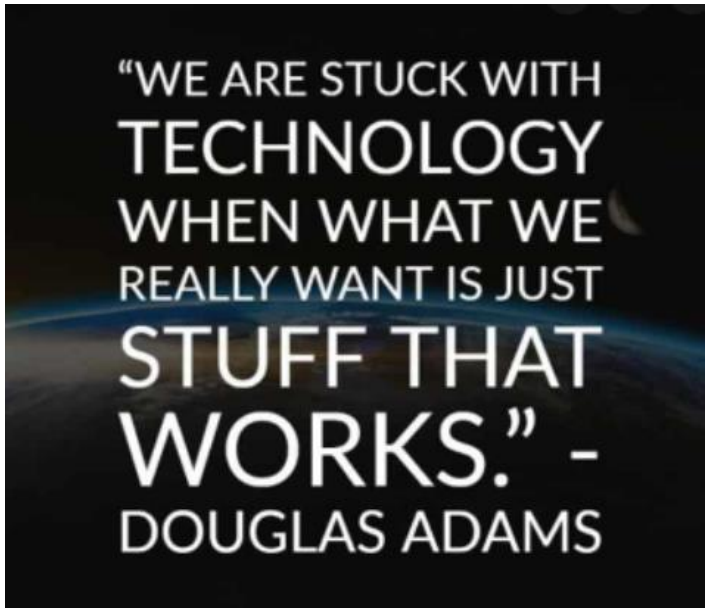


Eplet Matching - Kidney Now or Later

Nicole M Valenzuela, PhD, D(ABHI)

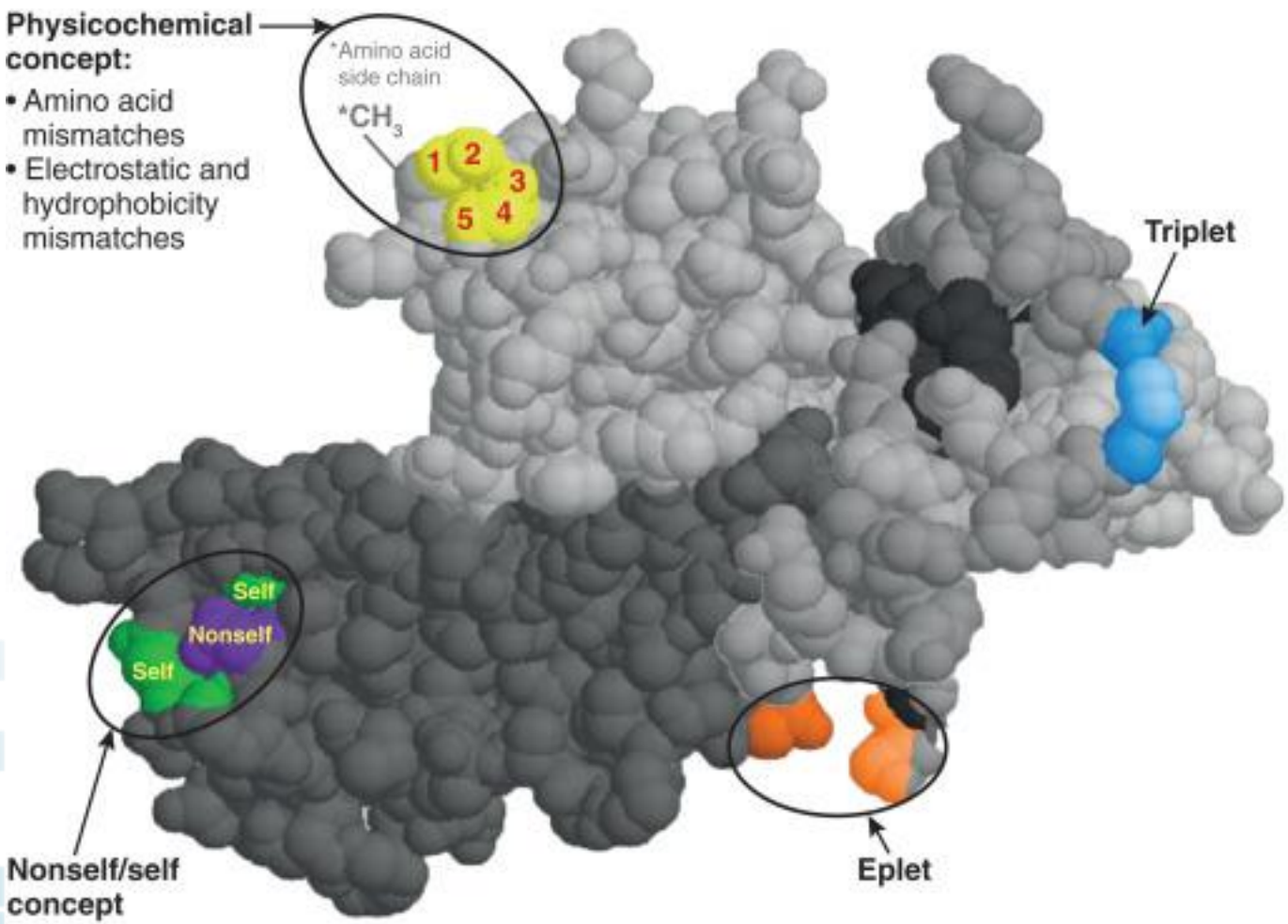
Department of Pathology and Laboratory Medicine, University of California, Los Angeles

14th Annual Living Donation Conference

Presented by the American Foundation for Donation and Transplantation

Overview

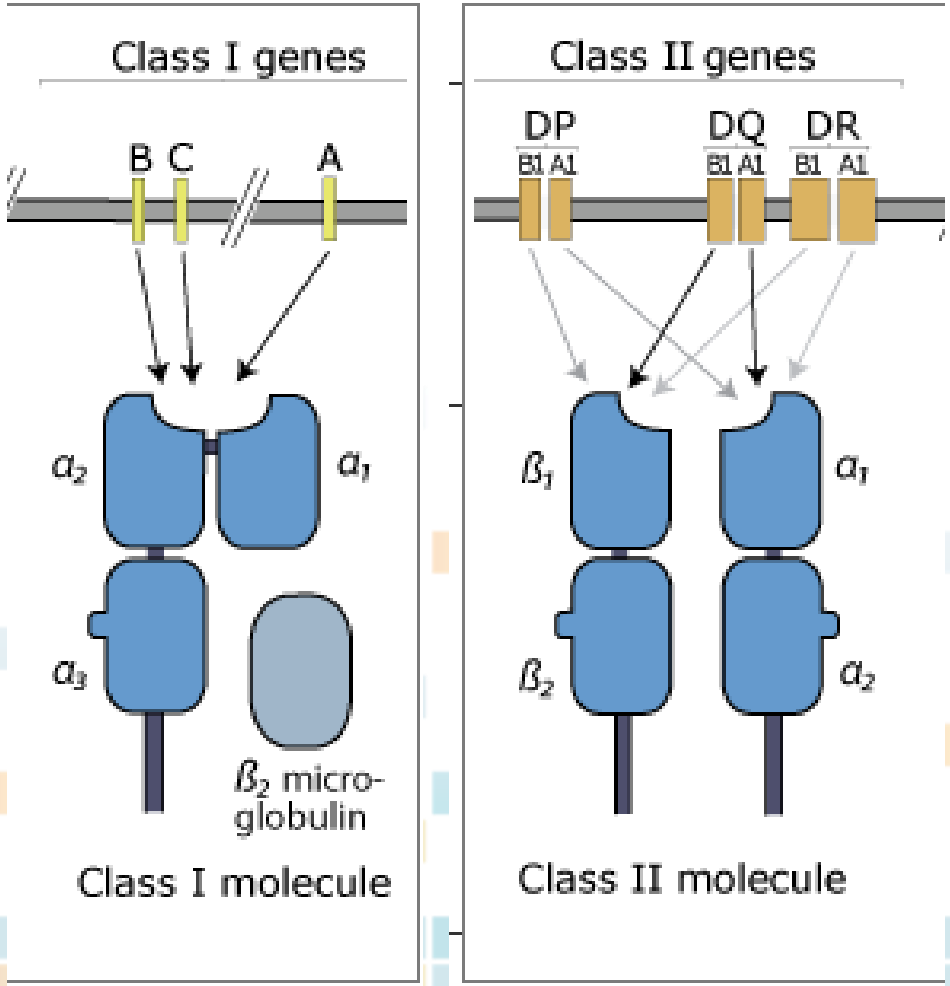
- HLA eplets
  - Premise
  - State of field
  - Potential application
  - Health Disparities



