Lecture 5

HLA Epitope Matching In Clinical Transplantation

HLA Matching for Transplantation: The Traditional Way

- Count the A, B, DR antigen mismatches of the donor (range: from 0 to 6)
  - Why do so many zero-antigen mismatches fail?
  - Why are many mismatches successful?
- Avoid unacceptable mismatches for highly sensitized patients
  - Why do we see so many graft failures?
  - Many patients are never transplanted
  - Desensitization with IVIG / plasmapheresis

HLA Antibodies Are Major Risk Factors for Graft Failure

Dealing with the HLA Antibody Problem

- Pre-transplant testing
  - Serum screening and cross-matching
  - Finding an acceptable mismatch
- Post-transplant monitoring
  - Donor-specific HLA antibodies
  - Biopsy pathology, C4d
- Desensitization
  - Plasmapheresis
  - IV-IgG
  - Bortezomib

Conventionally we talk about antibody specificities against HLA antigens

Examples: anti-HLA-A1
anti-HLA-B7
anti-HLA-DR1

But what do antibodies see?

HLA Antibodies Are Important in Transplantation

- HLA antibodies cause allograft rejection and transplant failure
- HLA antibodies recognize epitopes

Therefore

HLA epitopes are important in transplantation

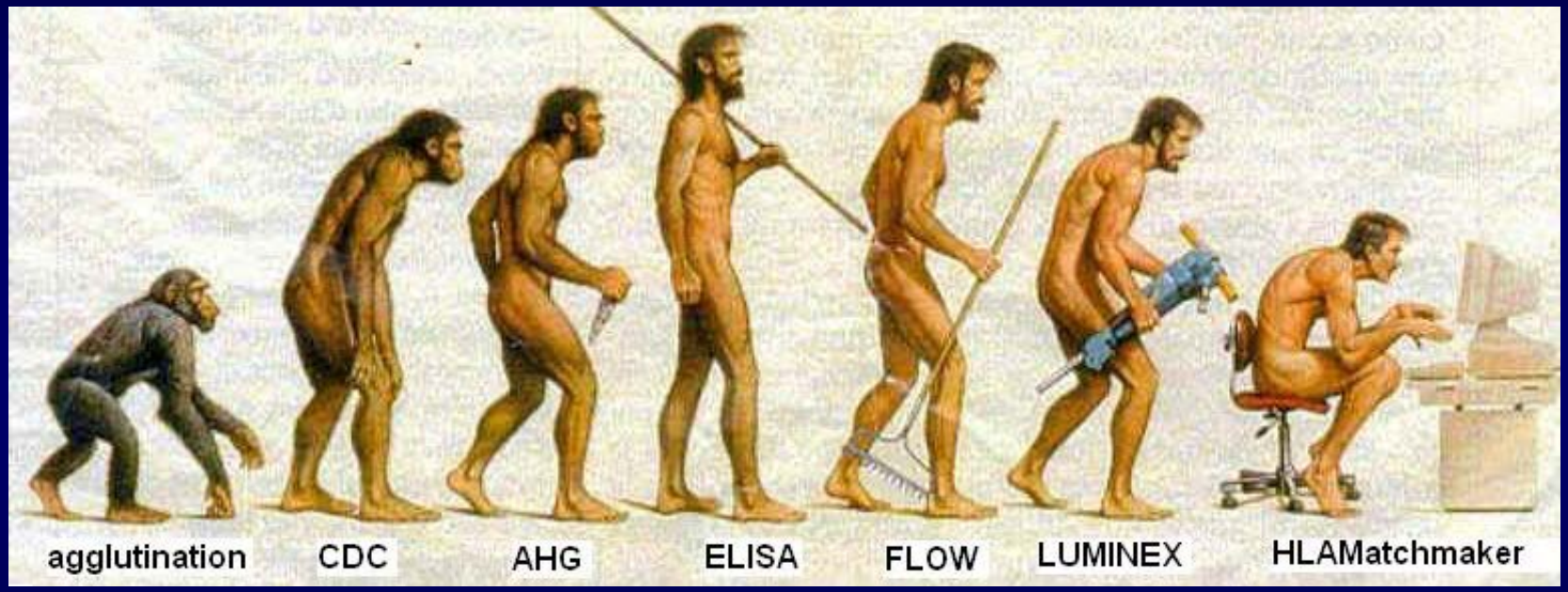
Clinical Applications of HLA Epitopes

- Epitope *antigenicity*: reactivity with antibody in different assays and *in vivo*
  - Epitope-based acceptable mismatching for sensitized patients (Eurotransplant)
- Epitope *immunogenicity*: ability to induce specific antibodies
  - Effects of epitope loads of antigen mismatches on HLA antibody responses and transplant outcome

Lecture Outline

- HLA-epitope based antibody analysis of transplant patients
- Acceptable mismatching for sensitized patients
- Effect of epitope loads on HLA antibody responses and transplant outcome
- Need for allele level typing of patients and donors

Evolution of HLA antibody testing methods



Epitope Specificity Analysis with HLAMatchmaker

- HLA-ABC epitopes
- HLA-DRB1/3/4/5 epitopes
- DQA1-DQB1 epitopes
- DPA1-DPB1 epitopes
- MICA epitopes

Purpose of Serum Screening

- Identify unacceptable HLA antigens (UNOS)

- Identify acceptable mismatches
  - Represents a direct approach of finding compatible donors for a transplant candidate
  - Eurotransplant uses the Acceptable Mismatch program in conjunction with HLAMatchmaker

(Claas et al. Transplantation 78:190-193, 2004
Doxiadis et al. Curr. Opin. Immunol. 17:536-540, 2005)

Eurotransplant's Acceptable Mismatch Program Now Includes HLAMatchmaker

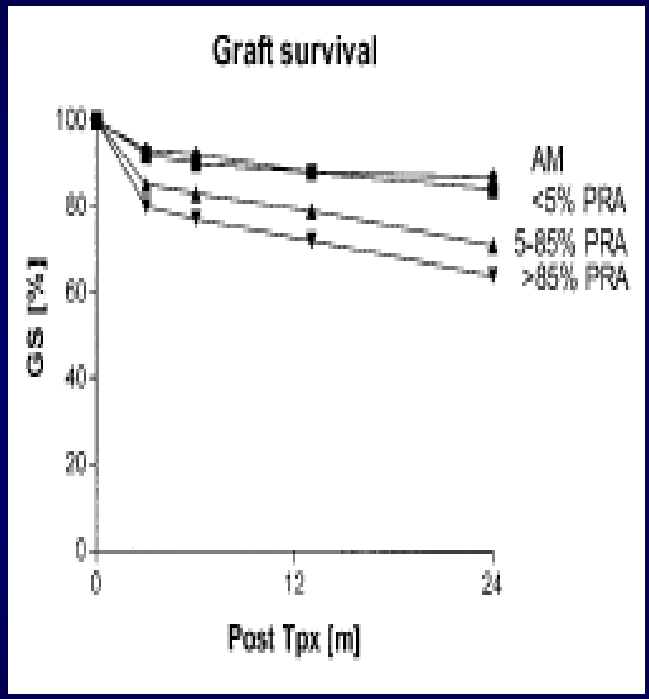
Validation of HLAMatchmaker by direct cross-matches

HLA-A mismatch

	Expected negative	Observed negative
0 triplet	**18**	**18**
1 triplet	**25**	**25**

HLA-B mismatch

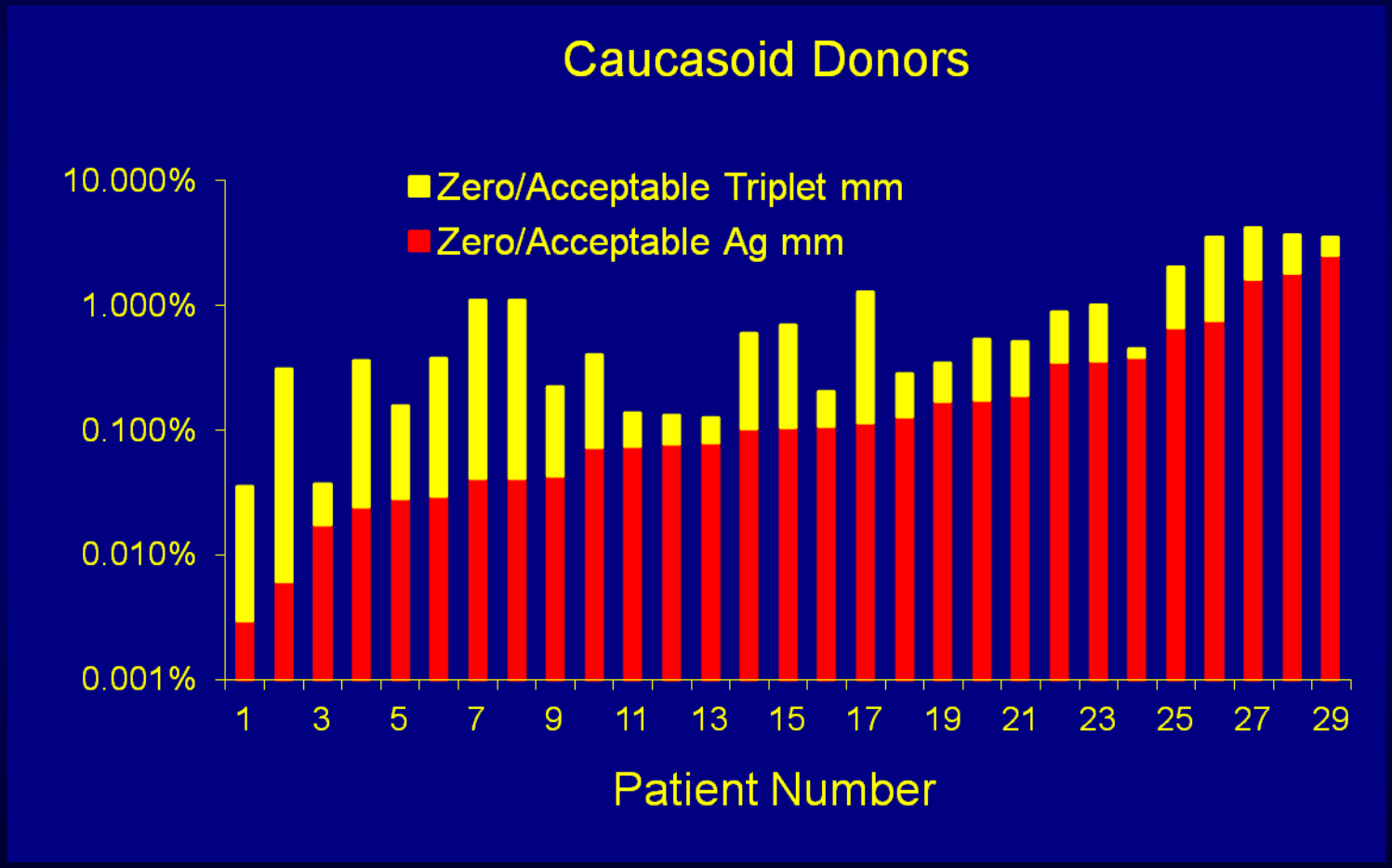
	Expected negative	Observed negative
0 triplet	**54**	**54**
1 triplet	**133**	**131**



