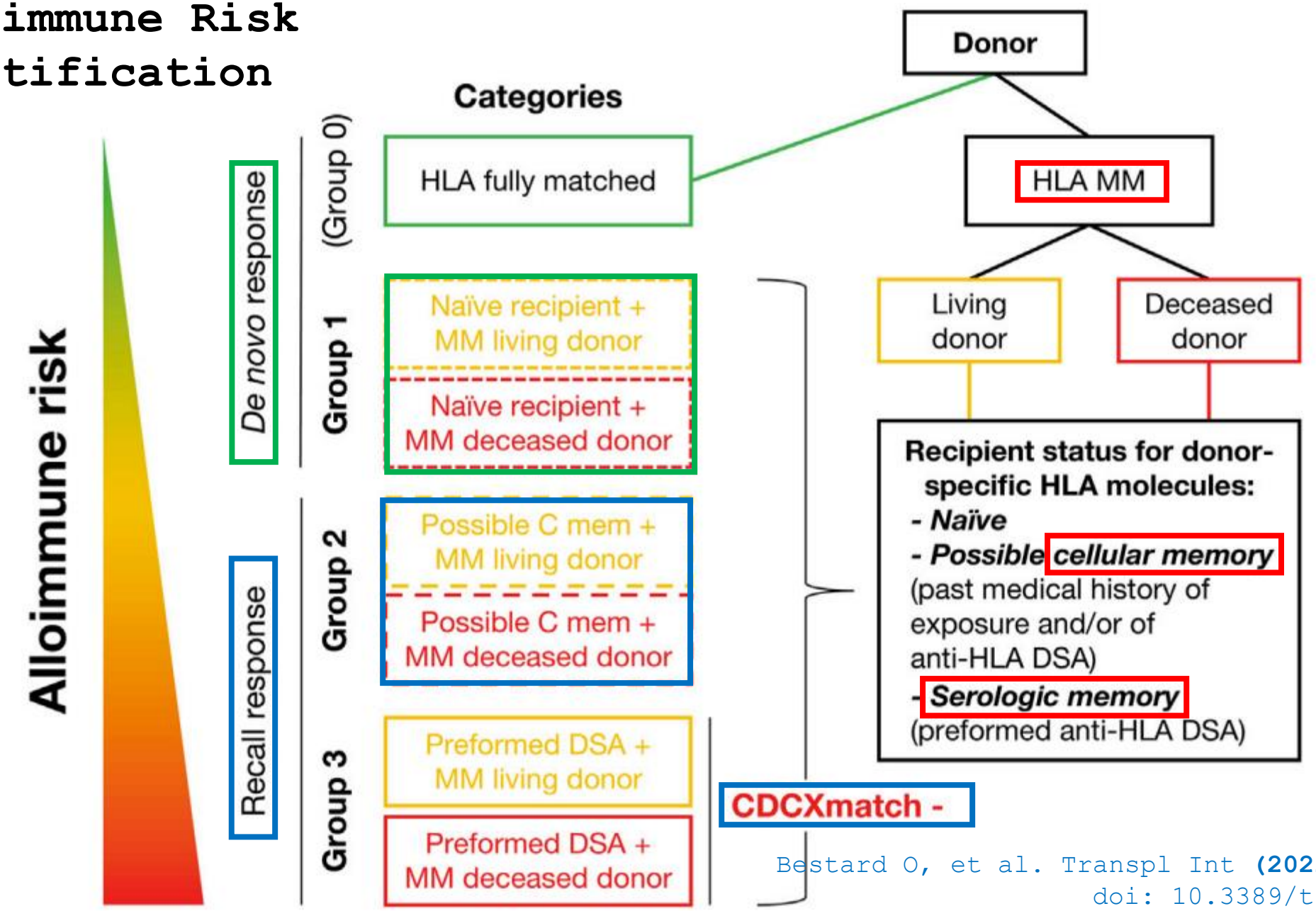
Vinson, 2022

CONCLUSION: When the donor was male, female recipients 0–44 and ≥60 years had higher excess mortality risks than male recipients of the same age. When the donor was female, there were no significant differences in any age interval.



Hamilton AJ, et al. BMC Nephrol
(2020) 21,492
Doi.org/10.1186/s12882-020-02156-2

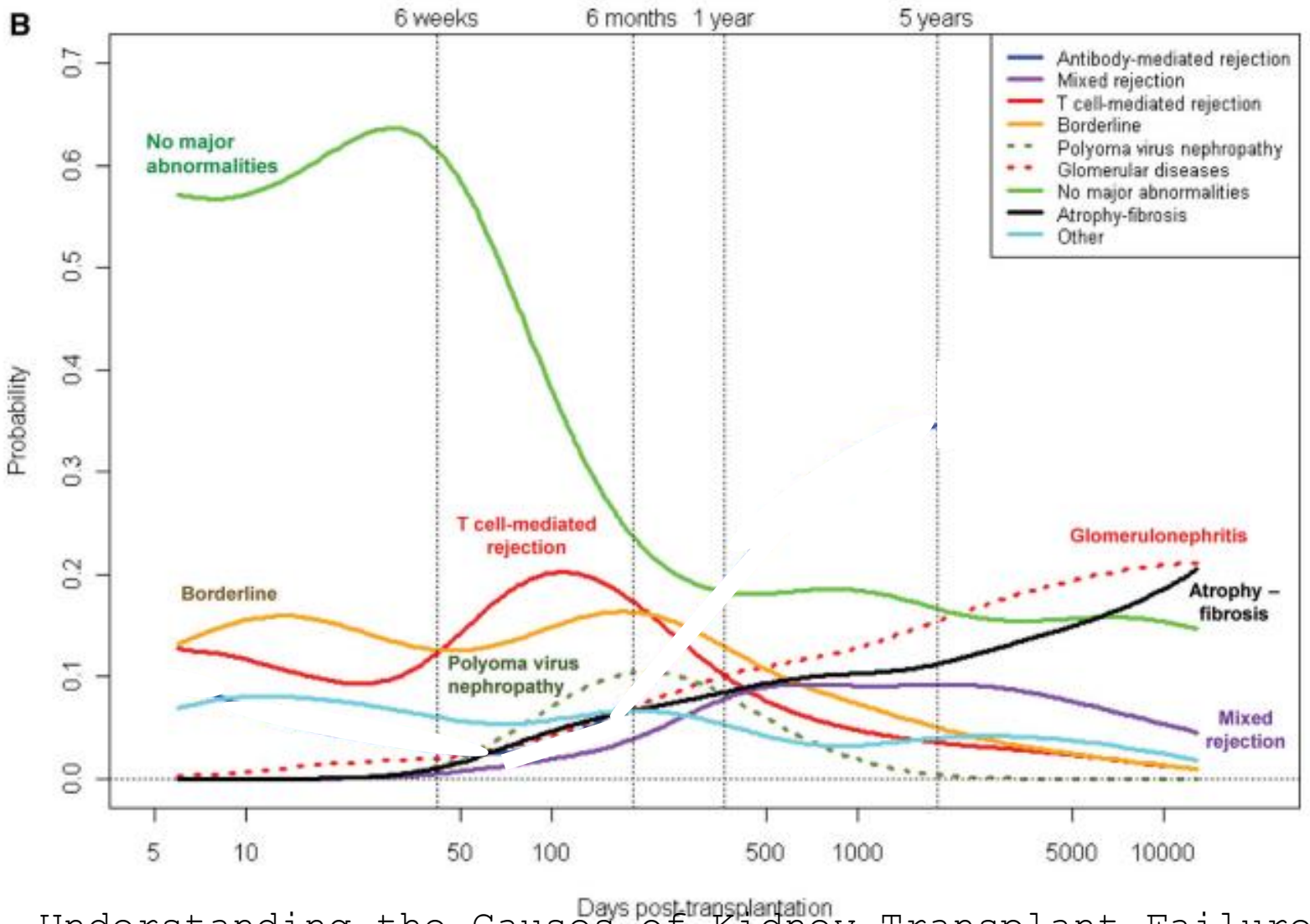
Alloimmune Risk Stratification



Bestard O, et al. Transpl Int **(2022)** 35: 10138
doi: 10.3389/ti.2022.10138

	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

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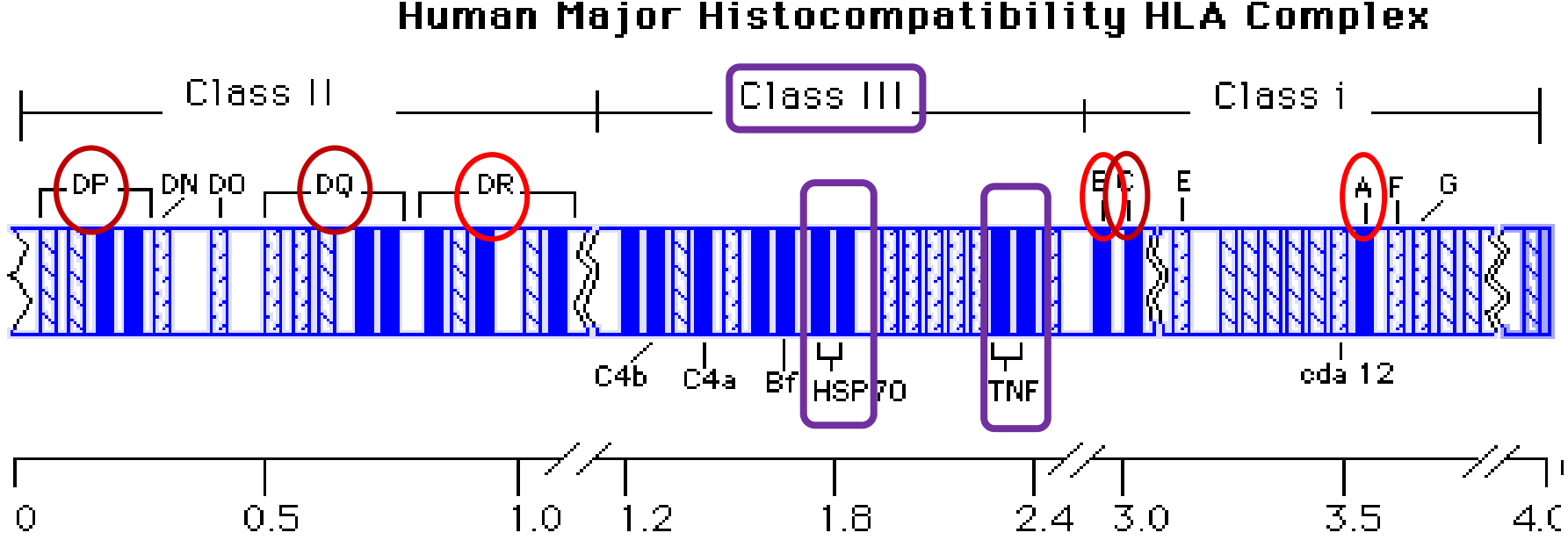