Lim *Kid Int* 2017

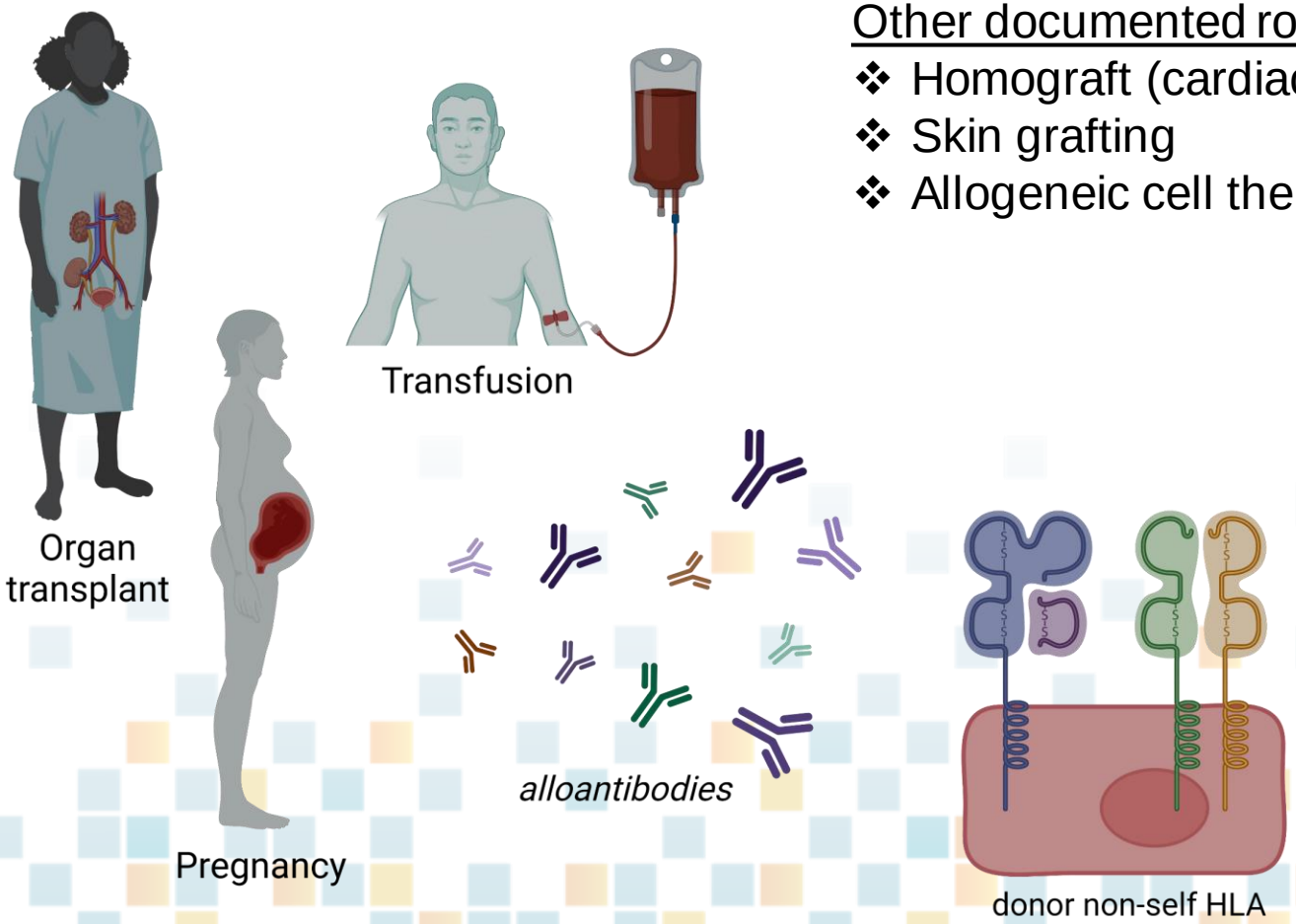
Structure and Polymorphism of HLA

- HLA molecules present peptides to T cells
- HLA class I is formed by a continuous polypeptide encoded by one gene
  - Expressed on every nucleated cell
- HLA class II proteins are heterodimers formed by the gene products of alpha (α) and beta (β) genes
  - Only expressed on antigen presenting cells and activated/inflamed cells like endothelium



Numbers of HLA Alleles	
HLA Class I Alleles	25,228
HLA Class II Alleles	10,592

Sources of HLA Allosensitization



Other documented routes:

- Homograft (cardiac repair)
- Skin grafting
- Allogeneic cell therapy

Nonsensitizing clinical events:

- Immunization
- Infection
- Surgery
- Changes in immunosuppression

Evolution of HLA Typing and Compatibility

DNA (intermediate)