AM are Acceptable Mismatches
5-85% and >85% PRA are conventional ET-KAS cases

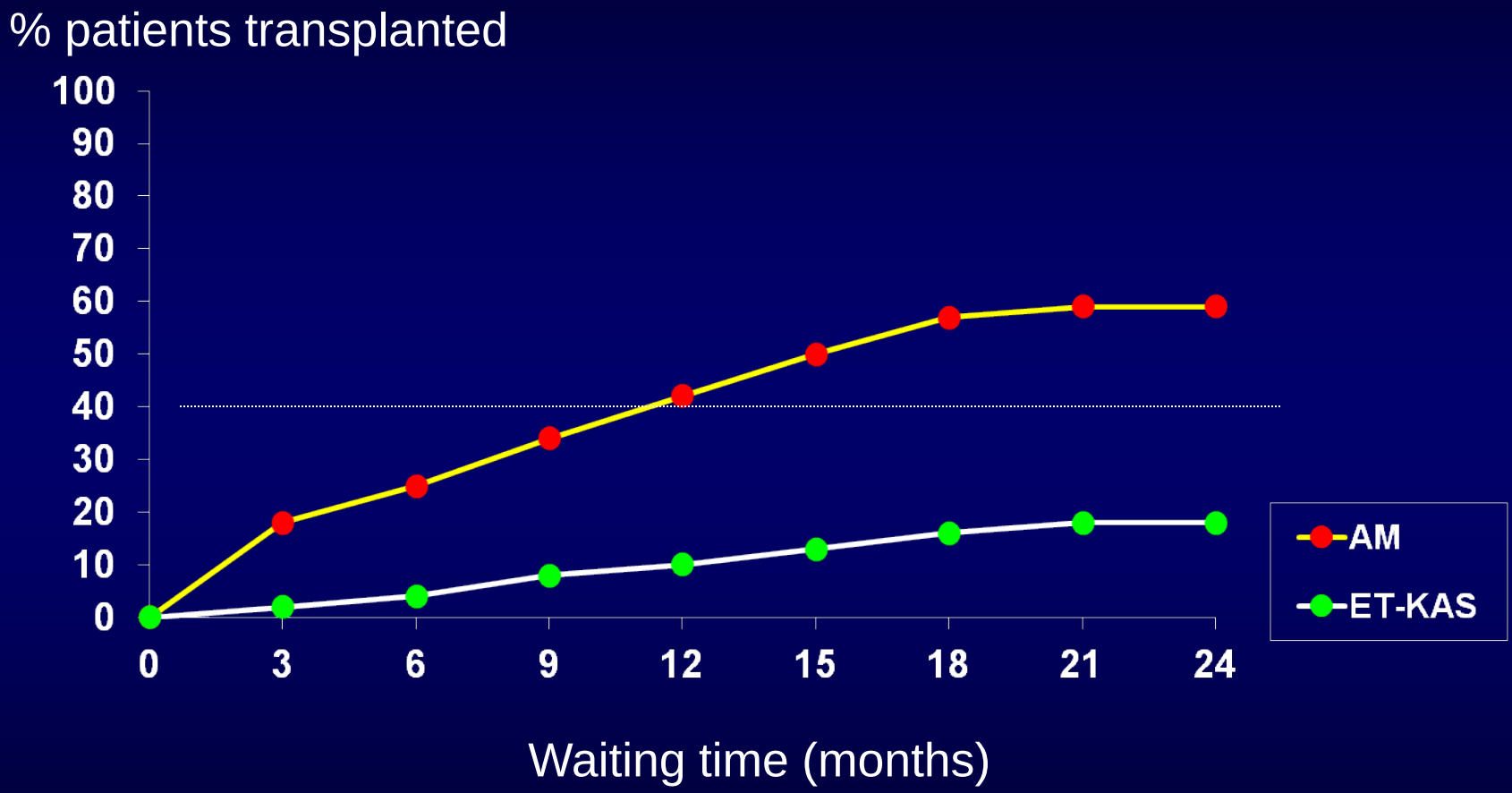
Claas et al.: The Acceptable Mismatch Program as a Fast Tool to Transplant Highly Sensitized Patients Awaiting a Post-Mortal Kidney: Short Waiting Time and Excellent Graft Outcome. Transplantation 78:190-193, 2004

Chances of Finding a Zero or Acceptable HLA-A,B Mismatch for a High PRA Patient (30 patients)



Eurotransplant Patients, Leiden, The Netherlands

Chance to Receive a Suitable Crossmatch Negative Organ



ET-KAS and UNOS strategies are similar

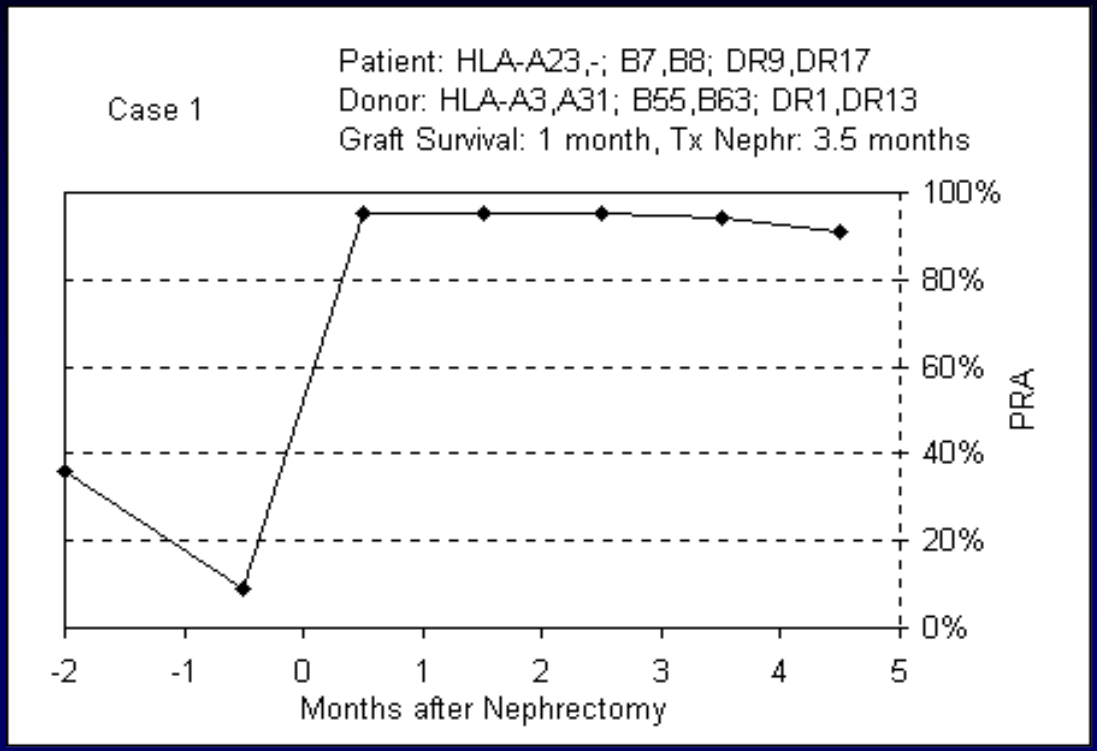
Slide provided by Frans Claas (Leiden University Medical Center, The Netherlands)

Post-transplant monitoring for epitope-specific antibodies

- Diagnostic tool for humoral rejection
- Does the transplant affect antibody detection?

Effect of Allograft Nephrectomy on HLA Antibody Detection in Serum

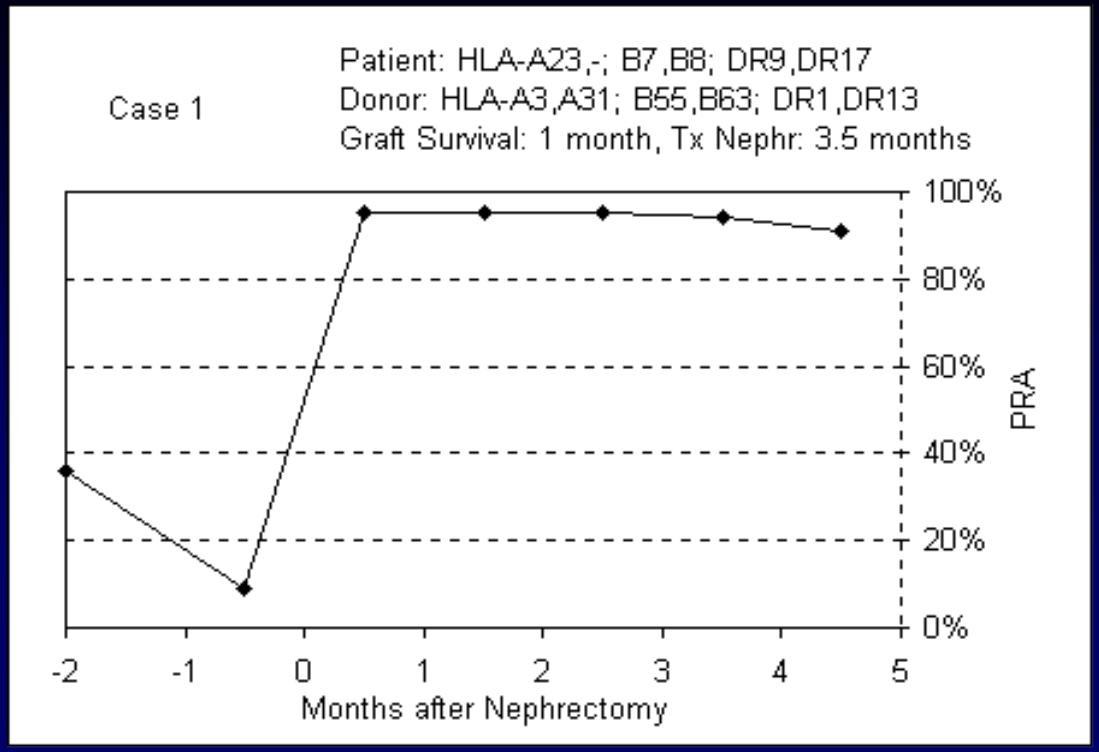
Allograft Nephrectomy Case



Donor Epitopes:

A3: 62QE,66RNV,70AQS,76VD,80GTL,144TKR,149AAH,151AHE,163DT
A31: 56R,62QE,66RNV,74ID,76VD,80GTL,193AvV
B55: 131S
B63: 45MA,66RNM,70ASA,74Y,131S

Adeyi et al. Transplant Immunology, 14: 53-62, 2005



Donor Epitopes (CDC-reactive epitopes are shown in underlined bold font)