Expressed genes

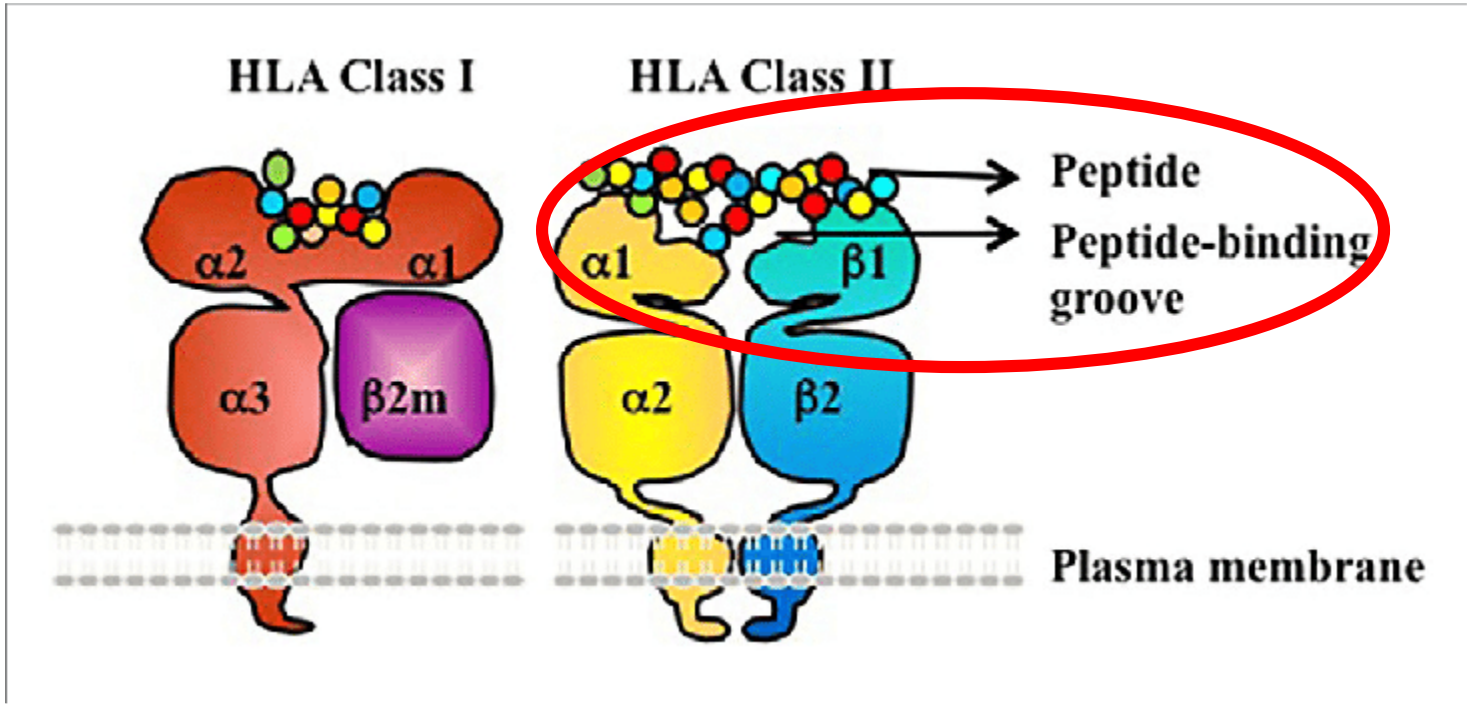
Unknown Status

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



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

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

Analysis of OPTN/UNOS registry suggests the number of HLA matches and not mismatches is a stronger independent predictor of kidney transplant survival

	Recipient	Donor 1	Donor 2	Donor 3
A	24	2	-	24
	2	-	2	2
B	18	18	37	37
	37	-	18	18
DR	2	2	7	2
	7	-	2	7
Match levels		3	5	6
Mismatch levels		0	0	0

Yacoub R, et al. Kidney International **(2017)**.
doi.org/10.1016/ j.kint.2017.07.016

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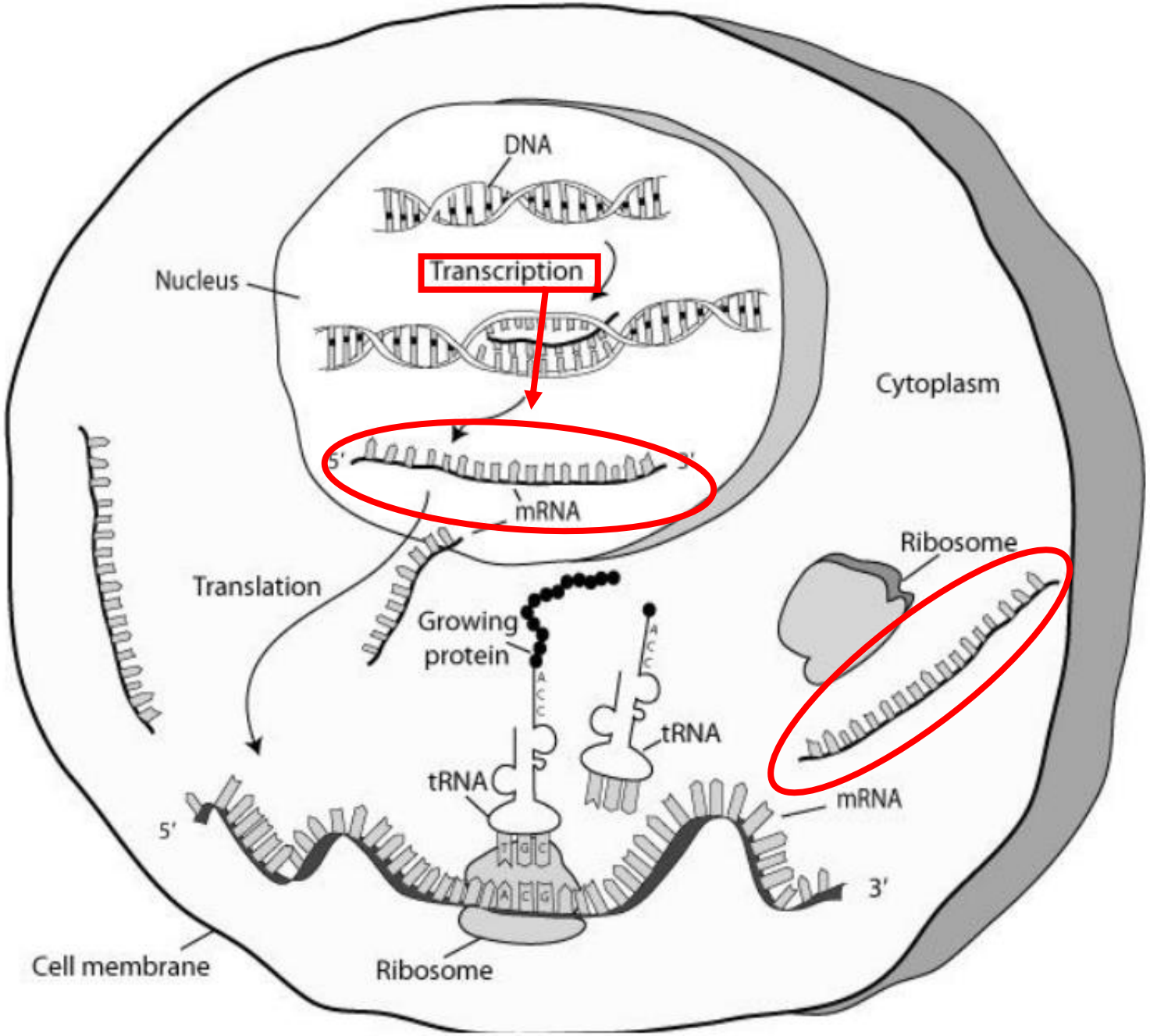
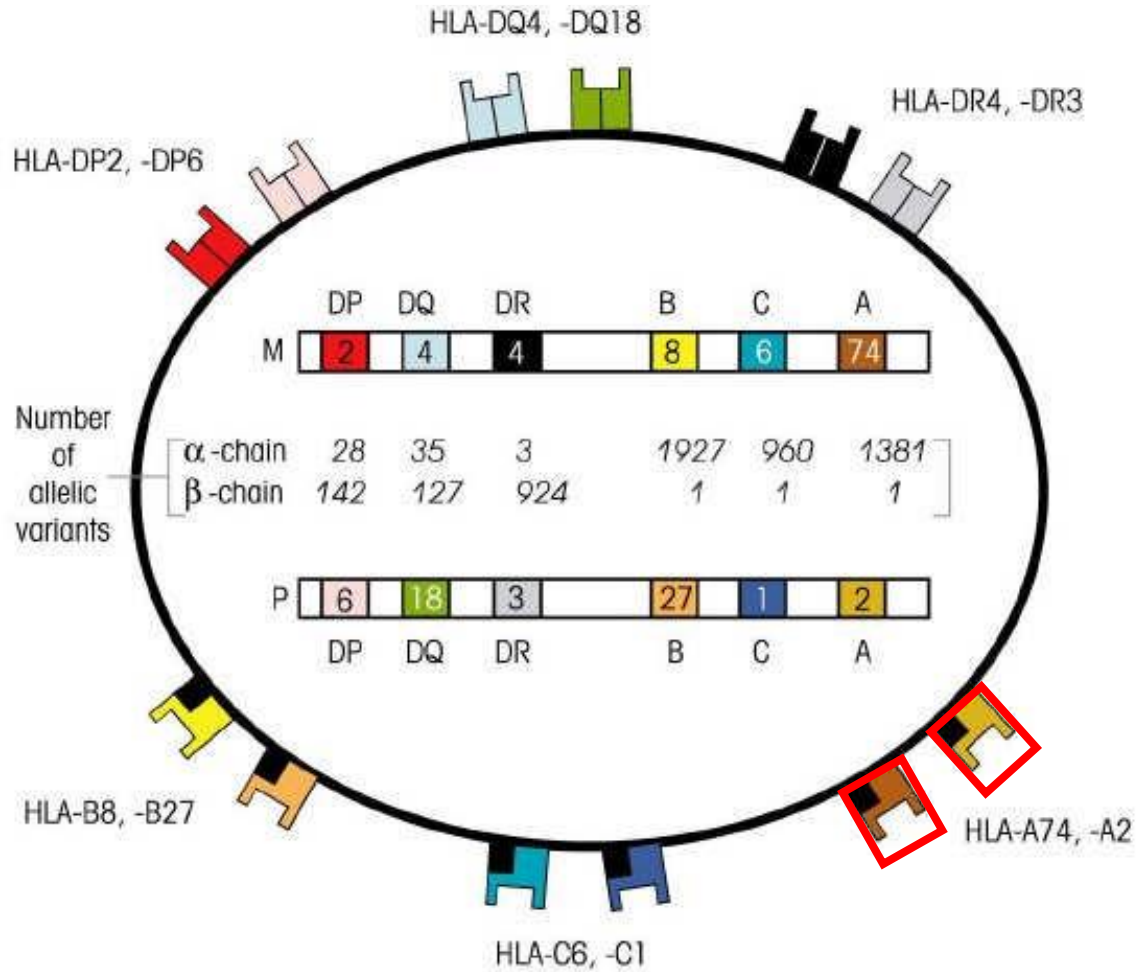
KDRI / KDPI & EPTS