Antigen level

A36 vs. A2

What an antibody sees

A1 CREG vs. A2 CREG

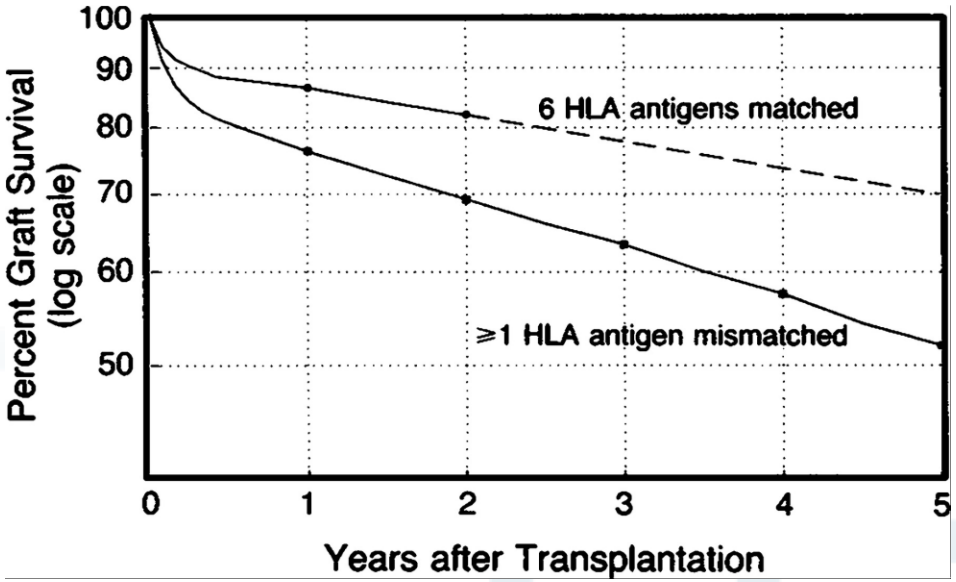
Serology

Cross-reactive groups

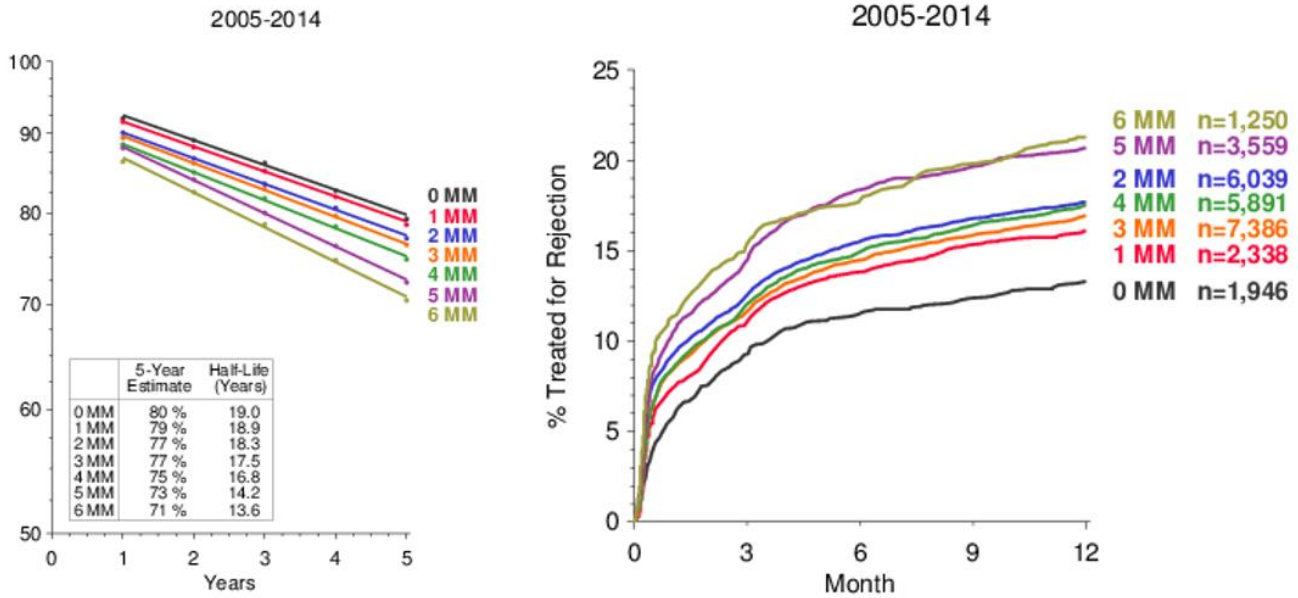
	A	B	C	DRB1	DQB1
Patient	1	8	7	17	2
	24	45	6	14	7
Donor	1	8	7	17	2
	2	64	8	1	5
Mismatch	***1***	***1***	***1***	***1***	***1***

3/6 ABDR Mismatch

Association of antigen level mismatching with outcome



Gjertson NEJM 1991



Collaborative Transplant Study; Williams *Transplant* 2016

Evolution of HLA Typing and Compatibility

DNA (intermediate)

Antigen level

A36 vs. A2

A1 CREG vs. A2 CREG

Serology

Cross-reactive groups

What an antibody sees

DNA (high resolution)