A3: 62QE,66RNV,**70AQS**,**76VD**,80GTL,144TKR,149AAH,**151AHE**,**163DT**
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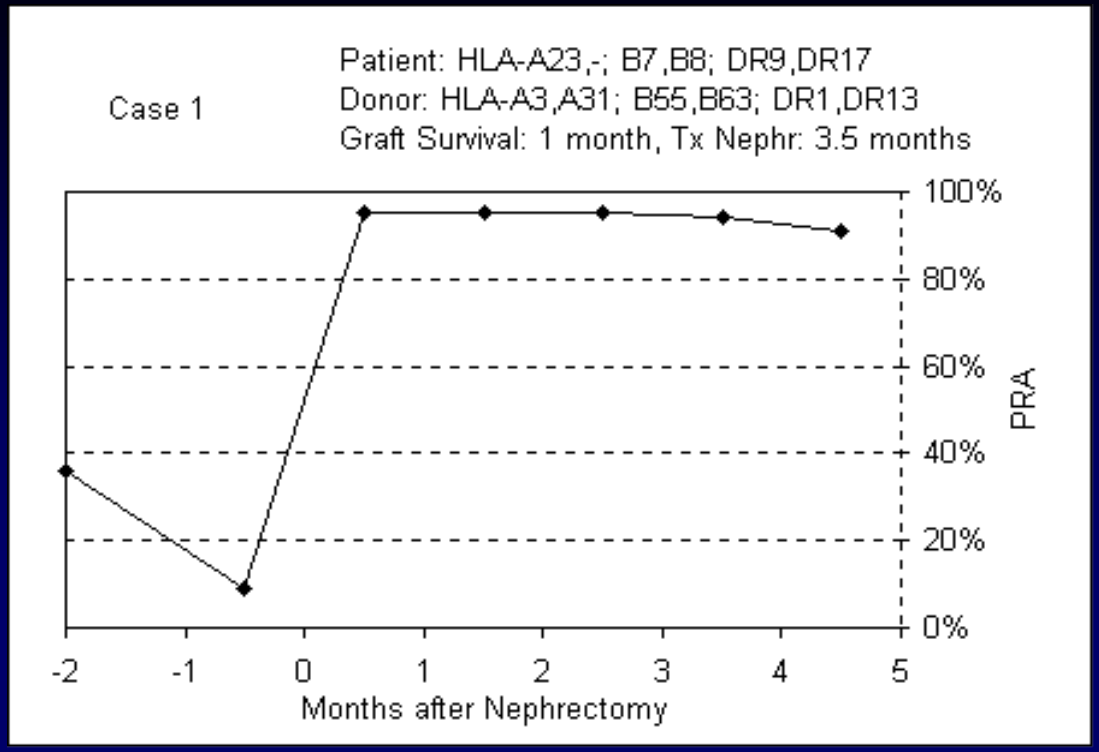

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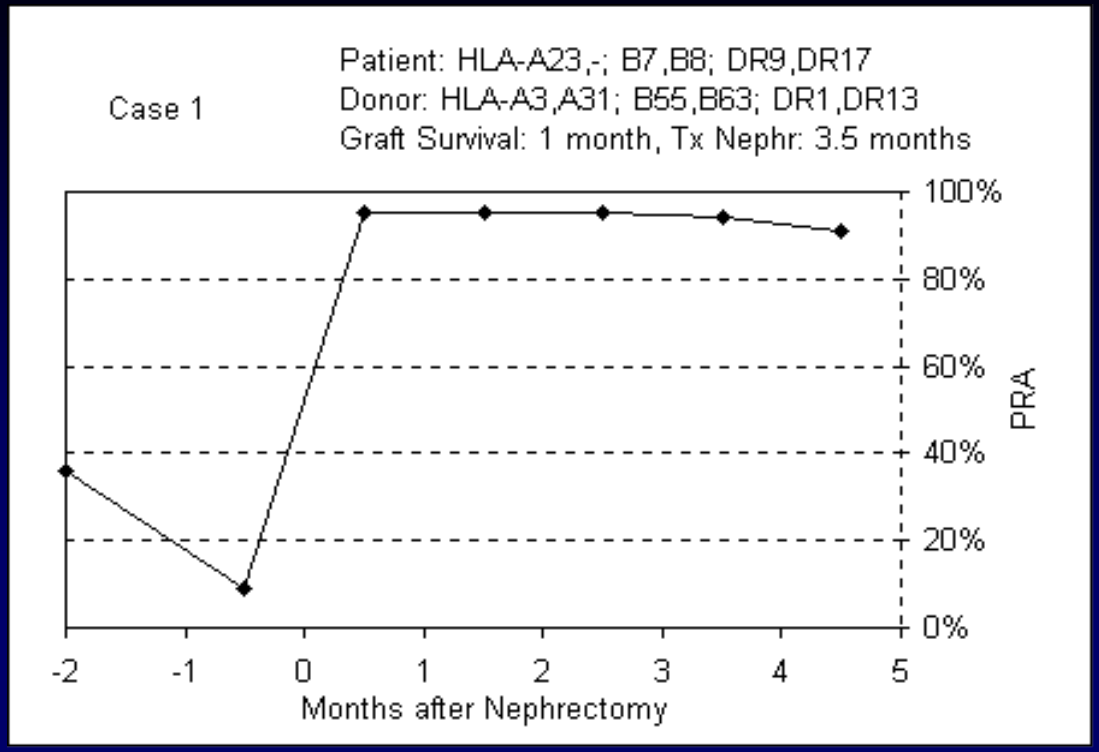
HLAMatchmaker predicts unacceptable mismatches
e.g. 45MA is present on B13, B46, B57,B62, B63. B75, B76, B77

Adeyi et al. Transplant Immunology, 14: 53-62, 2005



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B55: 131S
B63: **45MA**,66RNM,**70ASA**,74Y,131S

Challenging Question:
What about the non-immunogenic epitopes?
Did they induce specific B-cell tolerance?

Adeyi et al. Transplant Immunology, 14: 53-62, 2005

- 15th workshop project: 65 allograft nephrectomy cases contributed by 16 laboratories worldwide
- Antibody testing done with single allele panels on a Luminex platform

DSA	Pre-allonx	Post-allonx	Significance
HLA-AB	64%	87%	P=0.0033
DRB1	57%	86%	P=0.001
DRB3/4/5	65%	78%	P=0.22
DQB	76%	87%	P=0.18

Conclusion: although the Luminex antibody assay can detect anti-donor antibodies in the presence of a rejected transplant, it is apparent that the antibody specificity pattern is often incomplete especially against the HLA-A, -B and DR mismatches.

Marrari M and Duquesnoy RJ: Transplant Immunology, 22:105-109, 2010

HLAMatchmaker and Platelet Transfusions

HLAMatchmaker-driven analysis of responses to HLA-typed platelet transfusions in alloimmunized thrombocytopenic patients

Ashok Nambiar, Rene J. Duquesnoy, Sharon Adams, Yingdong Zhao, Jaime Oblitas, Susan Leitman, David Stroncek, and Francesco Marincola

BLOOD, 15 FEBRUARY 2006 • VOLUME 107, NUMBER 4

Validation of HLAMatchmaker algorithm in identifying acceptable HLA mismatches for thrombocytopenic patients refractory to platelet transfusions

Erin G. Brooks, Bruce R. MacPherson, and Mark K. Fung

TRANSFUSION 2008;48:2159-2166

A RETROSPECTIVE REVIEW OF THE EFFICACY OF HLA MATCHED PLATELETS TRANSFUSION USING HLAMATCHMAKER DEFINED TRIPLET AND EPLET EPITOPES FOR APLASTIC ANAEMIA PATIENTS

D. M. Kallon, C. J. Brown, J. Marsh, C. V. Navarrete (Abstract 2007 EFI meeting)

TRANSFUSION PRACTICE

Structural epitope matching for HLA-alloimmunized thrombocytopenic patients: a new strategy to provide more effective platelet transfusion support?

Duquesnoy: Transfusion, 48: 221-227, 2008

HLA Epitope-Based Matching Protocol (steps 1 and 2)

1. Perform HLA-A, B, C typing of patients and donors by DNA methods at the high-resolution (4-digit allele) level.
2. Screen patient sera with HLA typed panel
   - Complement-dependent methods: direct and/or antiglobulin-augmented lymphocytotoxicity
   - Antigen-binding assays such as Luminex, Flow Cytometry and ELISA preferably with single HLA class I alleles
   - HLAMatchmaker-based analysis of serum reactivity pattern to identify acceptable mismatches

HLA Epitope-Based Matching Protocol (step 3)

3. Conduct a platelet donor search
   - Establish a computerized platelet donor registry that incorporates an HLAMatchmaker-based search engine
   - Enter the HLA type of the patient and the non-reactive mismatched alleles in this database and the computer will generate a list of donors with matches and acceptable mismatches at the eplet level
   - No need for platelet cross-match testing for HLA incompatibility

HLA Epitope-Based Matching Protocol (step 4)

4. Evaluate the outcome of the platelet transfusion, if increment is low then:
   - Determine whether serum reactivity patterns have improperly been interpreted in terms of HLA mismatch acceptability
   - Look for antibodies against platelet-specific antigens and blood groups, or autoimmune phenomena and drug reactions
   - Consider clinical conditions such as coagulopathy, infection and hepatosplenomegaly

Transfusions grouped by different HLA matching criteria

Pai et al. TRANSFUSION 50: 2318-2327, 2010

	Zero AB matches	CREG Matches	Epitope-Based Matches	P value
N=142	61	38	43	
Median CCI	14.55 (10.38-22.17)	10.12 (2.11-26.32)	22.03 (9.85-30.87)	0.034*
Successful Transfusions	52 (85.2%)	24 (63.2%)	36 (83.7%)	0.021 **

* Kruskal-Wallis test; ** Chi-square test
EBM versus CREG p=0.004 (proportion test)

In follow-up studies, no emerging HLA-specific antibodies were detected after receiving eplet-matched platelets

Class I HLA Epitope Matching in Stem Cell Transplantation

HLAMatchmaker-Defined Triplet Matching for Patients with Class I HLA Allele Mismatched Hematopoietic Cell Transplants from Unrelated Donors

National Marrow Donor Program (NMDP) Study

2431 cases including ALL (N=581), AML (N=676), CML (N=954) and MDS (N=223)

Compare 10/10 allele matches with 9/10 allele matches with different numbers of mismatched triplets

Analyze engraftment, GVHD incidence and patient survival

Duquesnoy et al. Biol. Blood Marrow Transplant. 14: 1064-1071, 2008

Should We Try to Prevent or Minimize HLA Sensitization?

Causes of Sensitization

- Pregnancy (acts of nature)
- Transfusion (human procedure)
- Transplantation (human procedure)

How to prevent or minimize sensitization?