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



Polymorphic HLA specificities and their inheritance



Delves *et al. Roitt's Essential Immunology, 12th ed.*
© 2011 Delves *et al.* Published 2011 by Blackwell Publishing Ltd.

Allo-typing

- CDC Serologic: Terasaki's antihuman leukocyte antigen (HLA) antibodies from sera of sensitized patients
- Oligotyping
- DNA Sequence based typing

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

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

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 serologicall

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

TABLE 3.3 Cross-reactive antigen groups

On serologic basis

Broad and Split …. Specified molecularly

A LOCUS													
1.	A1	A3	A11	A36									
2.	A9	A23	A24	A34	A66	A43							
3.	A10	A25	A26										
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											

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

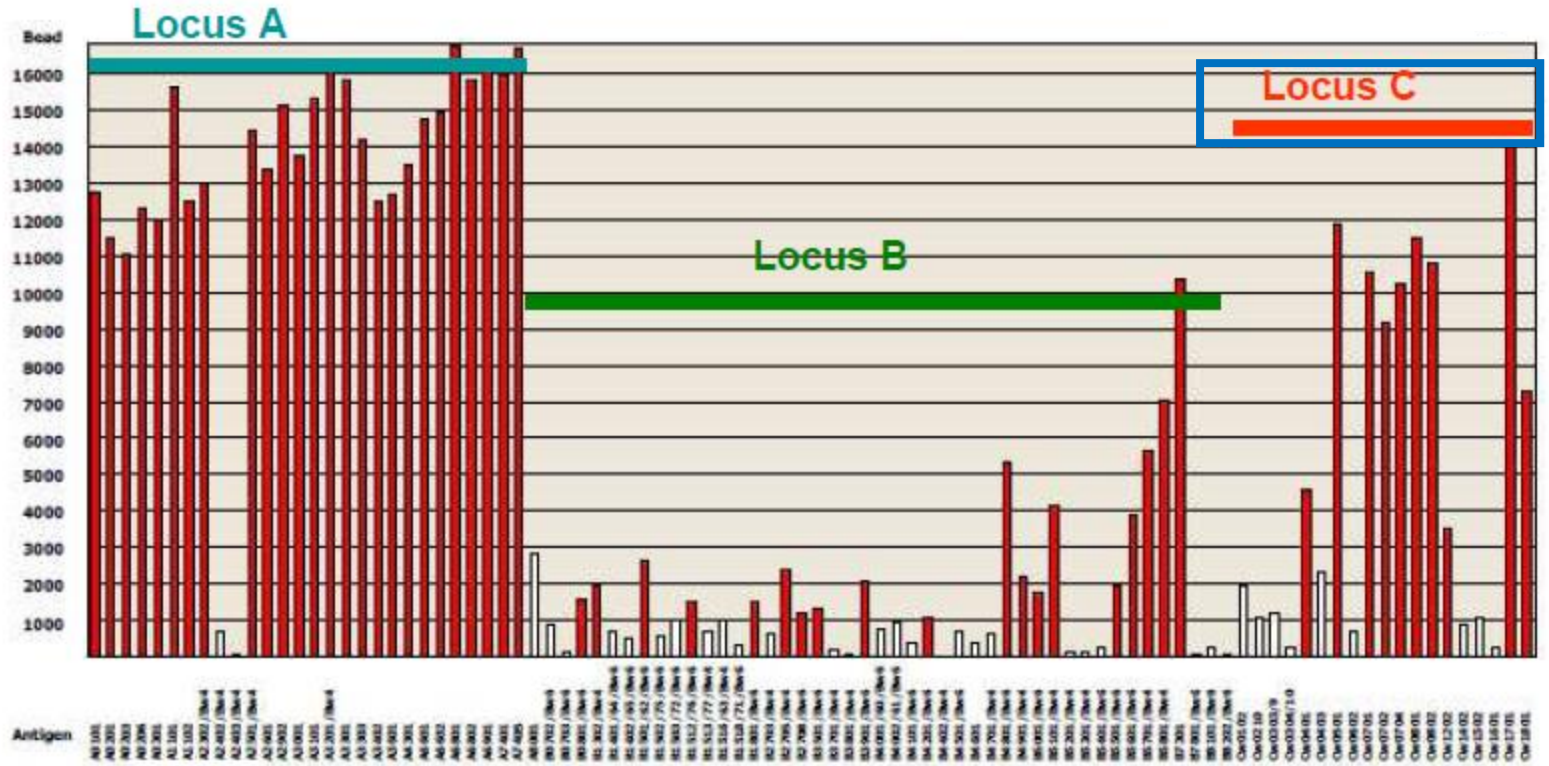
Human Leukocyte Antigen Examination Using Luminex®- Based Assays for Donor-Recipient Compatibility Assessment in Kidney Transplantation: Our Preliminary Experience

Table 2. The HLA Typing Results in all the Subjects

Subject	HLA-A	HLA-B	HLA-C	HLA-DR	HLA-DP	HLA-DQ
1	11, 24	15, 40	04, 08	B1 04, 12	A1 01:03, 02:02; B1 02:02, 105:01	A1 01, 03; B1 04, 05
2	11, 24	15, 51	08, 14	B1 04, 14	A1 02:01, 02:02; B1 05:01, 13:01	A1 01, 03; B1 04, 05
3	11, 24	18, 18	07, 07	B1 15, 15	A1 02:02, 02:02; B1 02:02, 05:01	A1 01, 01; B1 05, 06
4	11,33	38:02, 40	07, 08	B1 12, 14	A1 02:01, 02:02; B1 01:01, 13:01	A1 01, 06:01; B1 03, 05
5	11, 24	39, 40	07, 08	B1 04, 12	A1 02, 02; B1 01:01, 03:01	A1 03, 06:01; B1 03, 03
6	02:03, 11:01	15:139, 53:14	04:01, 08:01	B1 15:02, 16:02	A1 02,02: 02,02; B1 05:01, 05:01	A1 01:01, 01:02; B1 05:01, 05:02
7	02:01, 11:01	18:01, 56:02	03:03, 07:04	B1 14:04, 15:02	A1 02:01, 02:02; B1 05:01, 13:01	A1 01:01, 01:01; B1 05:01, 05:03

Birowo P, et al. Nephro - Urol Mon. **2020** November; 12(4): e104635
doi: 10.5812/numonthly.104635.

Antibodies anti HLA by SA-BAAssays
Definition of non acceptable mismatches
"Virtual Crossmatch"



ONE LAMBDA
A Thermo Fisher Scientific Brand

PRODUCT INSERT

LABScreen™

REF

Catalog ID	Product Name
LS1PRA*	LABScreen™ PRA Class I
LS2PRA*	LABScreen™ PRA Class II
LS12PRA*	LABScreen™ PRA Class I & II
LSM12*	LABScreen™ Mixed Class I & II
LS1A04*	LABScreen™ Single Antigen HLA Class I - Combi
LS1ASP01*	LABScreen™ Single Antigen HLA Class I Supplement - Group 1
LS2A01*	LABScreen™ Single Antigen HLA Class II - Group 1
LS2ASP01*	LABScreen™ Single Antigen HLA Class II Supplement - Group 1
LS1AEX01*	LABScreen™ Single Antigen HLA Class I ExPlex
LS2AEX01*	LABScreen™ Single Antigen HLA Class II ExPlex
LSMICA001	LABScreen™ MICA Single Antigen - Group 1
LSPWABUF	LABScreen™ Wash Buffer

IVD In Vitro Diagnostic Medical Device.

INTENDED USE

LABScreen products are intended for use in detection of HLA antibody using flow cytometric technology

SUMMARY AND EXPLANATION

LABScreen products use microbeads coated with purified Class I or Class II HLA antigens and pre-optimized reagents for the detection of Class I or Class II HLA antibodies in human sera. LABScreen products utilize the LABScan™ 100 (Luminex® 100/200) or LABScan3D™ (Luminex® FLEXMAP 3D®) for analysis of up to 100 or 500 bead regions, respectively, in a single test.

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**

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.