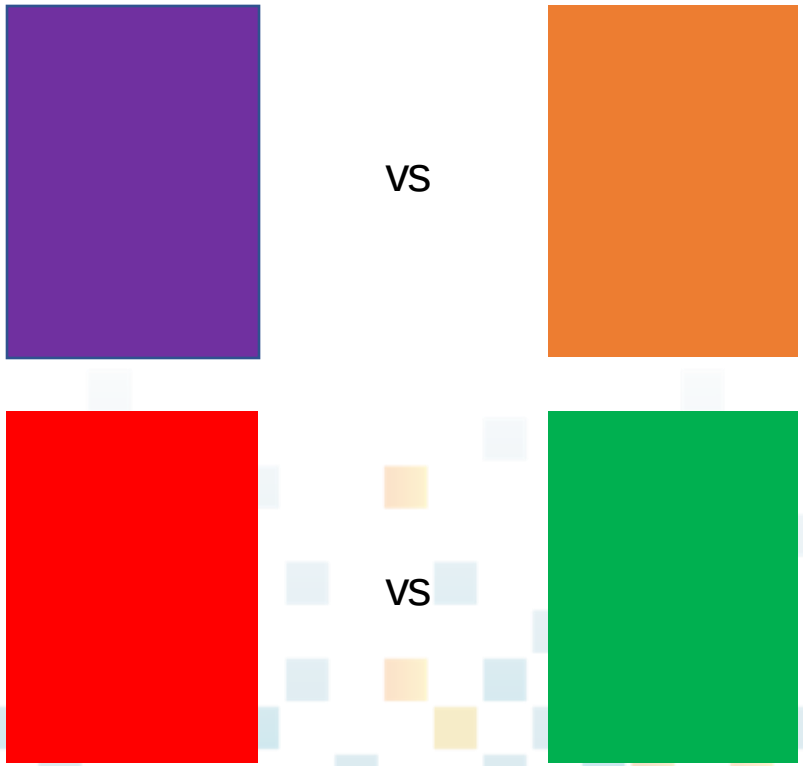
Allele level

A*36:01 vs. A*02:01 vs. A*02:05

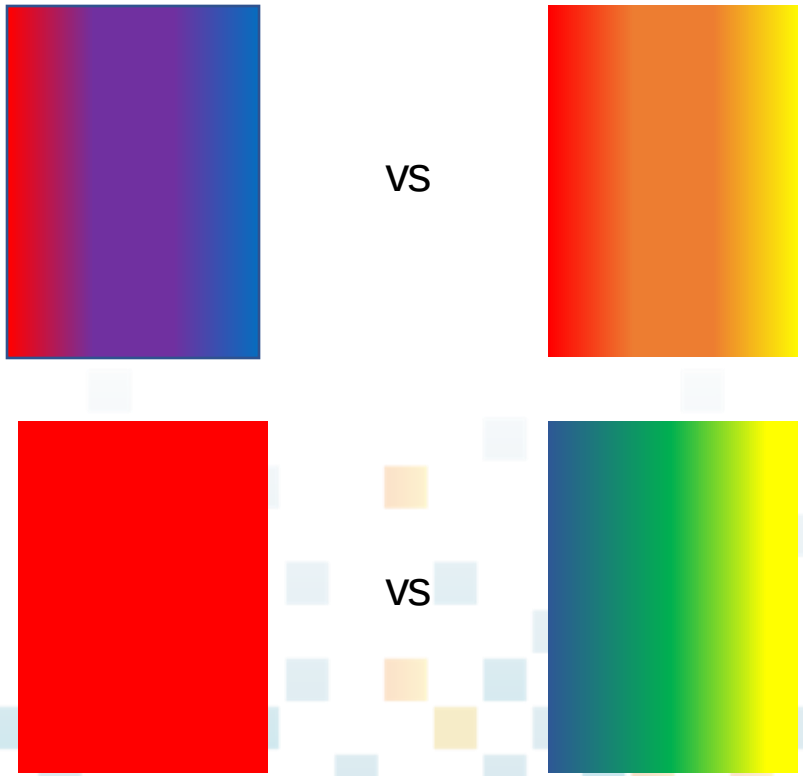
What a T cell sees

*What a **discriminating** antibody sees*

Degrees of difference

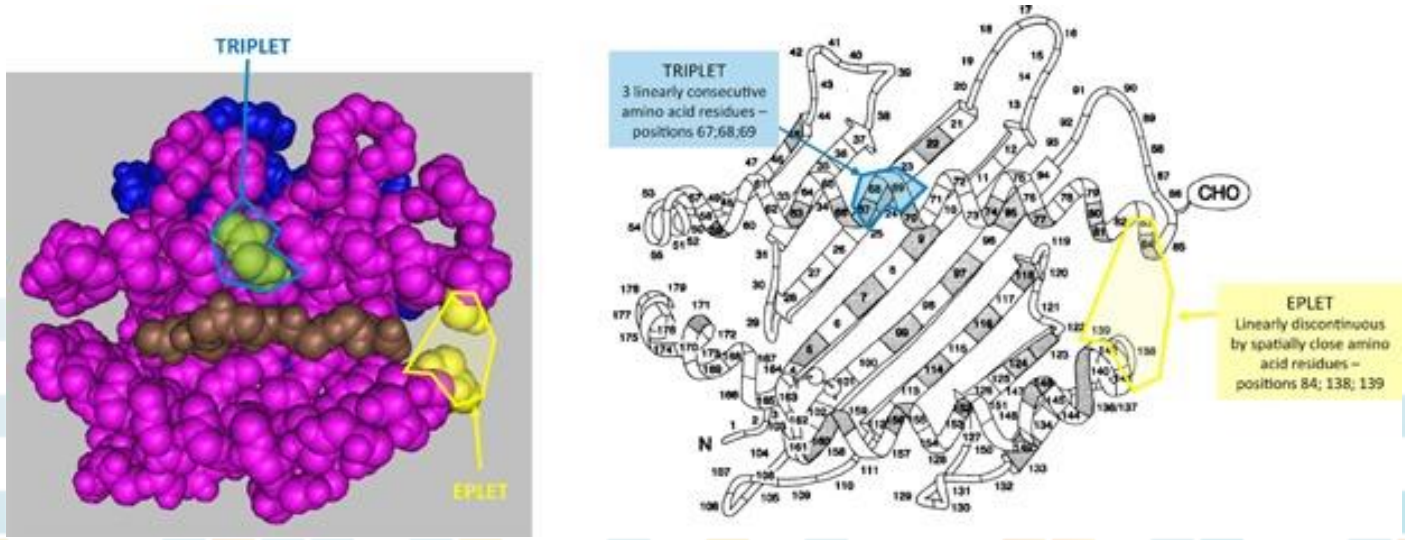


Degrees of difference



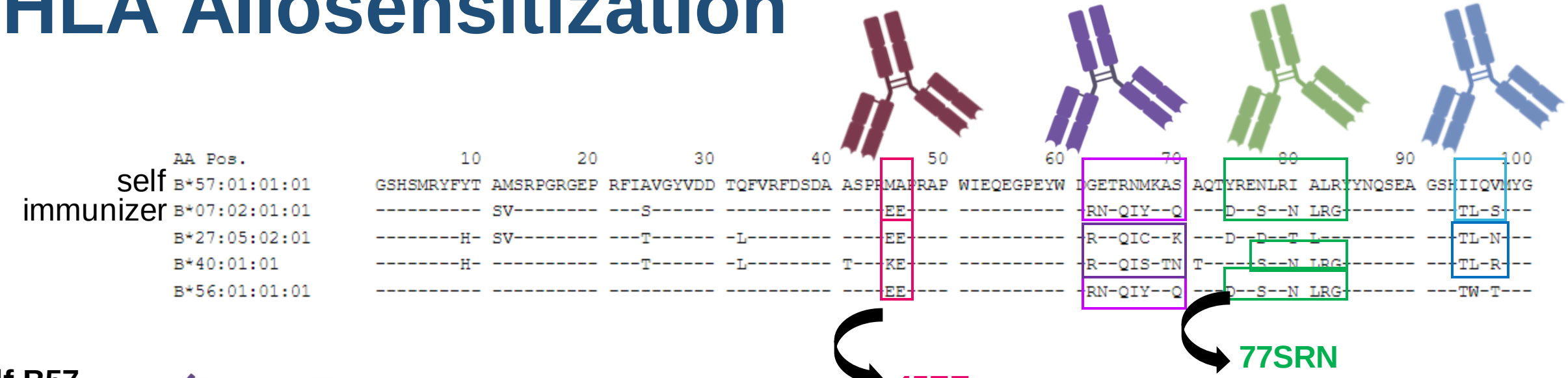
What are HLA Eplets?

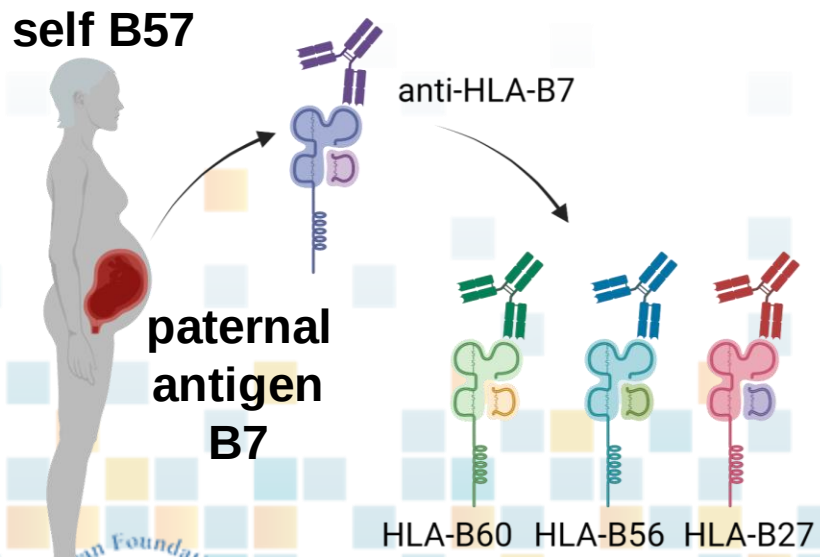
HLA eplets are amino acid residues that are different between two alleles, that *may* represent a target for an antibody or T cell



Tambur and Claas *AJT* 2015

HLA Allosensitization





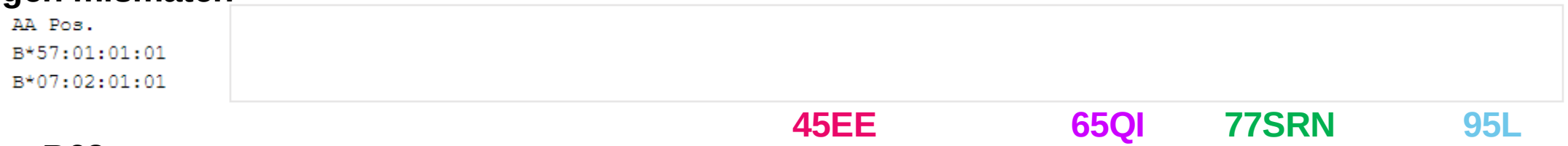
Luminex Alleles of Epitope: 45EE

B*07:02, B*07:03, B*08:01, B*14:01, B*14:02, B*14:05, B*14:06, B*15:03, B*15:10, B*15:18, B*27:03, B*27:05, B*27:08, B*38:01, B*39:01, B*39:05, B*42:01, B*48:01, B*55:01, B*56:01, B*59:01, B*67:01, B*73:01, B*81:01, B*82:01, B*82:02