Reduce HLA Sensitization

- Transplants and blood transfusions from HLA matched donors

- Permissible HLA mismatching
  - Consider low epitope loads
  - Minimize exposure to immunogenic epitopes

HLA Mismatches and Epitope Loads

The Epitope Load of a HLA Mismatch Depends on HLA Type of Recipient

Example: a single B51 mismatch

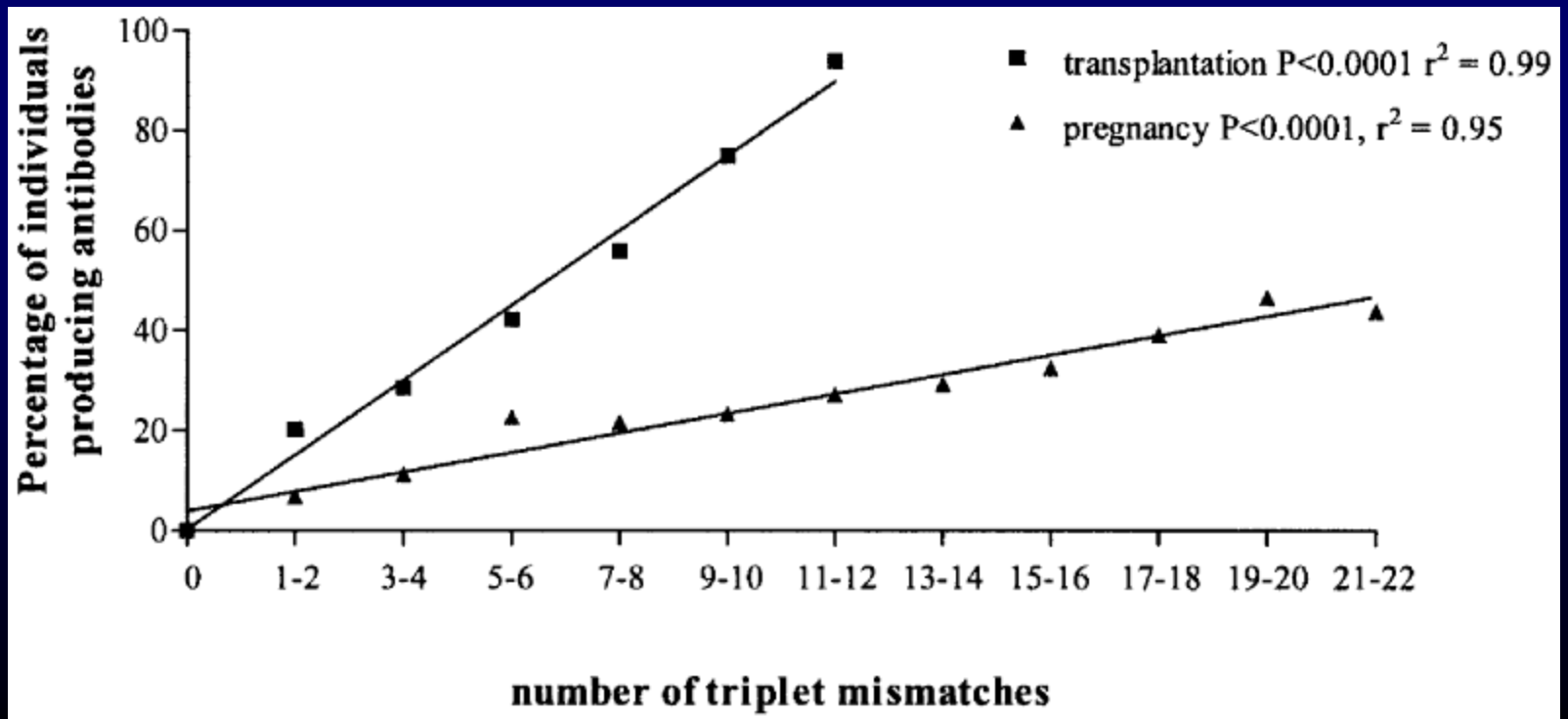
								B51 (B*5101)
Case	Phenotype						#Ep	Mismatched Eplets
1	A*0101	A*0201	B*1402	B*0702	Cw*0701	Cw*0702	7	11AMR, 44RTE, 76ERI, 82ALR, 113HN, 163L, 193PV
2	A*0101	A*0201	B*0702	B*0801	Cw*0701	Cw*0702	6	44RTE, 76ERI, 82ALR, 131S, 163L, 193PV
3	A*0101	A*0201	B*0702	B*4501	Cw*0701	Cw*0702	5	44RTE, 76ERI, 82ALR, 113HN, 193PV
4	A*0101	A*2501	B*0702	B*0801	Cw*0701	Cw*0702	4	44RTE, 131S, 163L, 193PV
5	A*0101	A*0201	B*0702	B*4403	Cw*0501	Cw*0702	3	44RTE, 76ERI, 113HN
6	A*0101	A*0201	B*4501	B*3901	Cw*0501	Cw*1701	3	44RTE, 76ERI, 82ALR
7	A*0101	A*2501	B*5501	B*3701	Cw*0602	Cw*0702	2	116Y, 163L
8	A*0101	A*2501	B*3501	B*4101	Cw*0602	Cw*0401	0	none

Wolters Kluwer Health | Lippincott Williams & Wilkins
Transplantation
THE OFFICIAL JOURNAL OF THE TRANSPLANTATION SOCIETY

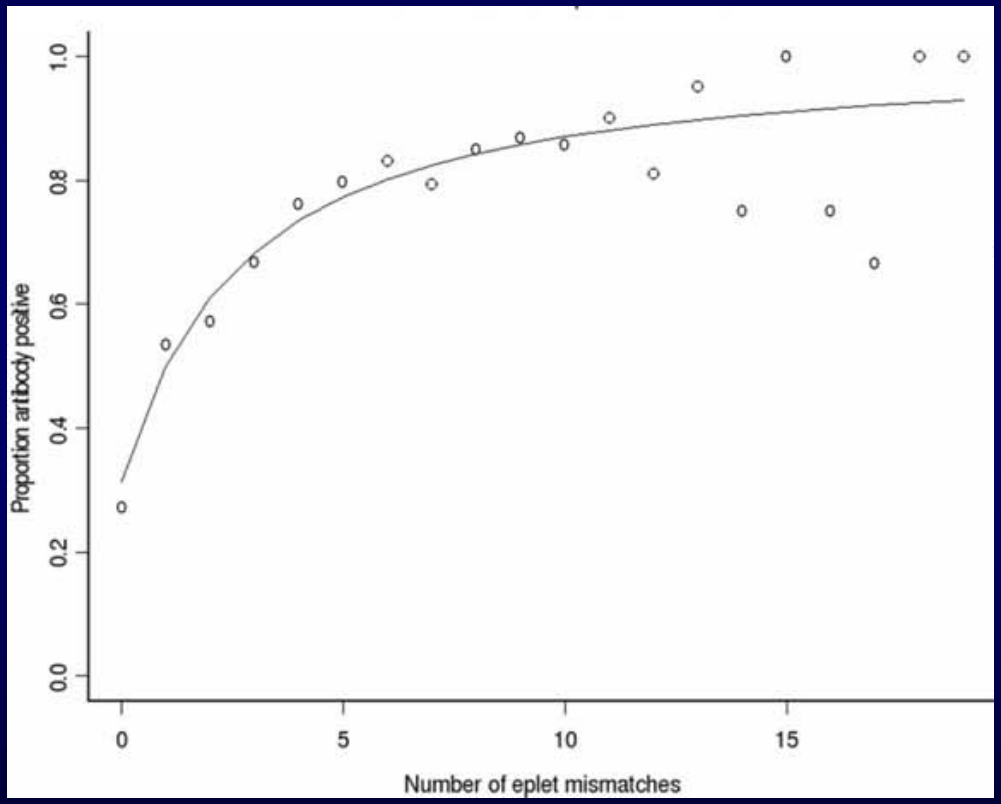
Volume 77(8) 27 April 2004 pp 1236-1239

THE NUMBER OF AMINO ACID TRIPLET DIFFERENCES BETWEEN PATIENT AND DONOR IS PREDICTIVE FOR THE ANTIBODY REACTIVITY AGAINST MISMATCHED HUMAN LEUKOCYTE ANTIGENS

Dankers, Witvliet, Roelen, De Lange, Korfage, Persijn, Duquesnoy, Doxiadis, and Claas
Department of Immunohematology and Blood Transfusion, Leiden University Medical Center