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

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

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

Anti-HLA Antibody Detection Techniques for Solid Organ Transplantation

Panel Donors

Specific Donor

Solid Pha

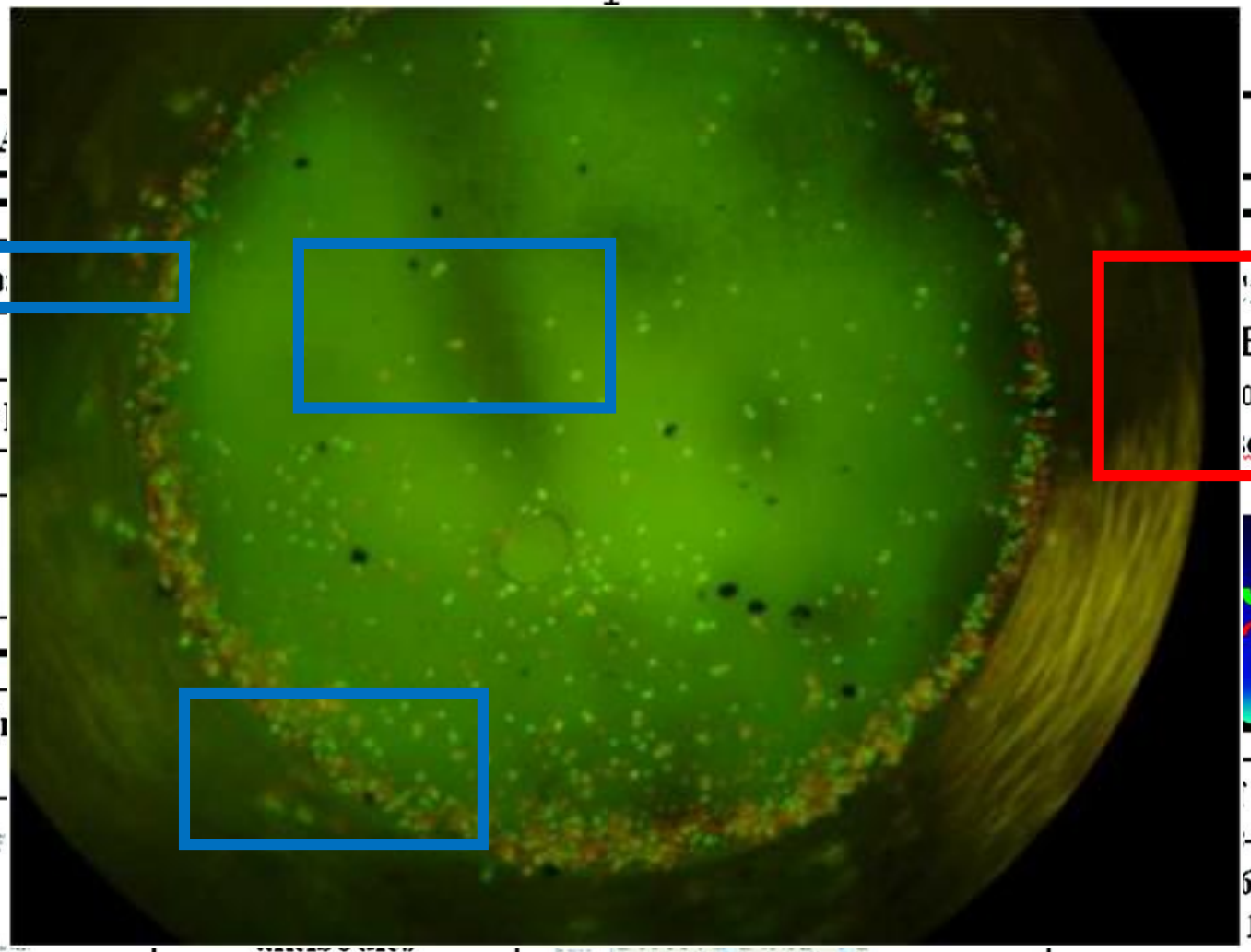
ELISA-P

Flow Screeni
PRA

ross-match
B cell (IgG)
or without
e)

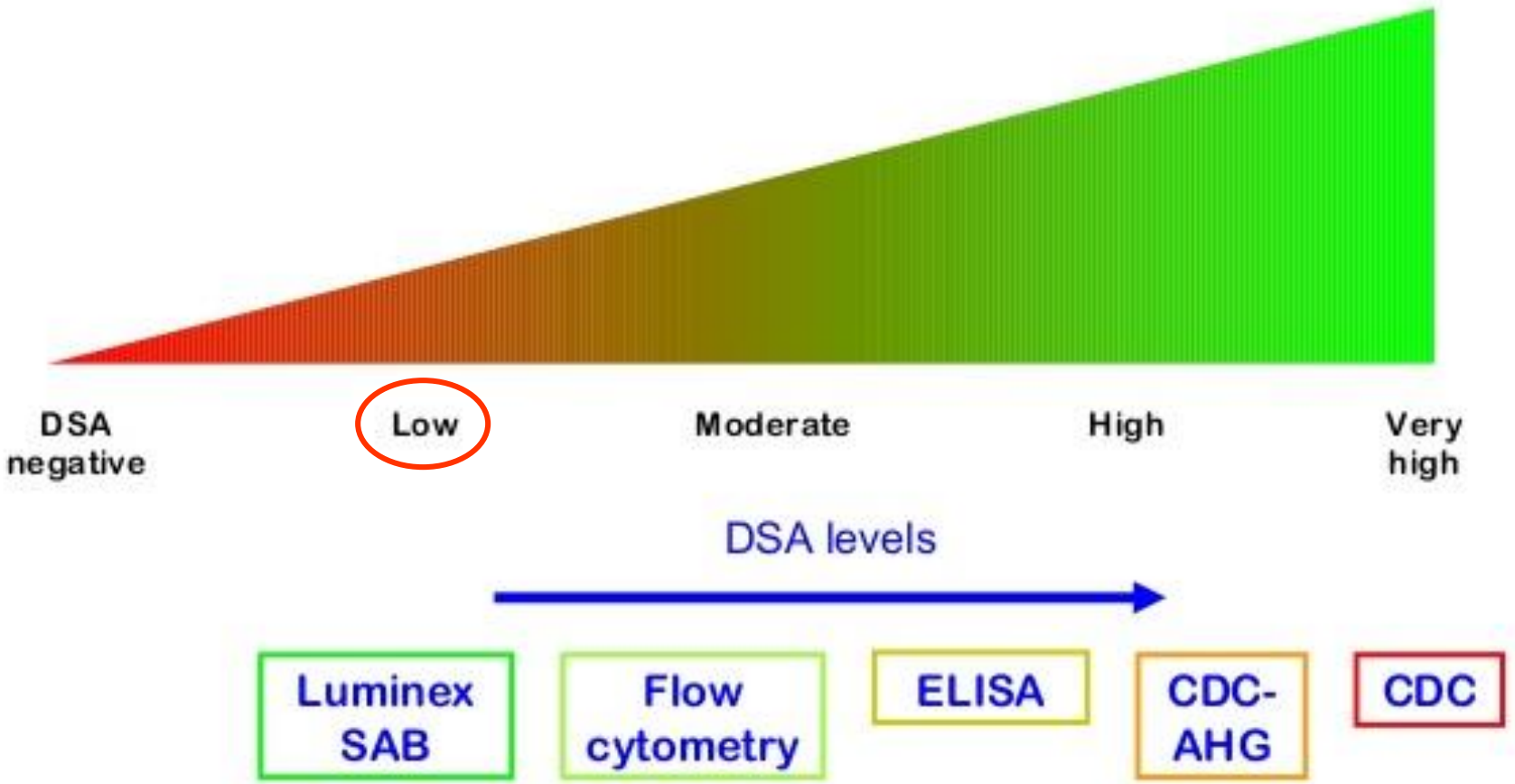
T and B cell
-match:
50 MCS
100 MCS are
negative cross-match.

MFI; SFI; MESF



Marfo K et al. CJASN 2011;6:922-936

Sensitivity of DSA identification methods

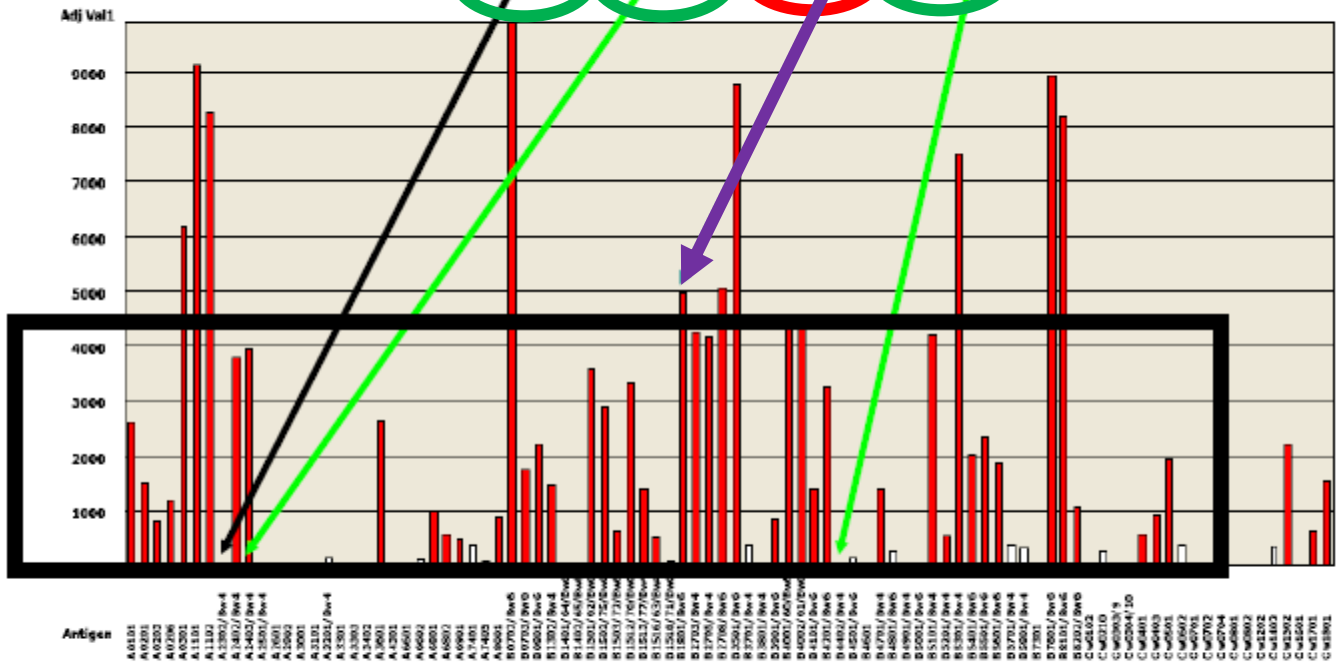


Gebel and Bray, Transplantation Reviews 20: 189-194, 2006

Detection of HLA antibodies

- Class II HLA [illegible] Possible
- [illegible] I Abs (of less significance) as well as **low titres of class-I Abs**
- **T cell +ve / B cell –ve** cross match is indicative of **non HLA Abs**