HLA Eplet vs. Antigen Mismatching

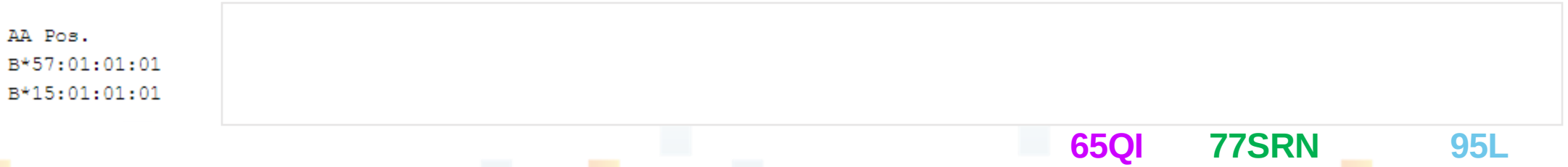
B57 vs. B7
1 antigen mismatch

= 4 eplet mismatch



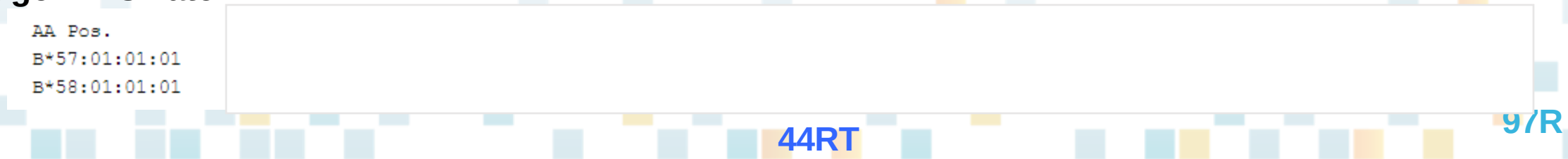
B57 vs. B62
1 antigen mismatch

= 3 eplet mismatch



B57 vs. B58
1 antigen mismatch

= 2 eplet mismatch



Clinical Utility of HLA Eplet Mismatching

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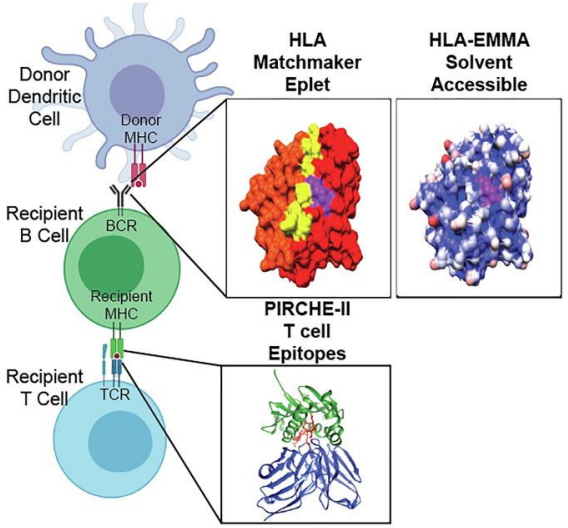
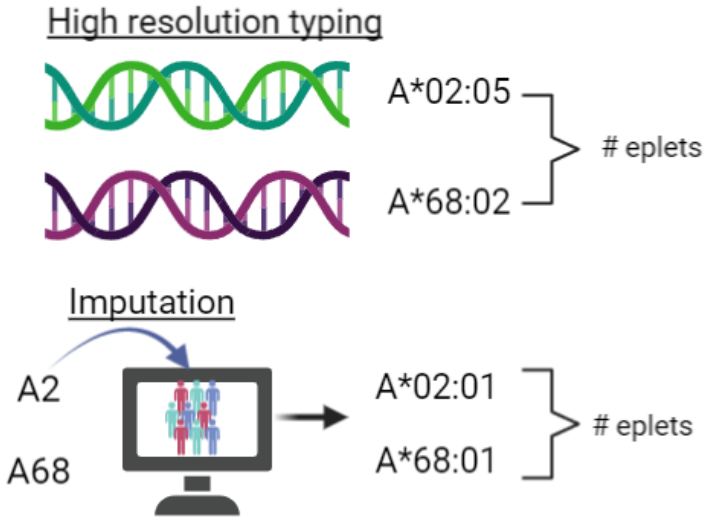
HLA Eplet Mismatching: Keep in mind



Ladowski *Hum Immun* 2021



Multiple algorithms

- Some *in silico*, others based on antibody verification
- Literature is a mix of approaches
- No consensus yet on which is the “best”

Requires high resolution typing

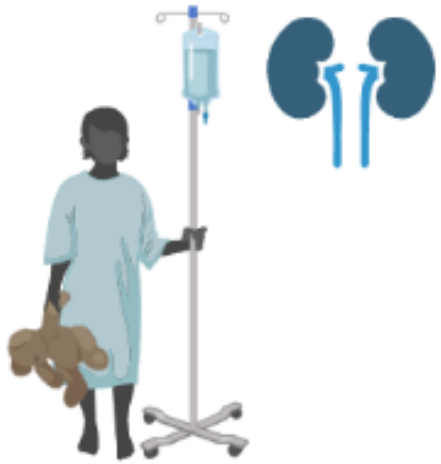
- Can be imputed from intermediate but imperfect accuracy, especially in non-Europeans [Engen *AJT* 2021]
- Larger studies from SRTR are based on imputation

Thresholds and relative risk are not fully defined

- How many is too many?
- Which mismatches are worse?
- Which outcome is more important?

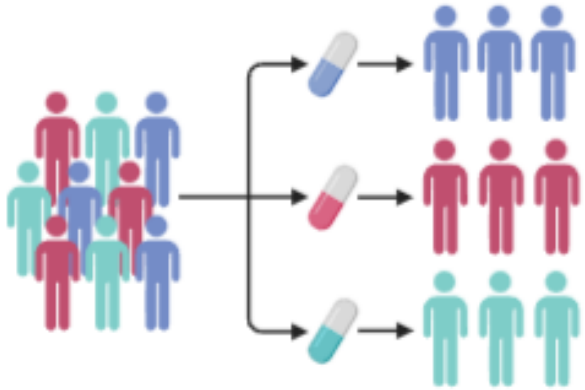
Clinical Utility of HLA Eplet Mismatching