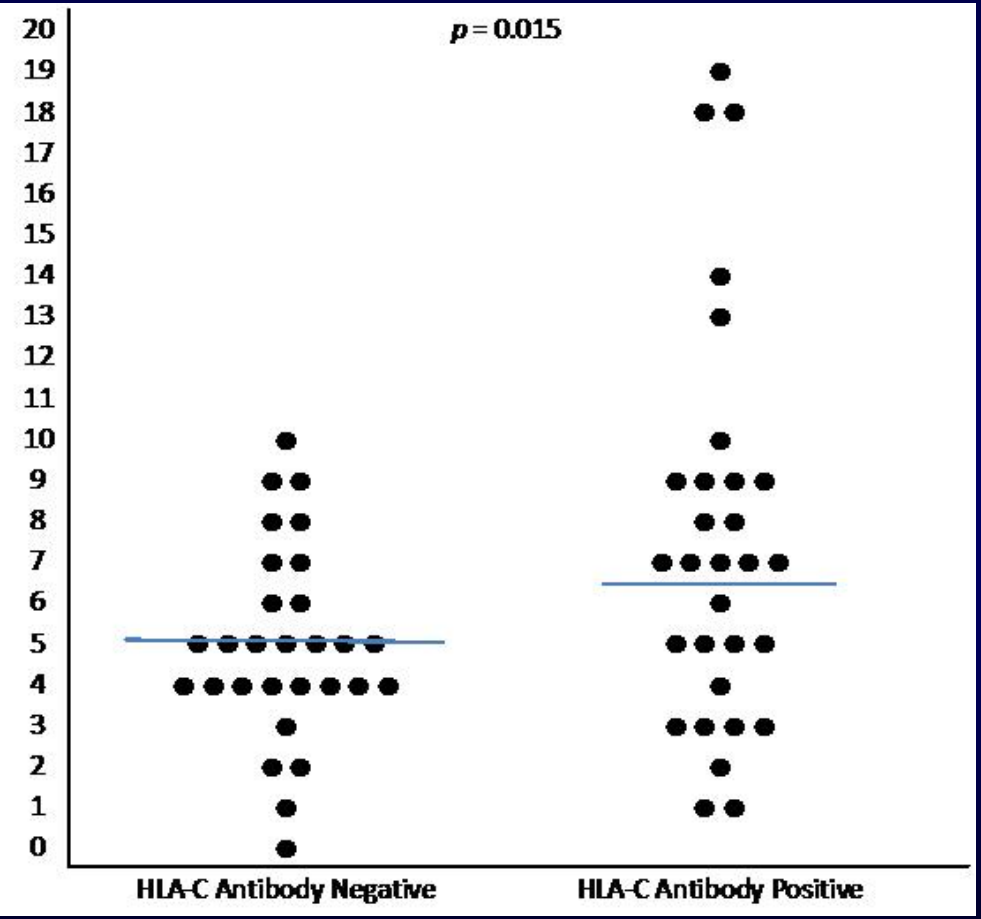


Donor HLA-A,B Specific Antibody Responses and Mismatched Eplet Loads



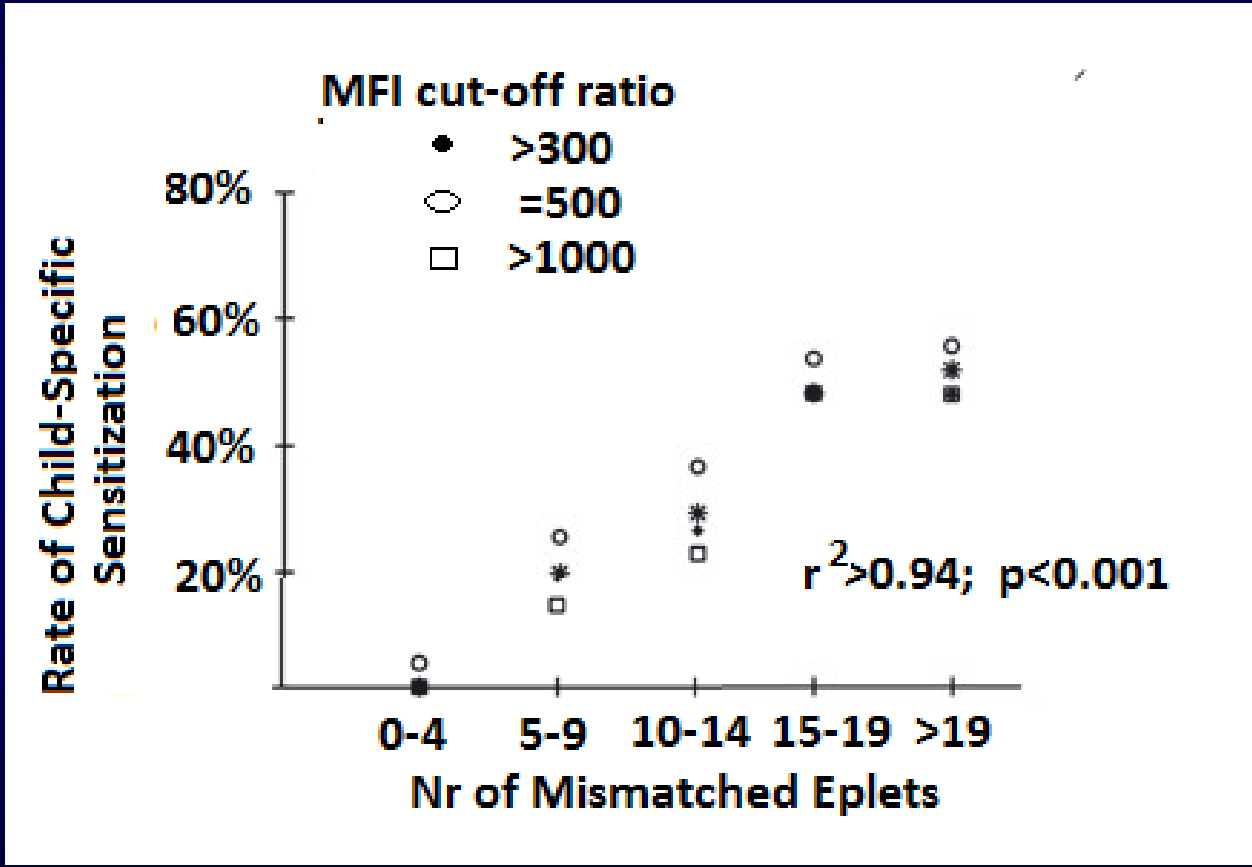
*Kosmoliaptsis, Bradley, Sharples, Chaudhry, Goodman and Taylor. (*Cambridge University Hospitals, UK) *Transplantation 85: 1817–1825, 2008*

Correlation between Eplet Load and Antibody Responses to HLA-C Mismatches



Marrari and Duquesnoy: Detection of Antibodies against HLA-C Epitopes in Patients with Rejected Kidney Transplants, Transplant Immunology. 24:164-171, 2011

Correlation between mismatched eplet numbers and frequency of child-specific HLA-ABC sensitization during pregnancy



Honger, Fornaro, Granado,Tiercy, Hosli and **Stefan Schaub**: (Basel, Switzerland)
Frequency and Determinants of Pregnancy-Induced Child-Specific Sensitization,
American Journal of Transplantation, Nov 2012

Epitope Loads and HLA Antibody Development

Year	Investigators	Reported Observation
2002	Lobashevsky et al. (Birmingham, AL) *Human Immunol.* 63: 364	Numbers of epitope (triplet) mismatches predict flow cytometry cross-match results with sera from highly sensitized renal patients ($p<0.00009$)
2004	Dankers et al. (Leiden, Netherlands) *Transplantation* 77: 1236	Correlation between the number of mismatched epitopes (triplets) and the incidence of humoral sensitization induced by a kidney transplant ($r^2=0.99$, $p<0.0001$) or developed during pregnancy ($r^2=0.95$, $p<0.0001$)
2006	Goodman et al. (Cambridge, UK) *Transplantation* 81: 1331	Correlation between the number of mismatched epitopes (triplets) and the presence of HLA antibodies detected in Luminex assays with single class I alleles
2008	Kosmoliaptsis et al. (Cambridge, UK) *Transplantation* 85: 1817	Analysis of recipient HLA type and mismatched HLA antigens using the HLAMatchmaker algorithm allows prediction of immunogenic donor HLA types.
2009	Kosmoliaptsis et al. (Cambridge, UK) *Transplantation* 88: 791	Close correlation between increasing number of amino acid polymorphisms and the presence and magnitude of the HLA antibody response ($p<0.0001$)
2011	Duquesnoy et al. (Pittsburgh, PA) *Transplant Immunol.* 24:164	More HLA-C antibody responses by transplant patients who have been exposed to greater HLA-C eplet loads ($p<0.001$).
2013	Schaub et al. (Basel, Switzerland) *Amer J Transplant* 13: 746	Number of mismatched HLA-ABC eplets strongly correlates with the rate of child-specific class I sensitization ($p<0.001$)

Mismatched Epitope Loads and Transplant Outcome

TRANSPLANTATION
Vol. 75, 884–889, No. 6, March 27, 2003
Printed in U.S.A.

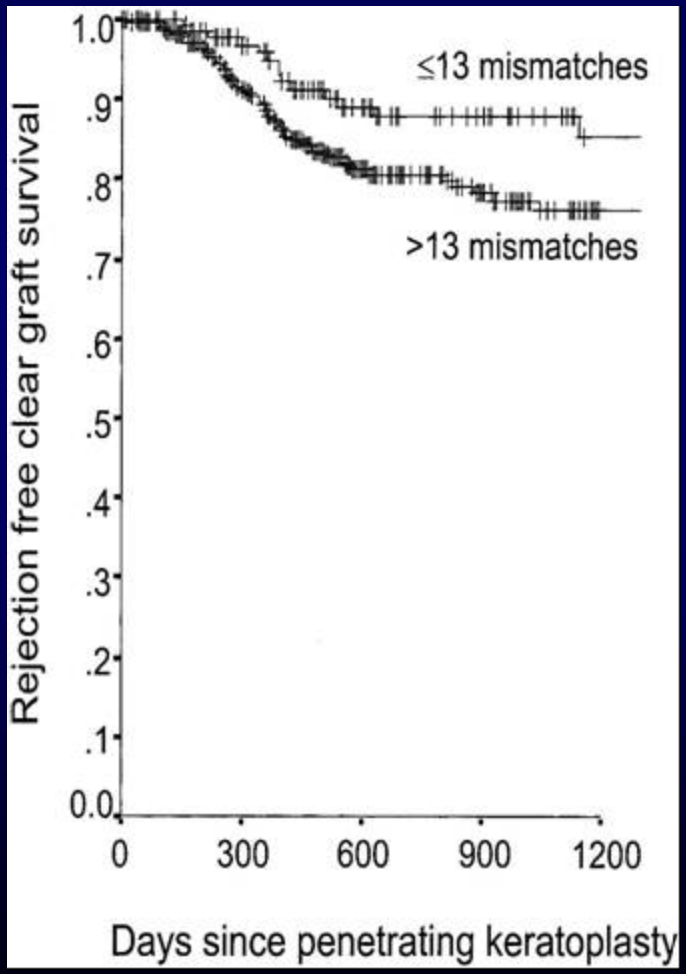
HLAMatchmaker: A MOLECULARLY BASED ALGORITHM FOR HISTOCOMPATIBILITY DETERMINATION. III. EFFECT OF MATCHING AT THE HLA-A,B AMINO ACID TRIPLET LEVEL ON KIDNEY TRANSPLANT SURVIVAL[1]

RENE J. DUQUESNOY,[2,6] STEVE TAKEMOTO,[3] PETER DE LANGE,[4] ILIAS I. N. DOXIADIS,[4]
GEZIENA M. TH. SCHREUDER,[4] GUIDO G. PERSIJN,[5] AND FRANS H. J. CLAAS[4]

Effect of HLA-A,B Mismatched Epitope Load on Graft Survival of Zero-HLA-DR Mismatched Kidneys in Eurotransplant

Mismatch Group:	0 AB Ag	0 Trp	1 trp	2 trp	3 trp	4 trp	5-9 trp	10-19 trp	20-29 trp
Number of Recipients:	3426	231	218	377	450	629	3448	6078	943
% Graft Survival after									
1 year	89.3	89.2	86.7	85.7	89.1	88.4	85.1	85.4	84.3
2 years	87.1	85.1	84.3	82.1	86.7	84.1	81.5	80.6	80.0
3 years	83.7	82.3	80.7	78.3	82.4	81.1	77.4	76.7	76.1
5 years	**76.8**	**75.6**	**76.4**	**72.9**	**75.4**	**75.9**	69.9	69.8	68.5

Mismatched Epitope (Triplet) Loads and Corneal Graft Survival



147 cases with up to 13 mismatched triplets
398 cases with more mismatched triplets

Kaplan-Meier estimation: 85% vs. 76%
rejection-free clear graft survival
log-rank test, p=0.047

Daniel Boehringer et al.
(University Hospital, Freiburg, Germany),
Transplantation 77, 417–421, 2004

Class II HLA Antibodies Decrease Transplant Success

- DRB1 is “standard” for donor-recipient matching
- DRB3/4/5
  - Antibodies to DR51, DR52 and DR53
- DQB1 and DQA1
  - Relevance of DQB matching in transplantation
  - Patients make antibodies to DQB and DQA epitopes
- DPA1 and DPB1
  - Relevance of DP matching
  - Anti-DP antibodies in transplantation

Conventional DR Compatibility: “One Antigen Mismatch”

Patient
DR15
DR18
Donor
DR mismatch
DR1
DR4
DR7
DR8
DR9
DR10
DR11
DR12
DR13
DR14
DR15 (self)
DR16
DR17
DR18 (self)

A DR Antigen Mismatch Has an Extra Class II Epitope Load

DRB1+ DRB3/4/5+ DQB+DQA+DPB+DPA

Conventional DR Compatibility: “One Antigen Mismatch”

Patient
DR15
DR18
Donor
DR mismatch
DR1
DR4
DR7
DR8
DR9
DR10
DR11
DR12
DR13
DR14
DR15 (self)
DR16
DR17
DR18 (self)

Common High-Resolution DR-DQ Haplotypes

Patient	**DRB1**	**DRB3/4/5**	**DQB1**	**DQA1**
DR15	DRB1*15:01	DRB5*01:01	**DQB1*05:02**	DQA1*01:02
DR18	DRB1*03:02	DRB3*01:01	DQB1*04:02	DQA1*04:01
Donor				
DR mismatch	**DRB1**	**DRB3/4/5**	**DQB1**	**DQA1**
DR1	DRB1*01:01	-	DQB1*05:01	DQA1*01:01
DR4	DRB1*04:01	DRB4*01:01	DQB1*03:01	DQA1*03:02
DR7	DRB1*07:01	DRB4*01:01	DQB1*02:02	DQA1*02:01
DR8	DRB1*08:01	-	DQB1*04:02	DQA1*04:01
DR9	DRB1*09:01	DRB4*01:01	DQB1*03:03	DQA1*03:02
DR10	DRB1*10:01	-	DQB1*05:01	DQA1*01:01
DR11	DRB1*11:01	DRB3*02:02	DQB1*03:01	DQA1*05:01
DR12	DRB1*12:01	DRB3*02:02	DQB1*03:01	DQA1*05:01
DR13	DRB1*13:01	DRB3*01:01	DQB1*06:03	DQA1*01:03
DR14	DRB1*14:01	DRB3*02:02	DQB1*05:03	DQA1*01:04
DR15 (self)	**DRB1*15:01**	**DRB5*01:01**	**DQB1*06:02**	**DQA1*01:02**
DR16	DRB1*16:01	DRB5*02:02	DQB1*05:03	DQA1*01:02
DR17	DRB1*03:01	DRB3*01:01	DQB1*02:01	DQA1*05:01
DR18 (self)	**DRB1*03:02**	**DRB3*01:01**	**DQB1*04:02**	**DQA1*04:01**