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



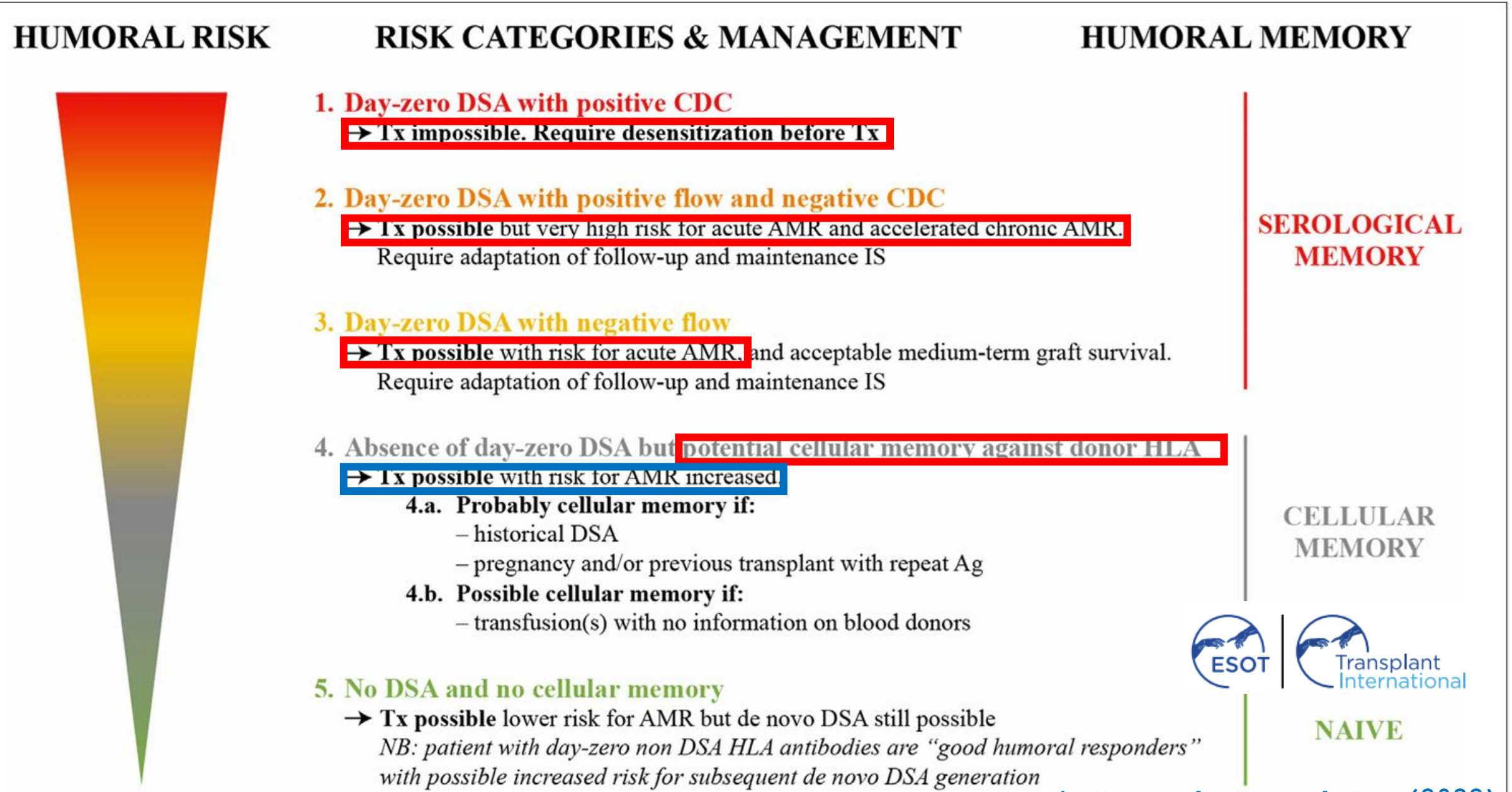
22/02/2007 PRA-CDC 56 % Anti- A3, B40, B51

Crossmatch: XM-CDC Negative; XM-FCM Negative

European Guideline for the Management of Kidney Transplant Patients With HLA Antibodies

- **To define the humoral risk in kidney transplantation, the use of the ENGAGE 5 strata system is recommended (1C).**

Mamode N, et al. Transpl Int **(2022)** 35: 10511
doi: 10.3389/ti.2022.10511



Mamode N, et al. Transpl Int (2022) 35: 10511
doi: 10.3389/ti.2022.10511

FIGURE 2 | Humoral risk stratification of kidney transplant candidates (adapted from reference (1)) AMR, antibody-mediated rejection; CDC, complement-dependent cytotoxicity; DSA, donor-specific antibodies; HLA, human leukocyte antigen; IS, immunosuppression; Tx, transplant.

The highly sensitized patient?

“Potential recipient of a graft with an amount of circulating anti-HLA antibodies which limits “notably” his/her access to transplantation”

Spain	**PRA CDC >90%** (only 1 of 10 donors would be compatible)
Eurotransplant	**PRA CDC >85%**
UNOS	**PRA CDC >80%** (2 of 10 donors would be compatible)

A Torio 2012

Comparison Between Phenotypic Bead Assay and Single Antigen Bead Assay for Determining Specificity of HLA Antibodies in Kidney Transplant Waiting List

Table 1. Performance of the Phenotypic Bead Assay for Determining Specificities of HLA Antibodies Using the SAB Assay as the Standard Method

Measure of Performance	HLA Class I	HLA Class II
Sensitivity (95% CI), %	53.9 (51.0-56.8)	57.3 (53.6-61.1)
Specificity (95% CI), %	93.0 (92.0-94.0)	94.9 (94.0-95.9)
Positive predictive value (95% CI), %	84.7(82.6-86.8)	79.4 (77.6-81.2)
Negative predictive value (95% CI), %	80.0 (77.2-82.9)	83.2 (81.0-85.5)
Accuracy (95% CI), %	78.1 (76.3-79.9)	81.4 (79.6-83.2)

Abbreviations: CI, confidence interval; SAB, single antigen bead.

Thammanichanond D, et al. Transplantation Proceedings (2020) XX: 1 - 5
doi.org/10.1016/j.transproceed.2020.01.153

Table 2. RR for acute AMR according to the MFI of highest pregraft ranked DSA detected by Luminex (logistic regression)

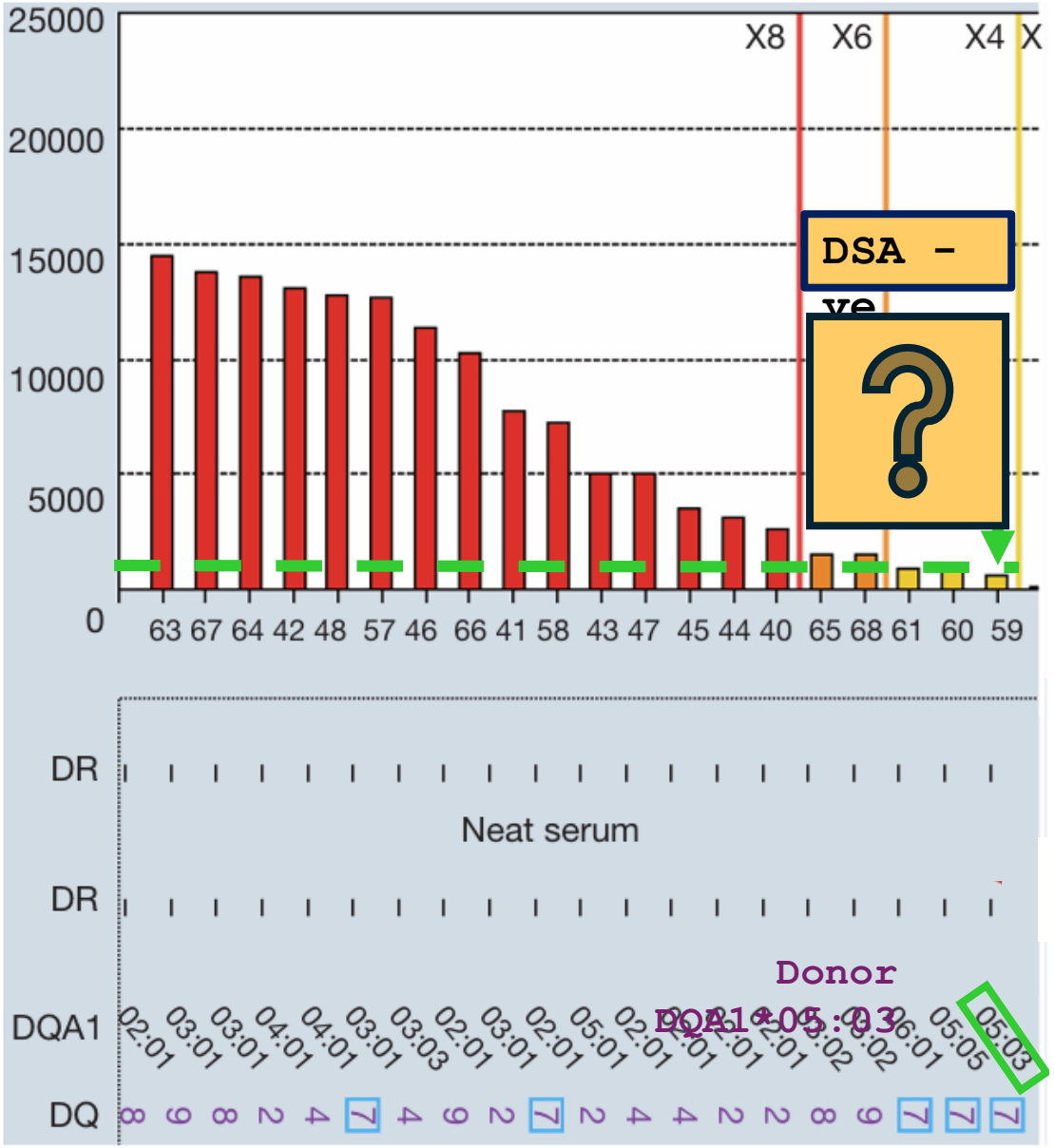
DSA MFI_{max} class	RR (95% CI)	*P*
≤465	1.0	
465 to 1500	24.8 (4.6 to 134.8)	<0.001
1500 to 3000	23.9 (3.5 to 160.8)	0.001
3000 to 6000	61.3 (11.5 to 327)	<0.001
>6000	113.0 (30.8 to 414)	<0.001