Pre-transplant

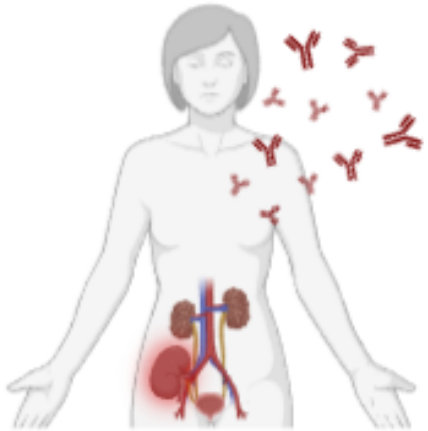


- equitable access
- DSA cross-reactivity
- unacceptable antigen

Post-transplant



- immunosuppression optimization



- rejection risk
- dnDSA risk
- graft longevity

Retransplant

WAITLIST

- cPRA after graft failure

Improving access to transplant

Pre-transplant

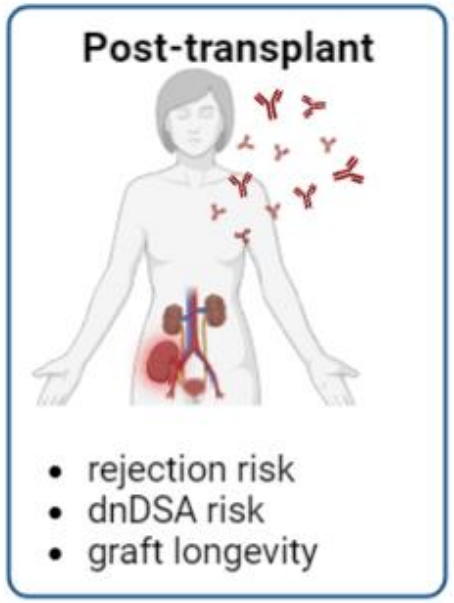
- equitable access
- DSA cross-reactivity
- unacceptable antigen

chance of finding a compatible donor for highly sensitized patients based on 0-2 triplet mismatches was much higher compared to matching on the antigen level [Duquesnoy *Transpl* 2003]

reduced transplant wait time for highly sensitized patients by 50% with comparable outcomes as non-sensitized patients [Eurotransplant; Lemieux Int Immuno 2021; Heidt Transpl 2019]

eplet based method of antibody analysis had a very high degree of **correlation with cell-based crossmatches** [Norin *Hum Immun* 2022]

Improving post-transplant outcomes



DR or DQ eplet mismatch was significantly correlated with production of **dnDSA** to that locus (OR 2.50 and 2.00 per 10MM) [Wiebe *JASN* 2017]

antibody-verified eplet mismatch load was associated with any type of **rejection**; but no threshold below which the risk of dnDSA occurrence was absent [Senev *JASN* 2020]

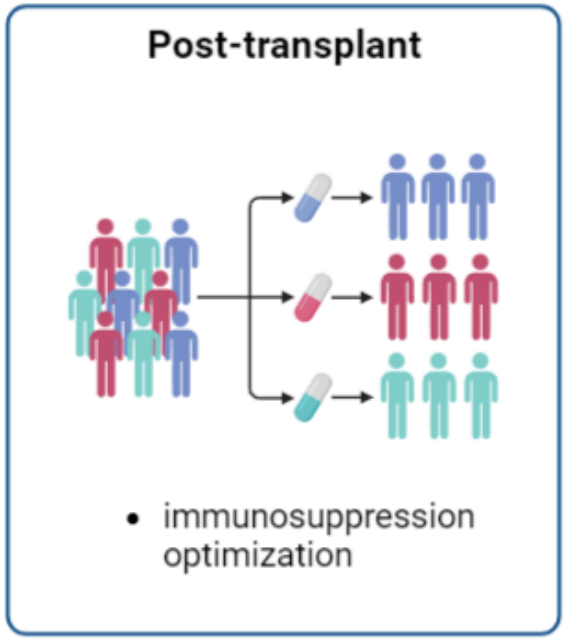
low-immunological risk recipients (0-2 ABDR mismatched kidneys) but with high eplet mismatches (≥20) had 2-fold increased **risk of acute rejection** [Nguyen *Transpl Dir* 2016]

significant relationship between eplet mismatches and **graft failure**; greater effect in unsensitized recipients [Sapir-Pichhadze *Kid Int* 2020]

discriminative performance for **graft failure** was low [Senev *JASN* 2020]

after dnDSA development in kidney transplant recipients, eplet mismatches did not correlate with ABMR or allograft loss [Wen *Hum Immun* 2021]

Tailoring immunosuppression based on risk



in CTOT-09 DQ mMM (>17) predicted dnDSA on **TAC minimization** despite patients being “low risk” (per sensitization) first 6 months post-transplant [Hricik *JASN* 2015]

recipients with high eplet mismatch load were **less likely to tolerate low tacrolimus** levels without developing de novo DSAs [Davis *AJT* 2021]

patients with high-risk DQ eplet mismatch score more frequently developed **acute rejection**, even if no pre-formed T cell alloimmunity was detected [Bestard *AJT* 2021]

Proposed Risk Stratification Model

Pretransplant donor–recipient HLA laboratory evaluation

CDC crossmatch	Flow crossmatch	Single antigen bead	History of sensitization	HLA molecular MM	HLA identical	Immune risk assessment
DSA positive	DSA positive	DSA positive				Active memory and at risk for hyperacute rejection
Negative	DSA positive	DSA positive				Active memory and at risk for ABMR and TCMR
Negative	Negative	DSA positive				Active memory and at risk for ABMR and TCMR
Negative	Negative	Negative	Pregnancy or prior transplant with repeat MM			At risk for latent memory with a recall B and T cell response
Negative	Negative	Negative	cPRA with unknown repeat MM			Potential risk for latent memory with a recall B and T cell response
Negative	Negative	Negative	No	High		Increased risk for de novo alloimmune response
Negative	Negative	Negative	No	Low		Baseline risk for de novo alloimmune response
Negative	Negative	Negative	No	0	Yes	Low risk for de novo alloimmune response

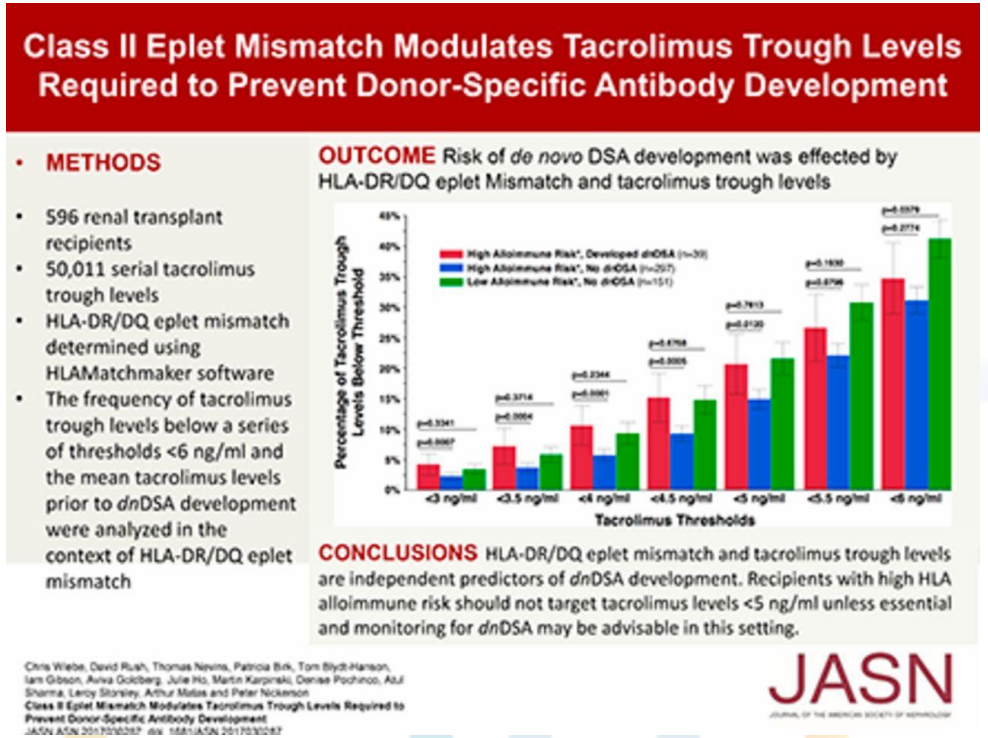
MM, Mismatch; DSA, donor-specific antibody; ABMR, antibody-mediated rejection; TCMR, T cell–mediated rejection.