Epitope-Based DR Compatibility

Patient	DRB1	DRB3/4/5	DQB1	DQA1					
DR15	DRB1*15:01	DRB5*01:01	DQB1*05:02	DQA1*01:02					
DR18	DRB1*03:02	DRB3*01:01	DQB1*04:02	DQA1*04:01					
Donor									
DR mismatch	**DRB1**	**DRB3/4/5**	**DQB1**	**DQA1**	**Epitope Total**	**DRB1**	**DRB3/4/5**	**DQB1**	**DQA1**
DR1	DRB1*01:01	-	DQB1*05:01	DQA1*01:01	9	5	0	2	2
DR4	DRB1*04:01	DRB4*01:01	DQB1*03:01	DQA1*03:02	42	8	14	9	11
DR7	DRB1*07:01	DRB4*01:01	DQB1*02:02	DQA1*02:01	41	10	14	10	7
DR8	DRB1*08:01	-	DQB1*04:02	DQA1*04:01	**4**	**4**	0	0	0
DR9	DRB1*09:01	DRB4*01:01	DQB1*03:03	DQA1*03:02	36	6	14	5	11
DR10	DRB1*10:01	-	DQB1*05:01	DQA1*01:01	12	8	0	2	2
DR11	DRB1*11:01	DRB3*02:02	DQB1*03:01	DQA1*05:01	22	3	2	9	8
DR12	DRB1*12:01	DRB3*02:02	DQB1*03:01	DQA1*05:01	26	7	2	9	8
DR13	DRB1*13:01	DRB3*01:01	DQB1*06:03	DQA1*01:03	12	2	0	7	3
DR14	DRB1*14:01	DRB3*02:02	DQB1*05:03	DQA1*01:04	11	4	2	2	3
DR15 (self)	DRB1*15:01	DRB5*01:01	**DQB1*06:02**	DQA1*01:02	**6**	0	0	**6**	0
DR16	DRB1*16:01	DRB5*02:02	DQB1*05:03	DQA1*01:02	**2**	0	**2**	0	0
DR17	DRB1*03:01	DRB3*01:01	DQB1*02:01	DQA1*05:01	17	0	0	9	8
DR18 (self)	DRB1*03:02	DRB3*01:01	DQB1*04:02	DQA1*04:01	0	0	0	0	0

Each DR Antigen Mismatch Has an Extra Class II Epitope Load

DRB1+ DRB3/4/5+ DQB+DQA+DPB+DPA

Donor mismatch for patient who types as DR13, DR18	Number of mismatched eplets
DR8 DR-DQ haplotype	4
DR1 DR-DQ haplotype	9
DR17 DR-DQ haplotype	17
DR7 DR-DQ haplotype	41
DR4 DR-DQ haplotype	42

Class II Epitope Loads and Antibody Responses

Epitope Loads and Anti-DRB1 and Anti-DRB3/4/5 Antibody Responses after Kidney Transplantation

Mismatch	Donor-Specific Antibody Frequency	Nr of Mismatched Eplets
DRB1	23/96 (24%)	6.8 ± 3.6*
DR51 (DRB*05)	4/8 (50%)	9.8 ± 2.5
DR52 (DRB*03)	6/13 (46%)	11.2 ± 1.0
DR53 (DRB*04)	15/18 (83%)	13.2 ± 2.3

*p< 0.01

Duquesnoy et al. Transplant Immunology 18: 352–360, 2008

Epitope Loads and Anti-DQ and anti-DRB1 Antibodies after Kidney Transplantation

Mismatch	Donor-Specific Antibody Frequency	Nr of Mismatched Eplets
DRB1 (N=96)	24%	6.8 ± 3.6*
DQB (N=62)	87%	10.2 ± 3.3
DQA (N=74)	64%	11.4 ± 4.9

*p< 0.01

Duquesnoy et al. Transplant Immunology 18: 352–360, 2008

American Journal of Transplantation 2013; XX: 1–9
Wiley Periodicals Inc.

doi: 10.1111/ajt.12478

Class II HLA Epitope Matching—A Strategy to Minimize *De Novo* Donor-Specific Antibody Development and Improve Outcomes

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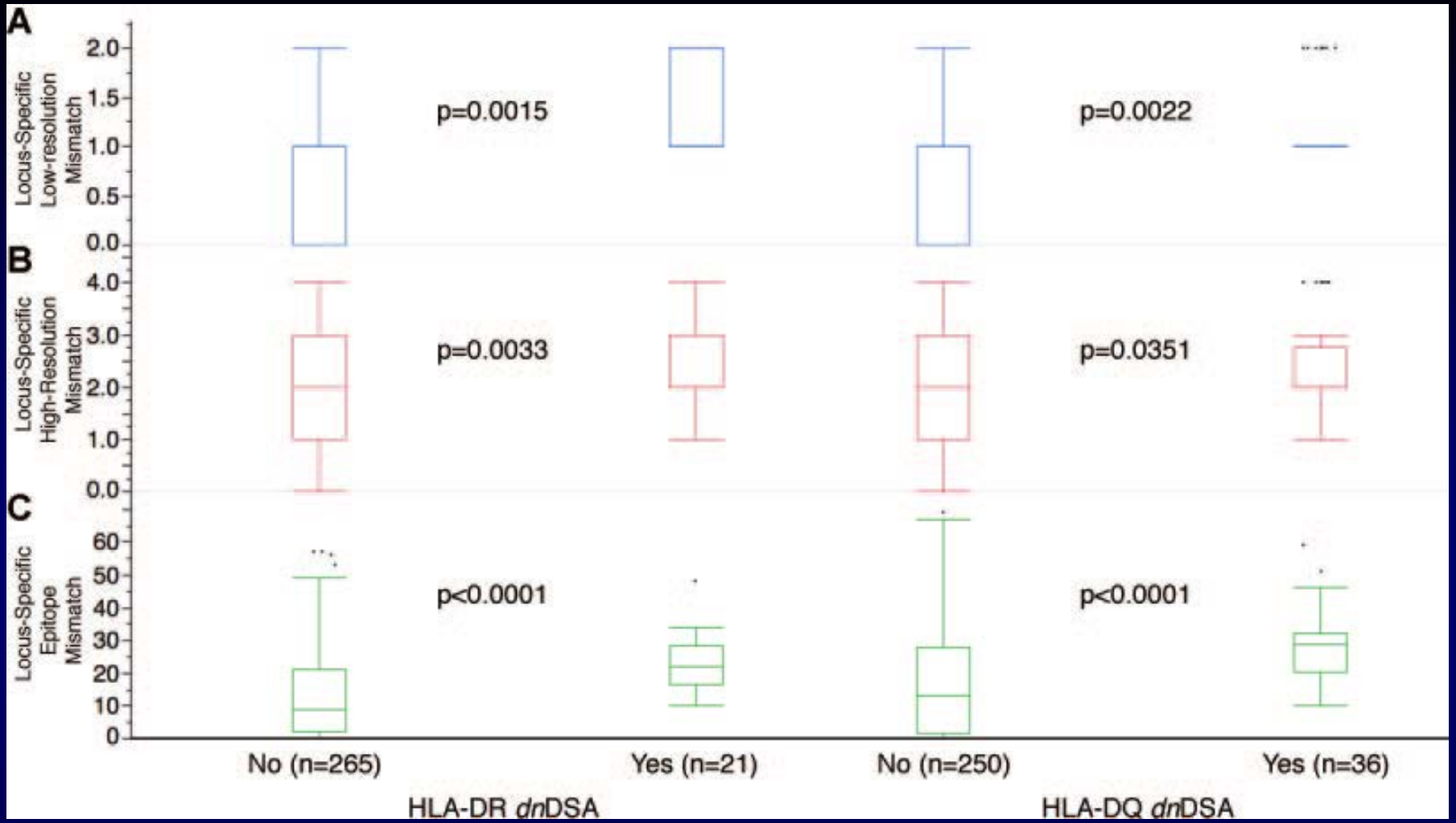
Keywords: Antibody-mediated rejection, donor-specific antibody, epitope, kidney transplant

Abbreviations: cAMR, chronic antibody-mediated rejection; cPRA, calculated panel reactive antibody; *dn*DSA, *de novo* donor-specific antibody; MFI, mean fluorescence intensity; TerEp, Terasaki epitope

Received 27 June 2013, revised 24 July 2013 and accepted for publication 25 July 2013

Conclusion: DR and DQ epitope mismatching outperforms traditional low-resolution antigen mismatching and high-resolution allele mismatching as a predictor of de novo Class II DSA development thereby improving long-term graft outcome



Prediction of *de novo* DSA post-transplant. A: how-resolution DRB,DQB mismatches, B: high-resolution DRB1/3/4/5, DQA,B mismatches; C: eplet-derived epitope mismatches

Wiebe et al. Amer. J Transplantation 20:1-9, 2013

HLA Class II Matching at the Epitope Level (Conclusions)

- Type for HLA-DRDQDP at the 4-digit allele level
- Determine mismatched epitopes on donor alleles
- Consider the epitope load of a donor mismatch and epitope immunogenicity as risk factors for antibody-formation in non-sensitized transplant patients

HLA **Epitope** Immunogenicity

How often do mismatched eplets induce specific antibodies?

This issue will be addressed in the next lecture

Determination of Epitope Specificities of Antibodies

Important to the Understanding of the Sensitization Process and the Clinical Management of the Sensitized Patient

Informative Case of HLA Antibody Reactivity in Luminex

HOW CAN A PATIENT WHO TYPES FOR HLA-B*4403 CAN DEVELOP ANTIBODIES THAT REACT WITH HLA-B*4402?