Lefaucheur C et al, JASN 21: 1398 –1406, 2010

Comparison of Methods for Abolishing Inhibitory Serum Factors in Luminex Single Antigen Assay. Abstract# D2330

de Marco, R.; De Lima, Gerbase M.. Comparison of Methods for Abolishing Inhibitory Serum Factors in Luminex Single Antigen Assay.: Abstract# D2330. Transplantation 98():p 387, July 15, 2014

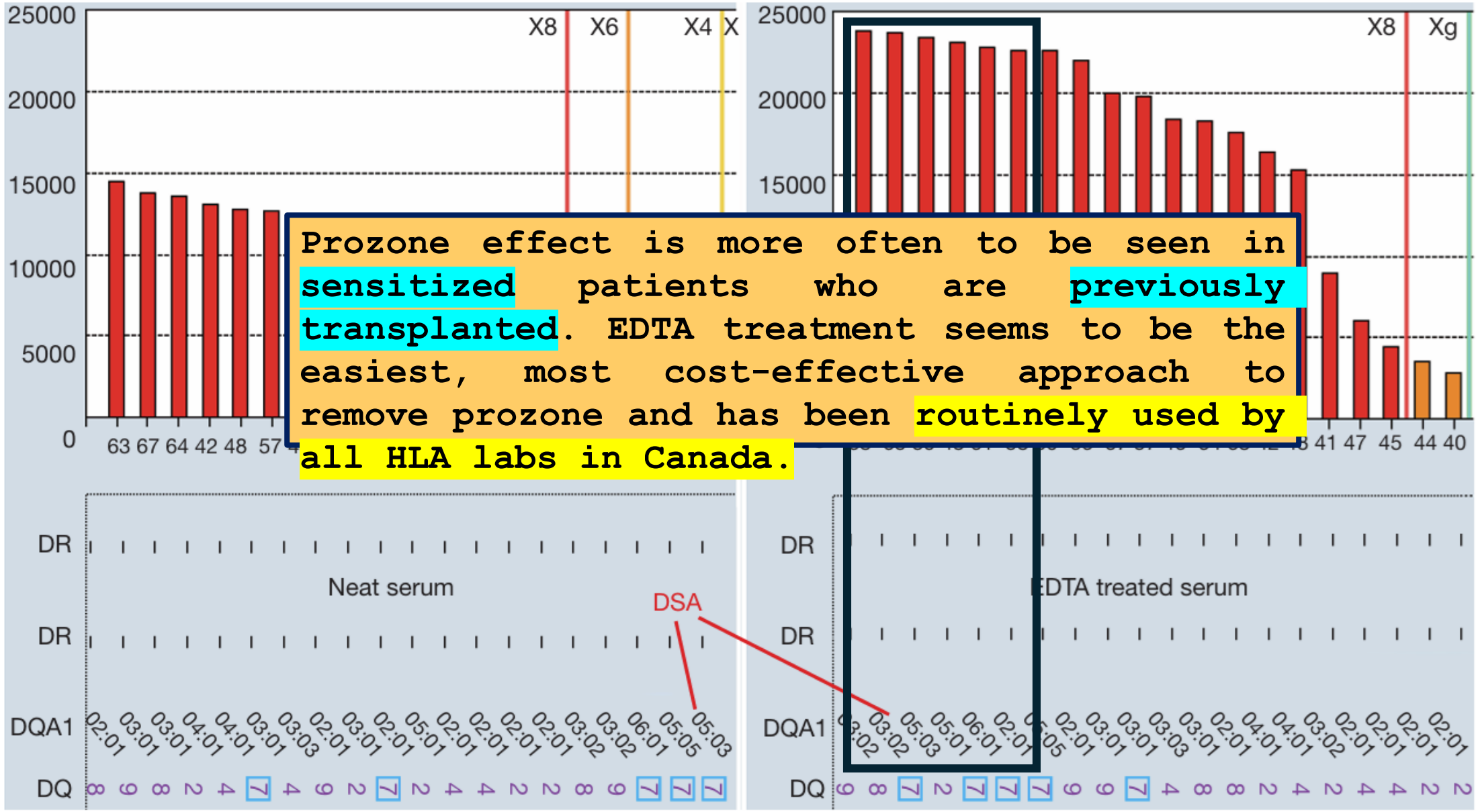
False-negative or weaker reactions may occur in the Luminex single-antigen bead assay (SAB), especially in fresh sera with high antibody titers, probably due to interference of the ligation of the anti-IgG antibody by complement factor 1 (C1) bound to the anti-HLA antibodies. We compared the strength (MFIs) of the reactions on One Lambda SAB of fresh not treated (NT) and treated sera with heat (sera in the Luminex plate were heated for 1 min at 56°C in a thermocycler) (H-1min), DTT and EDTA. Two sera with PRA I >80% and two sera with PRA II >80%, both with at least one reaction with >15,000 MFI, were tested. An MFI increase ≥ 50% in treated vs NT serum was considered relevant. One of the sera examined on HLA class I beads presented heightened reactions against 11 beads with HLA-A 2, 68, 69, 24, B57, 58 molecules, after any of the treatments, being the mean MFIs in NT and in H-1min, DTT and EDTA treated sera 4,684, 16,128, 18,768 and 19,577, respectively. With the other serum, there was no relevant increase in the reaction to any class I bead, with any treatment. Regarding the two sera analyzed on class II SAB: one showed increased MFIs with 6 beads with HLA-DQB1 2, 7, 8, 9 molecules only after H-1min or EDTA treatment and the respective mean MFI values in NT, H-1min, DTT and EDTA treated sera were 13,854, 22,116, 16,494 and 23,744; with the other serum, increased MFIs with any treatment occurred with 18 assorted beads, being the mean MFI values in NT, H-1min, DTT and EDTA treated sera 4,758, 13,857, 13,070 and 18,593, respectively. In addition, increased reactivity of this serum was observed with two other beads but only with H-1 min or DTT. This same serum also presented an unexpected inhibition of some reactions after treatment with EDTA. This inhibitory effect of EDTA was observed only against all the six beads coated with different HLA-DQB1*06 alleles, being the mean MFI values in NT, H-1min, DTT and EDTA treated sera 7,828, 9,288, 9,609 and 1,023, respectively. The same phenomenon was observed in a prior serum sample from the same patient. In conclusion, the inhibitory effect on reactions with some of the beads of Luminex-SAB that occur in some sera is in general equally reversed by treatment of the serum with heat, DTT or EDTA. In addition, we found that EDTA may cause inhibition of some reactions and this phenomenon deserves attention and further studies, since EDTA is currently the treatment of choice of sera in several laboratories.



patient experienced antibody-mediated rejection after kidney transplantation.
Illustrated Retrospectively that EDTA removed prozone and uncovered strong DSA to DQ7.

University Health Network, Toronto, Canada

McCaughan J, et al. HepatoBiliary Surg Nutr **2019**; 8(1): 37-52
doi: 10.21037/hbsn.2019.01.01



McCaughan J, et al. HepatoBiliary Surg Nutr
2019; 8(1): 37-52
doi: 10.21037/hbsn.2019.01.01