"There is a body of evidence in support of the utility of HLA mMM score as a basis for primary alloimmunity risk stratification"

STAR (Tambur *AJT* 2018; 2019)

Proposed prognostic and predictive biomarker for clinical trials
(under review by FDA)

The FDA Center for Drug Evaluation and Research agreed to evaluate the potential role of HLA-DR/DQ eplet mMM score as a strategy for **enrichment or risk stratification** in phase 2 and 3 kidney transplant clinical drug development trials and as a **prognostic biomarker** for de novo DSA, graft rejection, and graft failure.

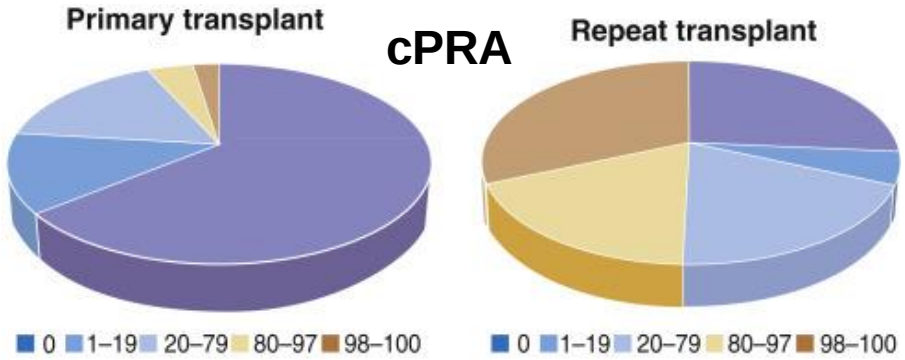


Decreasing breadth of sensitization after graft failure

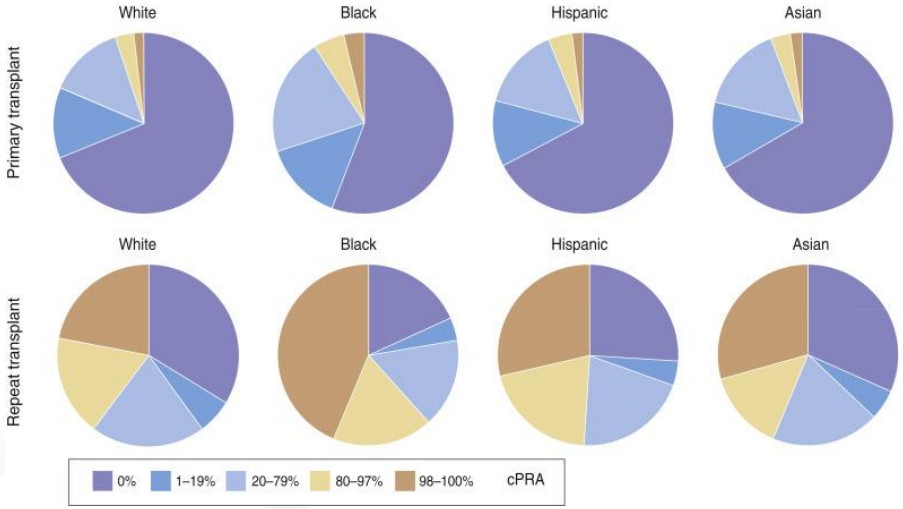
Retransplant

WAITLIST

- cPRA after graft failure



Tambur *Kid Int* 2021



highly sensitized patients had **lower rates of re-transplantation**, and higher rates of nephrectomy/graft intolerance syndrome [Singh *Clin Transpl* 2016]

after graft failure, **non-European recipients have a greater cPRA** than those of European ancestry [Tambur *Kid Int* 2021]

Decreasing breadth of sensitization after graft failure

Retransplant

WAITLIST

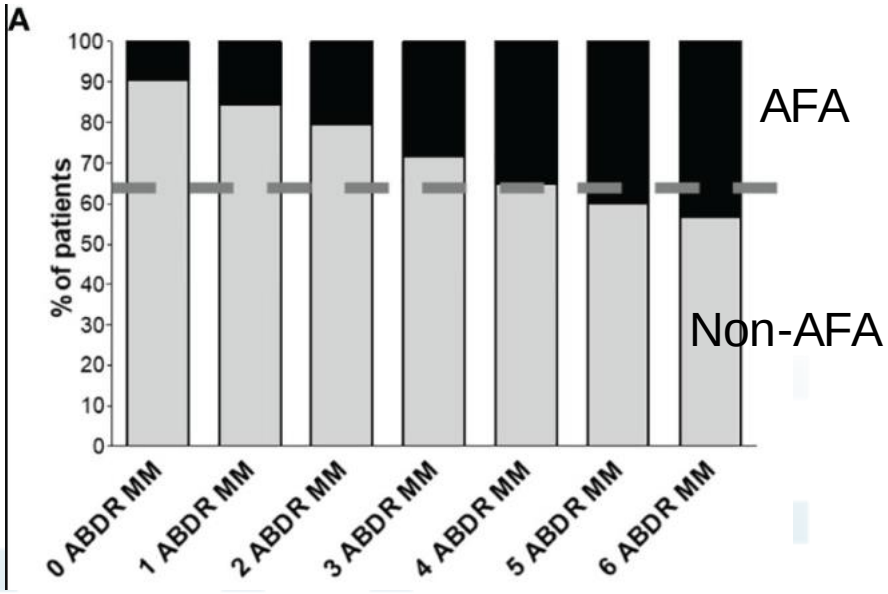
- cPRA after graft failure

clear relationship between the immunogenicity of donor HLA class I and class II mismatches and the development of HLA-specific antibodies **after graft failure and relisting for transplantation** [Kosmoliaptsis *AJT* 2016]

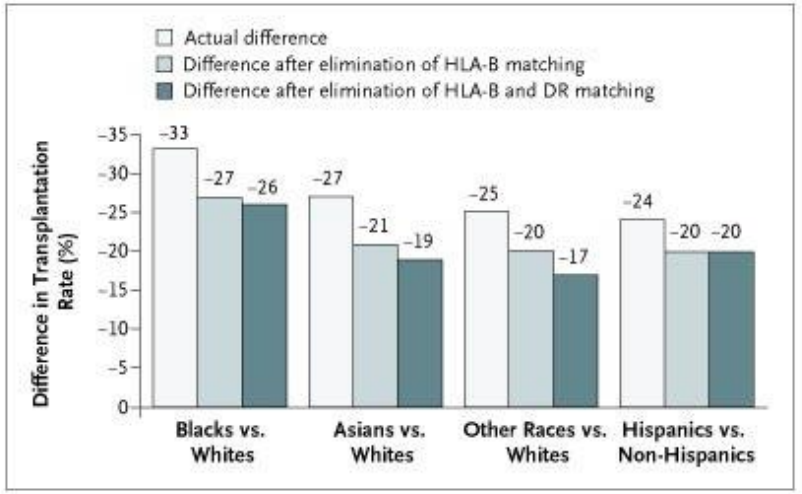
molecular mismatch scores were independently associated with degree of **sensitization after graft failure** [Kosmoliaptsis *AJT* 2016]

HLA eplet mismatch burden associated with higher risk of **higher cPRA** after renal allograft failure [Singh *Clin Transpl* 2016]