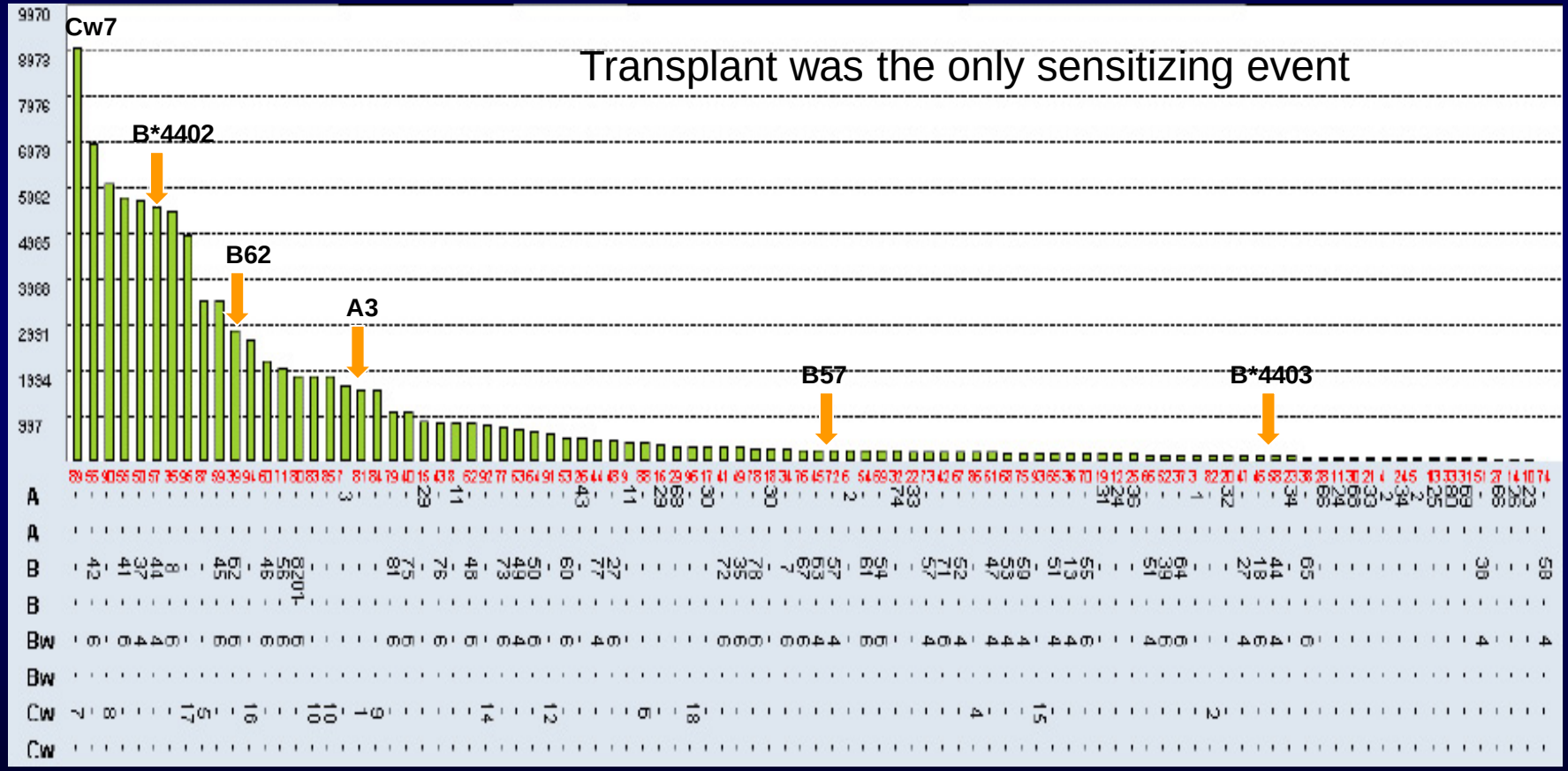
Jon Lomago, Larry Jelenik, Dwayne Zern, Judy Howe, Joan Martell, Adriana Zeevi and Rene J Duquesnoy

Tissue Typing Laboratory, Division of Transplantation Pathology, University of Pittsburgh Medical Center

Human Immunology, 71:176-178, 2010

Patient: HLA-A1,66; B44,58; Cw4,6
Donor: HLA- A1,3; B57,62; Cw6,7

61-yr old male, Kidney Transplant 2001, Failure 2005, Serum samples 2007-



Why does serum react with B*4402 but not with B*4403?

HLAMatchmaker shows that B*4402 has only one eplet difference from B*4403 ……namely 156DA !

Which alleles in the panel have 156DA?

HLAMatchmaker shows that B*4402 has only one eplet difference from B*4403 ……namely 156DA !

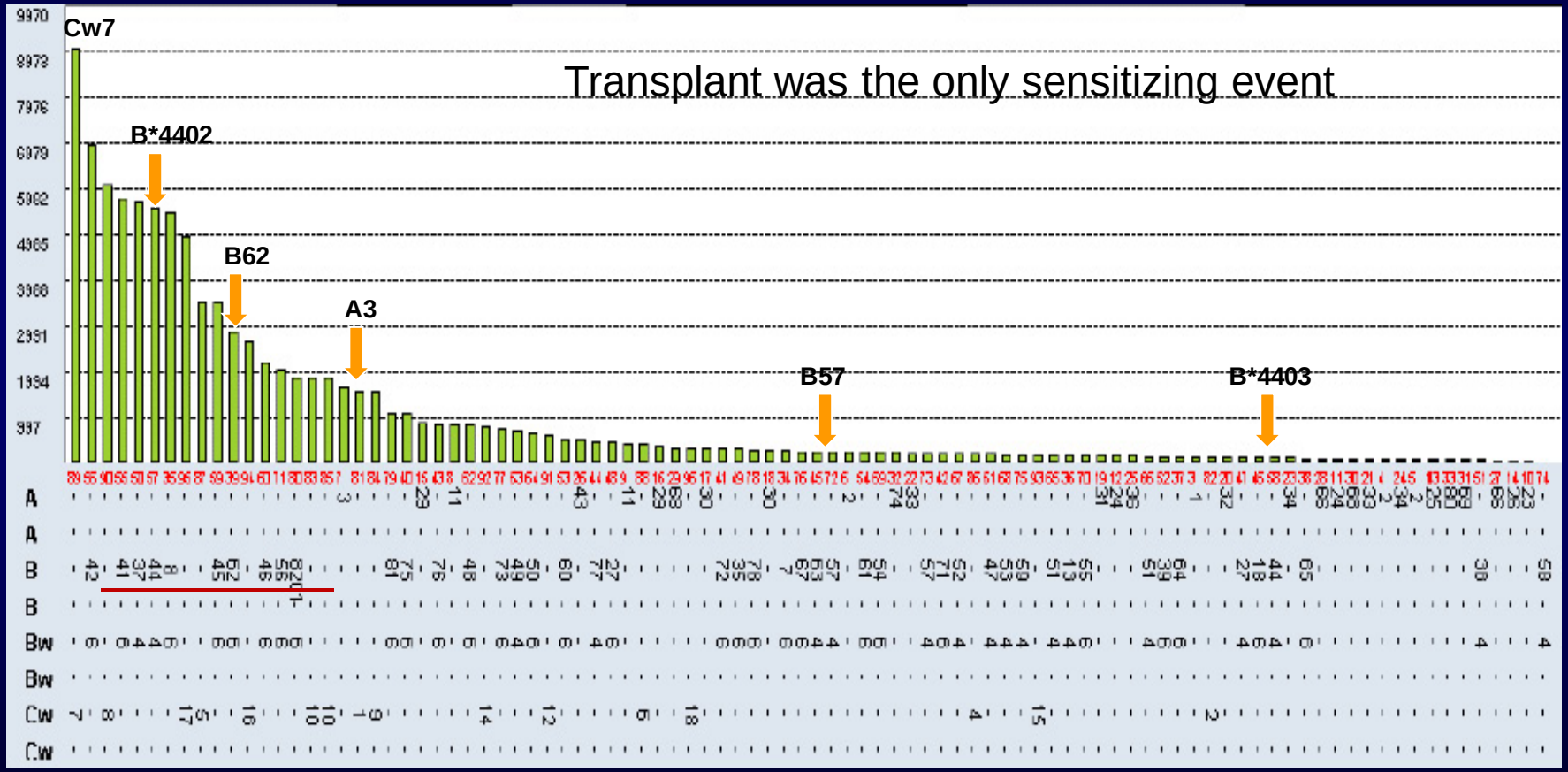
Which alleles in the panel have 156DA?

B*0801
B*3701
B*4101
B*4201
B*4402
B*4501
B*8201

They are all reactive!

Patient: HLA-A1,66; B44,58; Cw4,6
Donor: HLA- A1,3; B57,62; Cw6,7

61-yr old male, Kidney Transplant 2001, Failure 2005, Serum samples 2007-



Why does serum react with B*4402 but not with B*4403?

B*4402 has only one eplet difference from B*4403 .... namely 156DA !

Does the immunizing Cw7 have 156DA?

The common Cw*0701 and Cw*0702 alleles do not have 156DA

Hypothesis: donor type must have the uncommon Cw*0704 which has 156DA

High-Resolution Typing Results

Patient:
A*0101,*6601; B*4403,*5801;
Cw*0401,*0602

Donor:
A*0101,*0301; B*1501,*5701;
Cw*0602,**0704**

HLAMatchmaker analysis of Luminex results with two single allele kits

		OneLambda	Tepnel	
Neg Cont		38	252	-
Pos Cont		9273	2807	-
Average Self Reactivity		258±146	1174±518	
				Immunizer Eplets on Reactive Alleles
Neg Alleles	N=63	387±308	1160±527	none
Cw*0704	Imm	nt	9427	73AS, 77VSN, 79VRN, 152RA, **156DA**, 177KT, 193PL, 267QE
B*4402		5622	3202	**156DA**
B*0801		5770	8818	**156DA**
B*3701		5775	747	**156DA**
B*4101		5872	3237	**156DA**
B*4201		6967	3882	**156DA**
B*4501		3620	4468	**156DA**
B*8201		1997	nt	**156DA**
B*8202		nt	5062	**156DA**

Anti-156DA Specific Antibodies and Unacceptable Mismatches

- Immunizing allele Cw*0704 and 156DA-carrying alleles in the Luminex panel: B*0801, B*3701, B*4101, B*4201, B*4402, B*4501 and B*8201

- Other 156DA-carrying alleles not in the Luminex panel such as B*0704, B*4102, B*4405, B*5108 and B*8301

B44 Case Summary

- Serum reactivity with B*4402 but not B*4403 is due to antibody against the 156DA epitope
- A Cw*0704 mismatch induced anti-156DA antibodies
- This sensitization event rendered 156DA-carrying HLA-B alleles as unacceptable mismatches

- These findings illustrate the importance of high-resolution HLA typing in the interpretation of antibody reactivity patterns and the determination of HLA mismatch acceptability

Question about Retransplantation of this Patient

- How do you find a suitable donor under the current UNOS kidney allocation system based on HLA-A,B,DR antigen matching?
- How do you look at HLA-C compatibility ?
- Would you consider B44 a match or a mismatch?

Question about Retransplantation of this Patient

- How do you find a suitable donor under the current UNOS kidney allocation system based on HLA-A,B,DR antigen matching?
- How do you look at HLA-C compatibility ?
- Would you consider B44 a match or a mismatch?

Are we ready for this?

In Organ Allocation for Sensitized Patients:

HLA Typing Should Be at the Allele Level and Determine Antibody Reactivity with HLA Epitopes

Personal Viewpoint: Should HLA Mismatch Acceptability for Sensitized Transplant Candidates Be Determined at the High-Resolution Rather than the Antigen Level?

R. J. Duquesnoy, M. Kamoun,
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R. A. Bray, F. H. J. Claas, D. D. Eckels,
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American Journal of Transplantation, In Press, March 2015

Questions about the Traditional Approach of Antigen-Based Matching

- Multicenter transplant organizations have organ allocation protocols designed to give preference to highly sensitized patients to increase their chances of receiving a compatible organ.

- The selection of donors has traditionally considered mismatch acceptability at the HLA antigen level as determined from serum antibody reactivity patterns with HLA panels.

- Many laboratories currently use sensitive HLA antibody detection assays with single allele bead (SAB) panels but the reactive alleles are converted to antigen equivalents which are then recorded in registries as unacceptable mismatches.

- SAB panels have often two or more alleles corresponding to the same antigen. This can create a dilemma in the interpretation of mismatch acceptability at the antigen level when corresponding alleles have different reactivity with patient's serum antibodies.

- How does one handle the mismatch acceptability of an allele not present in the SAB panel? Would conversion of an HLA allele to a two-digit antigen be without any risk to the recipient?

New Approach: HLA Allele Matching Based on Epitopes

- It is well accepted that HLA antibodies specifically recognize epitopes rather than HLA antigens.
- Molecularly defined high-resolution alleles corresponding to the same antigen possess different epitope repertoires.
- Determination of HLA compatibility at the allele level represents a more accurate approach to identify suitable donors for highly sensitized patients.
- This approach would offer opportunities for increased transplant rates and improved long term graft survivals.