University Health Network, Toronto, Canada

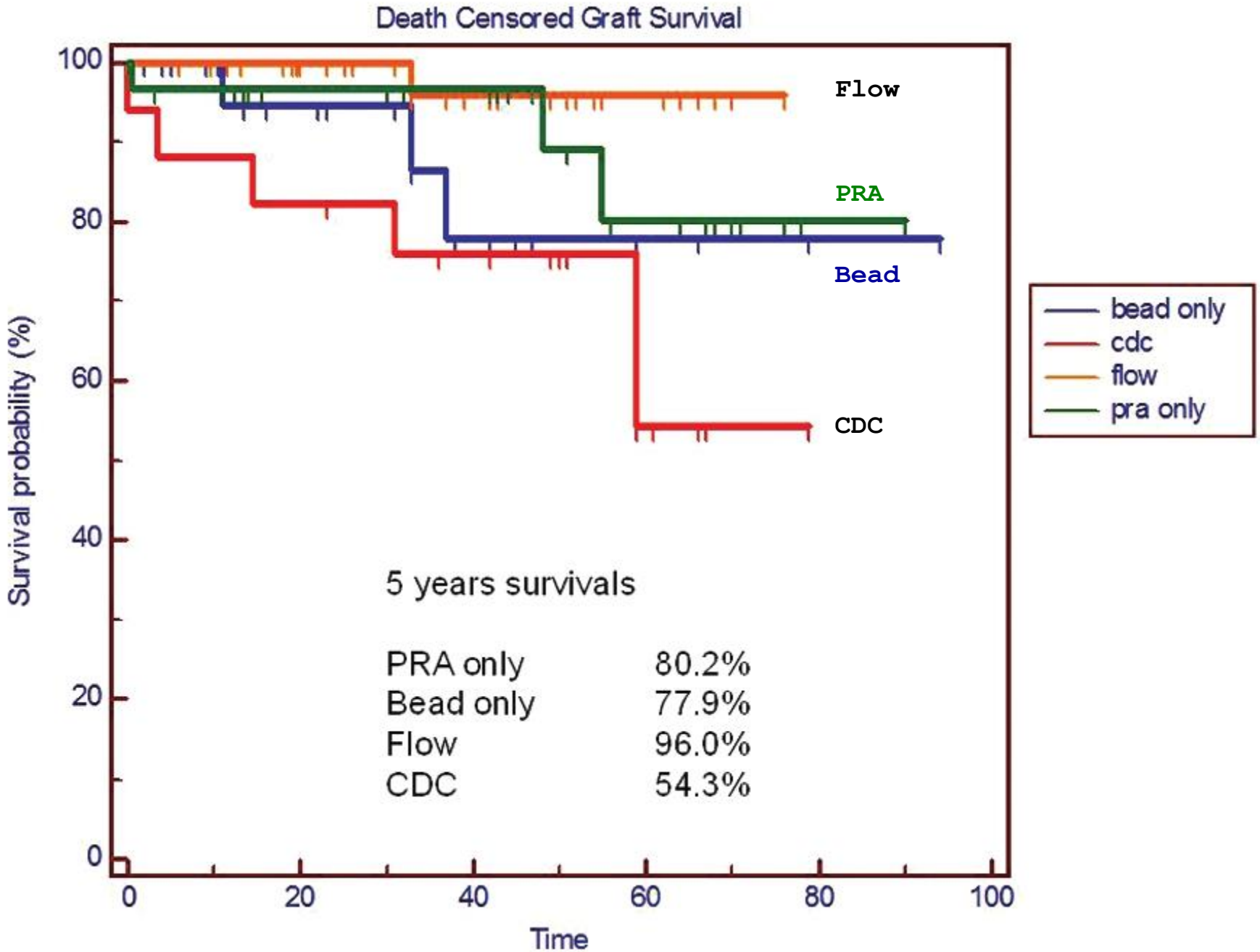
Detecting DSA: the importance of sorting the wheat from the chaff

Abstract:

Human leukocyte antigen (HLA) compatibility is very important for successful transplantation of solid organs. In this paper, we focused on the humoral arm of immunity in the clinical setting of organ transplantation: how HLA antibodies develop, how they can be detected, and what they can do to injure organ transplants. Specifically, we explore the technical perspectives of detecting donor-specific antibodies (DSA) in HLA laboratories, and use real-life clinical cases to explain the principles. Currently there are many tools in our HLA antibody detection toolbox: conventional cytotoxicity cross match, flow cross match, and solid phase assays using beads conjugated with single or multiple HLA antigens. Single antigen bead (SAB) assay is the most sensitive tool available for detecting HLA antibodies and assessing the immunological risk for organ transplant. However, there are **intrinsic limitations to solid-phase assays and they are prone to both false negativity and importantly, false positivity**. Denatured antigens on single antigen beads might be the most prominent source of false positive reactivity, and may have been underestimated by many HLA experts. ***No single assay is perfect and therefore multiple methods, including the less sensitive assays, should be employed to determine the clinical relevance of detected HLA antibodies.*** Thoughtful process,

University Health Network, Toronto, Canada

McCaughan J, et al. HepatoBiliary Surg Nutr 2019; 8(1): 37-52
doi: 10.21037/hbsn.2019.01.01


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

Outline

Risk Stratification

Risk weight of HLA antigens

HLA specification

AM Acceptable Mismatches

PM Permissive Mismatches

Eplet and Epitope Matching

c-PRA vs TS

KDRI / KDPI & EPTS

Highly Sensitized Patients Are Well Served by Receiving a Compatible Organ Offer Based on Acceptable Mismatches

Highly sensitized kidney patients accrue on the transplant waiting list due to their broad immunization against non-self Human Leucocyte Antigens (HLA). Although challenging, the best option for highly sensitized patients is transplantation with a crossmatch negative donor without any additional therapeutic intervention. The Eurotransplant Acceptable Mismatch (AM) program was initiated more than 30 years ago with the intention to increase the chance for highly sensitized patients to be transplanted with such a compatible donor. The AM program allows for enhanced transplantation to this difficult to transplant patient group by allocating deceased donor kidneys on the basis of a match with the recipient's own HLA antigens in combination with predefined acceptable antigens. **Acceptable antigens are those HLA antigens towards which the patients has never formed antibodies**, as determined by extensive laboratory testing. By using this extended HLA phenotype for allocation and giving priority whenever a compatible donor organ becomes available, organ offers are made for roughly 80% of patients in this program. Up till now, more than 1700 highly sensitized patients have been transplanted through the AM program. Recent studies have shown that the concept of acceptable mismatches being truly immunologically acceptable holds true for both rejection rates and long-term graft survival. Patients that were transplanted through the AM program had a similar rejection incidence and long-term graft survival rates identical to non-sensitized patients transplanted through regular allocation. However, a subset of patients included in the AM program does not receive an organ offer within a reasonable time frame. As these are often patients with a rare HLA phenotype in comparison to the Eurotransplant donor population, extension of the donor pool for these specific patients through further European collaboration would significantly increase their chances of being transplanted. **For those patients that will not benefit from such strategy, desensitization is the ultimate solution.**

Heidt S, et al. Front. Immunol. **(2021)** 12: 687254.
doi: 10.3389/fimmu.2021.687254

European Guideline for the Management of Kidney Transplant Patients With HLA Antibodies

- **The Eurotransplant Acceptable Mismatch program should be expanded to other European countries (that do not have this type of matching) to improve donor/recipient matching (1C).**

Mamode N, et al. Transpl Int **(2022)**
35: 10511
doi: 10.3389/ti.2022.10511

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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

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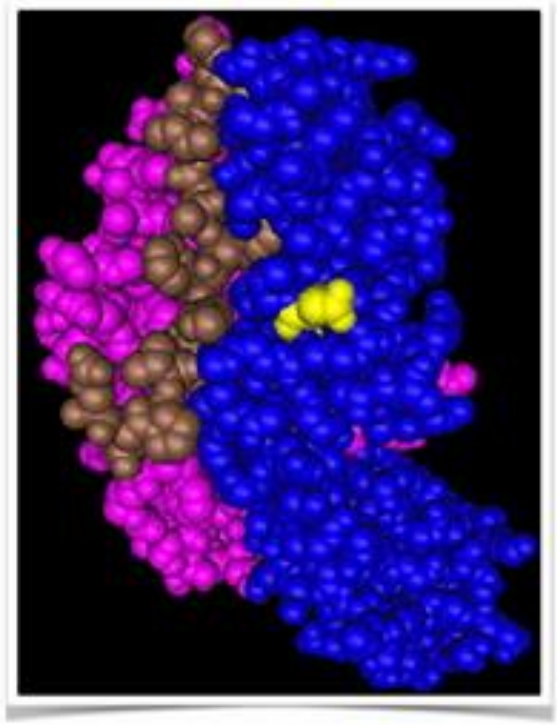
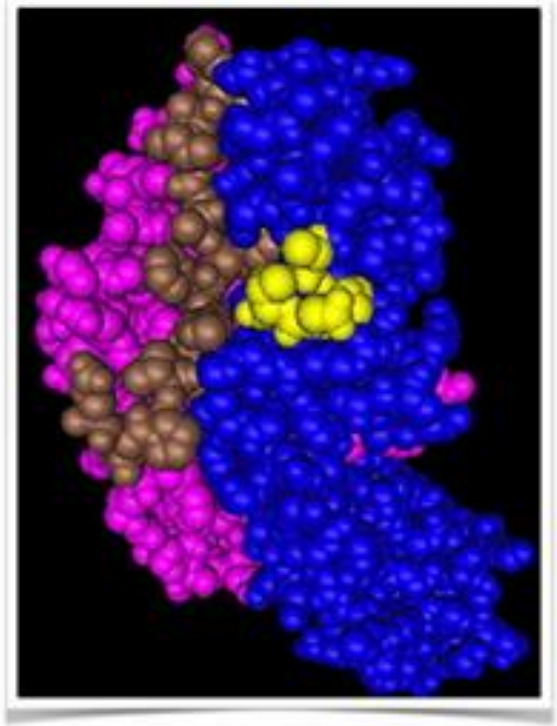
Antibody-Reactive Epitope Determinations With HLAMatchmaker and Its Clinical Applications[1]