Alleles in SAB Panels

Ag	Alleles	Ag	Alleles	Ag	Alleles	Ag	Alleles
A1	A*01:01	B7	B*07:02/03	B53	B*53:01	B78	B*78:01
A2	A*02:01/02/03/05/06	B8	B*08:01	B54	B*54:01	B81	B*81:01
A3	A*03:01	B13	B*13:01/02	B55	B*55:01	B82	B*82:01/02
A11	A*11:01/02	B14	B*14:01/02/05/06	B56	B*56:01	Cw1	C*01:02
A23	A*23:01/02	B18	B*18:01	B57	B*57:01/03	Cw2	C*02:02/10
A24	A*24:02/03	B27	B*27:03/05/08	B58	B*58:01	Cw3	C*03:02/03/04
A25	A*25:01	B35	B*35:01/08	B59	B*59:01	Cw4	C*04:01/03
A26	A*26:01	B37	B*37:01	B60	B*40:01	Cw5	C*05:01
A29	A*29:01/02	B38	B*38:01	B61	B*40:02/06	Cw6	C*06:02
A30	A*30:01/02	B39	B*39:01/05	B62	B*15:01	Cw7	C*07:01/02/04
A31	A*31:01	B41	B*41:01	B63	B*15:16	Cw8	C*08:01/02
A32	A*32:01	B42	B*42:01	B64	B*14:01	Cw12	C*12:02/03
A33	A*33:01/03	B44	B*44:02/03	B65	B*14:02	Cw14	C*14:02
A34	A*34:01/02	B45	B*45:01	B67	B*67:01	Cw15	C*15:02
A36	A*36:01	B46	B*46:01	B71	B*15:10/18	Cw16	C*16:01
A43	A*43:01	B47	B*47:01	B72	B*15:03	Cw17	C*17:01
A66	A*66:01/02	B48	B*48:01	B73	B*73:01	Cw18	C*18:01/02
A68	A*68:01/02	B49	B*49:01	B75	B*15:02/11		
A69	A*69:01	B50	B*50:01	B76	B*15:12		
A74	A*74:01	B51	B*51:01/02	B77	B*15:13		
A80	A*80:01	B52	B*52:01				

Table 1 Examples of antibody-reactive epitope expression on SAB alleles corresponding to the same antigen and predictions of unacceptable and acceptable non-SAB alleles

Antibody Reactive Epitope	Epitope-Carrying SAB Alleles	Potential Donor Antigen	Epitope-carrying Reactive SAB Alleles (Unacceptable)	Non-reactive SAB Alleles (Acceptable)	Predicted Unacceptable Non-SAB Alleles	Predicted Acceptable Non-SAB Alleles
145KHA	A*01:01,A*02:01,A*02:02,A*02:05,A*02:06,A*03:01,A*11:01,A*11:02, A*24:02,A*24:03,A*36:01,A*68:01,A*68:02,A*69:01,A*80:01	A2	A*02:01/02/05/06	A*02:03	A*02:07/10/12/13/14/16/17	A*02:19/25
166DG	A*01:01,A*23:01,A*23:02,A*24:02,A*80:01,B*15:12	A24	A*24:02	A*24:03	A*24:05/07/08/14/17/20	A*24:10/18/22
65QIA	B*07:02,B*27:03,B*27:05,B*27:08,B*42:01,B*54:01,B*55:01, B*56:01,B*67:01,B*73:01,B*81:01,B*82:01,B*82:02	B7	B*07:02	B*07:03	B*07:04/05/09/10	B*07:08/13/16
21H	C*02:02,C*02:10,C*03:02,C*03:03,C*03:04,C*04:03,C*15:02,	Cw4	C*04:03	C*04:01/02	C*04:06/16	C*04:04/05/07/08

Table 2 Prediction of mismatch acceptability of non-SAB alleles corresponding to selected donor HLA antigens for patients with antibodies specific for the 62GE, 76AN and 76ESN defined epitopes

Epitope	Epitope-Carrying SAB Alleles	Donor Antigen	Predicted Unacceptable or Acceptable Non-SAB Alleles
62GE	A*02:01,A*02:02,A*02:03,A*02:05,A*02:06,B*57:01,B*57:03,B*58:01	B58 (unacceptable?) A24 (acceptable?)	B*58:02/06 NOT B*58:04 A*24:04/05/06/07/10 NOT A*24:08
76AN	A*01:01,A*26:01,A*29:01,A*29:02,A*36:01,A*43:01,A*80:01	A26 (unacceptable?) A24 (acceptable?)	A*26:02/07/08/09 NOT A*26:03/05/06 A*24:05/06/07/08/10 NOT A*24:04
76ESN	B*07:02,B*07:03,B*08:01,B*14:01,B*14:02,B*15:01,B*15:02,B*15:03,B*15:10, B*15:11,B*15:12,B*15:18,B*18:01,B*27:08,B*35:01,B*35:08,B*39:01,B*39:05, B*40:01,B*40:02,B*40:06,B*41:01,B*42:01,B*45:01,B*48:01,B*50:01,B*54:01, B*55:01,B*56:01,B*67:01,B*78:01,B*81:01,B*82:01,B*82:02	B8 (unacceptable?) B44 (acceptable?)	B*08:04/05/07/09/10 NOT B*08:02/03 B*44:04/05/06/08/10 NOT B*44:09

Table 3 Amino acid residue differences between HLA-A2, -A24, -B27 and -B35 alleles with greater than 0.5% frequencies in one or more common population groups of potential transplant donors

position	9	43	66	73	74	95	97	99	107	149	152	156
A*02:01* (A2)	F	Q	K	T	H	V	R	Y	W	A	V	L
A*02:02*	-	R	-	-	-	L	-	-	-	-	-	W
A*02:03* (A203)	-	-	-	-	-	-	-	-	-	T	E	W
A*02:04	-	-	-	-	-	-	M	-	-	-	-	-
A*02:05*	Y	R	-	-	-	L	-	-	-	-	-	W
A*02:06*	Y	-	-	-	-	-	-	-	-	-	-	-
A*02:07	-	-	-	-	-	-	-	C	-	-	-	-
A*02:08	Y	R	N	-	-	L	-	-	-	-	-	W
A*02:10* (A210)	Y	-	-	-	-	-	-	F	G	-	-	-
A*02:11	-	-	-	I	D	-	-	-	-	-	-	-
A*02:12	-	-	-	-	-	-	-	-	-	-	-	Q
A*02:17	-	-	-	-	-	L	M	F	-	-	-	-
A*02:20	-	-	N	-	-	-	-	-	-	-	-	-

position	3	7	62	65	70	76	79	80	81	82	83	114	116	163	166	167
A*24:02* (A24)	H	Y	E	G	H	E	R	I	A	L	R	H	Y	T	D	G
A*24:03* (A2403)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	E	W
A*24:04	-	-	-	-	-	A	G	T	L	R	G	-	-	-	-	-
A*24:07	-	-	-	-	Q	-	-	-	-	-	-	-	-	-	-	-
A*24:08	Q	-	G	R	-	-	-	-	-	-	-	-	-	-	-	-
A*24:10	-	-	-	-	-	-	-	-	-	-	-	-	-	R	E	W
A*24:17	-	-	-	-	-	-	-	-	-	-	-	R	D	-	-	-
A*24:20	Q	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A*24:25	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-

position	59	77	80	81	82	83	97	113	114	116	131	152	211
B*27:05* (B27)	Y	D	T	L	L	R	N	Y	H	D	S	V	A
B*27:02	-	N	I	A	-	-	-	-	-	-	-	-	-
B*27:03*	H	-	-	-	-	-	-	-	-	-	-	-	-
B*27:04	-	S	-	-	-	-	-	-	-	-	-	E	G
B*27:06	-	S	-	-	-	-	-	-	D	Y	-	E	G
B*27:07	-	-	-	-	-	-	S	H	N	Y	R	-	-
B*27:08* (B2708)	-	S	N	-	R	G	-	-	-	-	-	-	-
B*27:09	-	-	-	-	-	-	-	-	-	H	-	-	-

position	67	94	95	97	103	109	114	116	152	156	194
B*35:01* (B35)	F	I	I	R	L	L	D	S	V	L	V
B*35:02	-	-	-	-	-	F	N	Y	-	-	-
B*35:03	-	-	-	-	-	-	-	F	-	-	-
B*35:04	-	-	-	-	-	-	N	Y	-	-	-
B*35:05	-	T	L	S	-	-	-	-	-	-	-
B*35:06*	-	-	-	-	-	-	-	-	-	R	-
B*35:12	-	-	-	-	V	-	N	Y	-	-	-
B*35:14	-	-	-	-	-	-	-	-	E	W	-
B*35:17	-	-	-	S	V	-	-	-	-	-	-
B*35:20	S	-	-	-	-	-	-	-	-	-	-

* Alleles in single allele bead (SAB) panels are annotated with an asterisk; the equivalent serological antigen is also listed. Antibody-accessible sequence positions on the molecular surface are in boxes

Application of Allele-Based Mismatch Acceptability in the Clinical Setting

- There is not surprisingly, disagreement within the transplant community on the practical clinical utility of this a strategy. The main arguments focus on cost, time constraints, and lack of funding to pilot the change.

- New technological advances readily permit high-resolution HLA types within a few hours after test setup. Molecular typing kits could be expanded to include commonly enough alleles.

- There is also some skepticism about the clinical usefulness of what amounts to a paradigm shift. The term “antigen” conveys an entity that generates an immune response whereas some transplant professionals believe that the term “allele” implies less antigenicity. The reality is that HLA antibodies specifically recognize epitopes and that alleles offer better descriptions of epitopes than antigens.

- Matching at the allele level applies not only to the HLA-A, -B, -DR (DRB1) and -DQB loci but also to HLA-C, -DRB3/4/5, -DQA and -DP mismatches which may lead to antibodies that are deleterious to the transplanted organ.

- The implementation of allele-based matching in the clinical transplant setting will raise many practical issues that require a great deal of community discussion and public comments.

Advantages of Allele-Based Determination of Mismatch Acceptability

- Greater diversities within populations of organ donors and recipients have led to an increase in the number of alleles seen for each antigen.

- Mismatch acceptability at the allele level will reduce the likelihood for errors when attempting to assess whereby sensitized patients might be denied a suitable organ or might receive a transplant with a higher risk of rejection and possible failure.

- Unacceptable alleles can be determined from epitope specificities of antibodies in patient sera.

- With broader geographic sharing and increased priority for the most highly sensitized recipients the predictability of virtual cross-matching for these patients is all the more vital.

Conclusion

- The science of histocompatibility testing has advanced considerably. Molecular typing at the allele level provides a better assessment of donor-recipient compatibility and the number of clinically relevant HLA gene loci has expanded. HLA antibodies are important risk factors for transplant rejection and it is now generally accepted that they specifically react with epitopes.

- Allele-based typing will be in the best interest of the highly sensitized patient and also will also offer new directions to increase our understanding of antibody responses to HLA mismatches and the clinical relevance of HLA epitope-specific antibodies in transplantation.

Clinical Relevance of HLA Epitope-Based Matching for Transplantation (Conclusions)

- Epitope antigenicity
  - Analyses of epitope specificities of antibodies enhance the determination of HLA mismatch acceptability for sensitized transplant candidates
  - Consider the antigen-antibody binding energy concept
- Epitope loads of HLA antigen mismatches
  - Clinically useful in the post-transplant management of patients at risk for antibody-mediated rejection
  - May lead to permissible mismatch strategies for non-sensitized patients to reduce humoral rejection and increase transplant success