HLA-DR
polymorphic amino acid

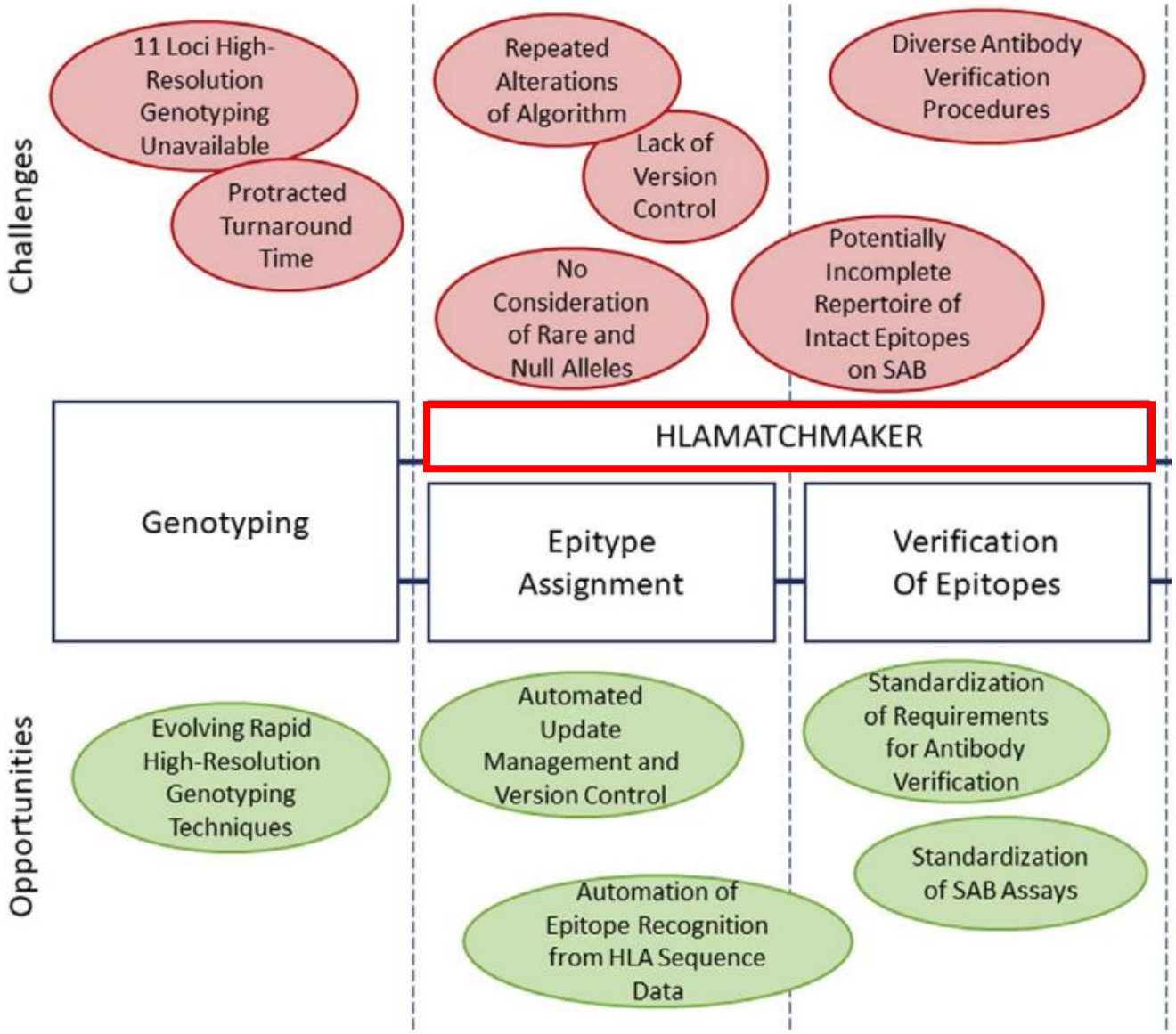


Eplet: Amino acids within a 3Å radius
(Epitope: Antibody binding site 15Å radius)



1. Duquesnoy RJ. *Tissue Antigens*. 2011;77:525-534.

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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Definitions of Transplantability Score and calculated combined PRA

A donor pool, based on 2000 recently HLA typed deceased donors registered in Scandiatransplant, has been made. The pool forms the basis of the Transplantability Score and calculated combined PRA.

	Calculated PRA (cPRA)	Transplantability Score (TS)
HLA information used in calculation	HLA antibody specificities defined on the patient. HLA-A, B, C, DRB1 and DQB1	HLA antigen typing and defined acceptable HLA mismatches on the patient. HLA-A, B, C, DRB1, DRB3, DRB4, DRB5, DQA1, DQB1, DPA1 and DPB1 antigens
AB0 used in calculation	No	Yes
Result based on the donor pool	Percentage of donors which the patient has antibodies against.	Percentage of donors which are AB0 identical/compatible and have HLA split level antigens that are acceptable to the recipient
Describes the probability of finding a suitable donor	No, AB0 is not included	Yes, dependent on the size of the donor pool

SCANDIA TRANSPLANT
Founded 1969 by the Nordic Council

Outline

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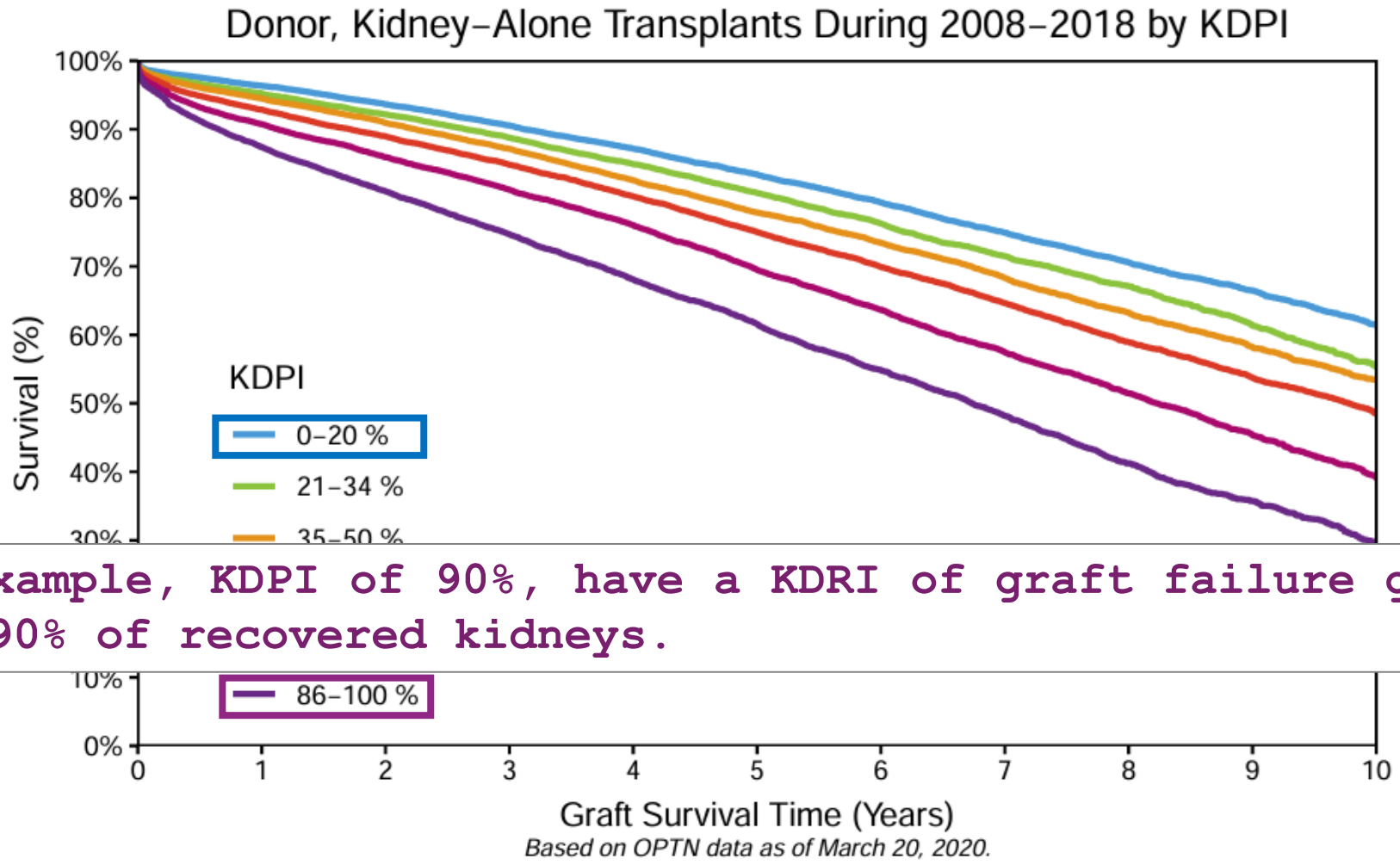
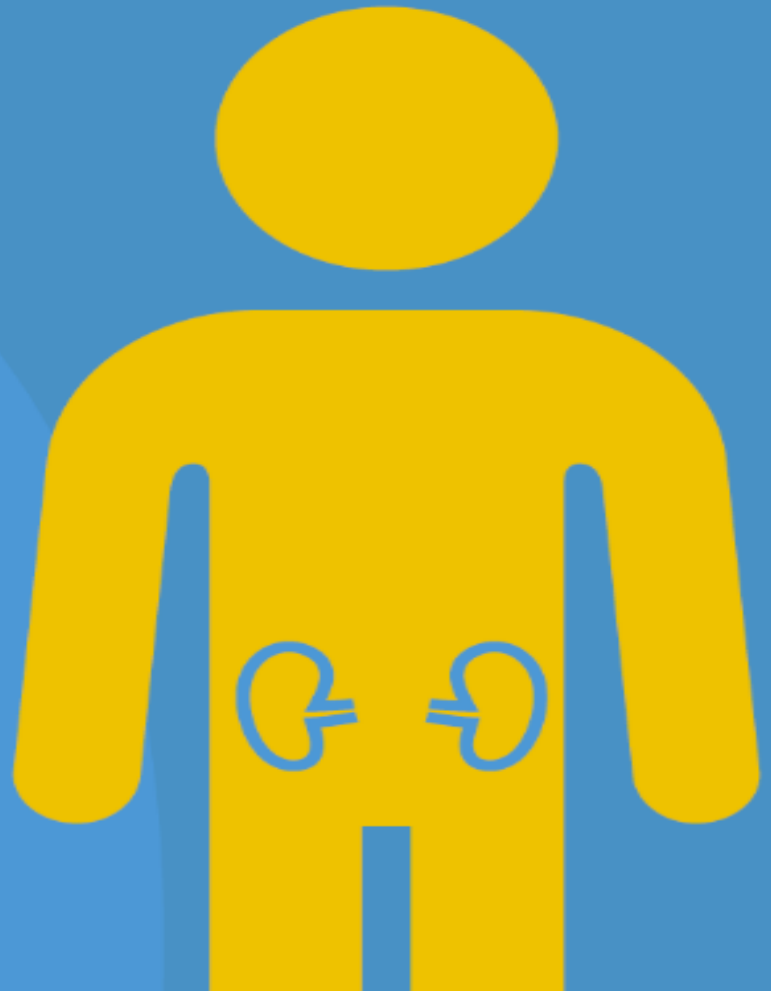


for example, KDPI of 90%, have a KDRI of graft failure greater than 90% of recovered kidneys.

IN FOCUS

A patient's EPTS can range from 0-100% and is an important factor in prioritization.

Estimated Post Transplant Survival



Immunosuppression and Adherence is the corner stone of maintaining graft survival

Allospecific matching is the Key stone of ensuring graft survival