Equitable allocation and addressing health disparities



Bekbolsynov *Front Immun* 2022



Roberts *NEJM* 2004

Table 4. Actual Number of Graft Failures in 2000 and Number Expected If Matching for HLA-B Alone or HLA-B and DR Was No Longer a Priority.*

Race	Actual No. of Graft Failures	Change Resulting from Elimination of HLA-B Matching	Change Resulting from Elimination of HLA-B and DR Matching
		no. of failures	
All	1779	+36	+142
White	1057	+21	+85
Black	631	+13	+51
Other	91	+2	+7

* Calculations are based on random matching of donors and recipients with compatible blood types. The rates are for graft failures occurring in 2000 after transplantation between March 6, 1995, and June 30, 2001. The average duration of follow-up was 2.7 years. One- and three-year rates of graft survival for the entire group were 87.2 percent and 77.1 percent, respectively. The relative risk of graft failure was 1.02 (P<0.001) with the elimination of HLA-B matching as a priority and 1.08 (P<0.001) with the elimination of HLA-B and DR matching as a priority, as compared with keeping the current allocation policy.

Higher rates of antigen-level mismatching in non-European recipients

Models eliminating antigen-level matching predicted increased rates of transplantation for non-European ancestry patients
…but quite offset by greater risk of rejection

Enhancing HLA matching for non-European patients

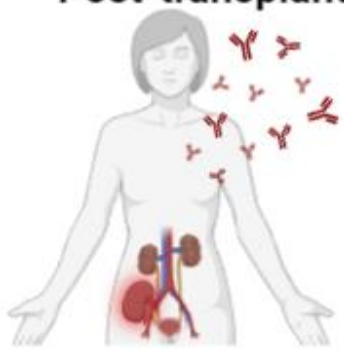
Pre-transplant

- equitable access
- DSA cross-reactivity
- unacceptable antigen

the current allocation system inadvertently matches Black patients to donors with significantly higher immunogenic transplants compared to other races

using race-adjusted immunogenicity thresholds in allocation would result in a net gain of thousands additional kidney life-years for all races [Bekbolsynov *Front Immun* 2022]

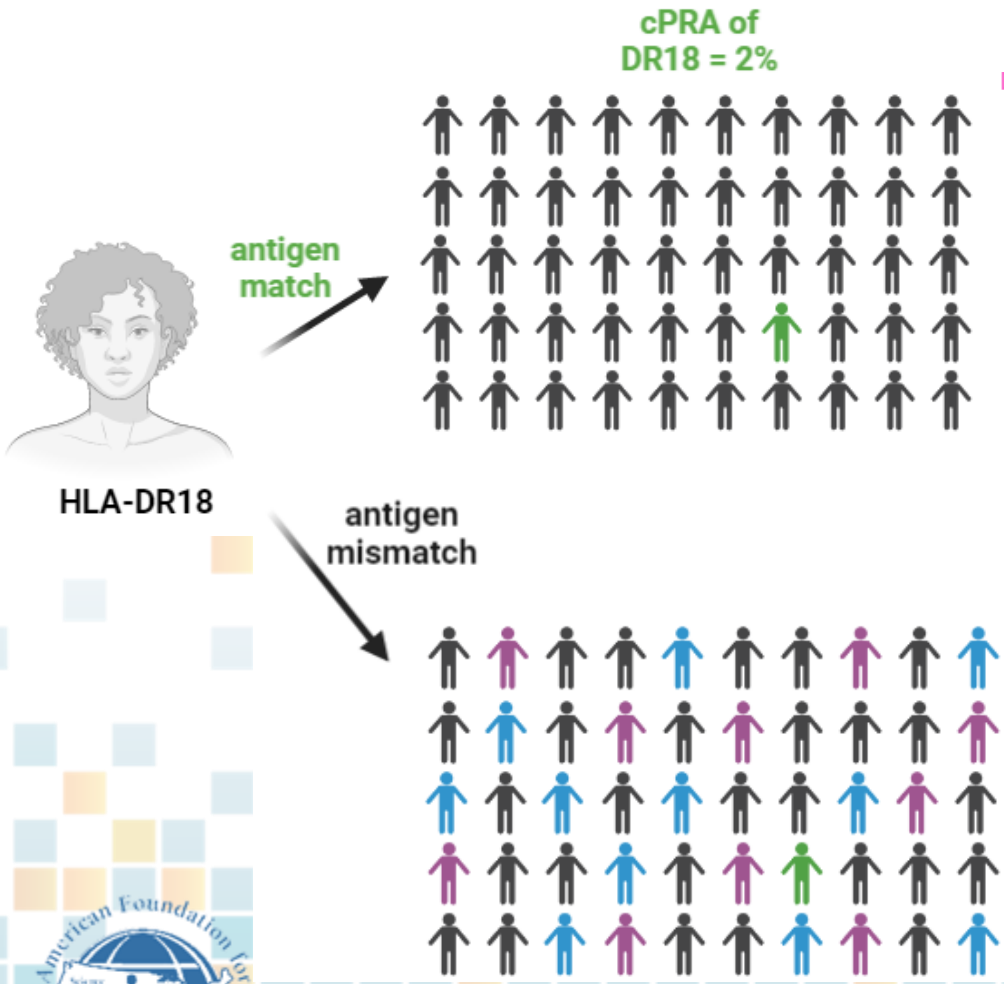
Post-transplant



- rejection risk
- dnDSA risk
- graft longevity

HLA class I eplet load greater than 70 resulted in a greater risk of rejection in the race-mismatched pediatric transplants vs. race-matched [Philogene *Ped Nephrol* 2019]

Selecting a “better matched” mismatched donor




```
                AA Pos.              10         20         30         40         50         60         70         80         90        100
Patient DR18    DRB1*03:02:01:01 GDTRPRFLEY STSECHFFNG TERVRFLERY FHNQEENVRF DSDVGEYRAV TELGRPDAEY WNSQKDLLEQ KRGRVDNYCR HNYGVGESFT VQRRVHPKVT
Donor 1 DR17    DRB1*03:01:01:01 ---------- ---------- -----Y-D-- ---------- ------F--- ---------- ---------- ---------- -----V---- ----------
Donor 2 DR7     DRB1*07:01:01:01 ---Q----WQ GKYK------ ----Q----L -Y----F--- ---------- ------V--S ------I--D R--Q--TV-- ---------- -------E--

                AA Pos.             110        120        130        140        150        160        170        180        190        200
                DRB1*03:02:01:01 VYPSKTQPLQ HHNLLVCSVS GFYPGSIEVR WFRNGQEEKT GVVSTGLIHN GDWTFQTLVM LETVPRSGEV YTCQVEHPSV TSPLTVEWRA RSESAQSKML
                DRB1*03:01:01:01 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
                DRB1*07:01:01:01 ---A------ ---------- ---------- ---------A --------Q- ---------- ---------- ---------- M--------- ----------
```

DR18 vs. DR17 (5 eplets)
26Y, 28D, 47F*, 85VV, 86V

DR18 vs. DR7 (23 eplets)
4Q*, 25Q*, 31FY, 32Y, 32YN, 37F, 37FV, 57V*, 67I, 70D*, 70DR, 71R, 73GQ, 77T*, 98E*, 98ES*, 104A*, 104AK, 140A, 140AV, 149Q, 180VMP, 181M*

By molecular analysis, the patient’s DR18 is more similar to DR17, which is very common across donor populations

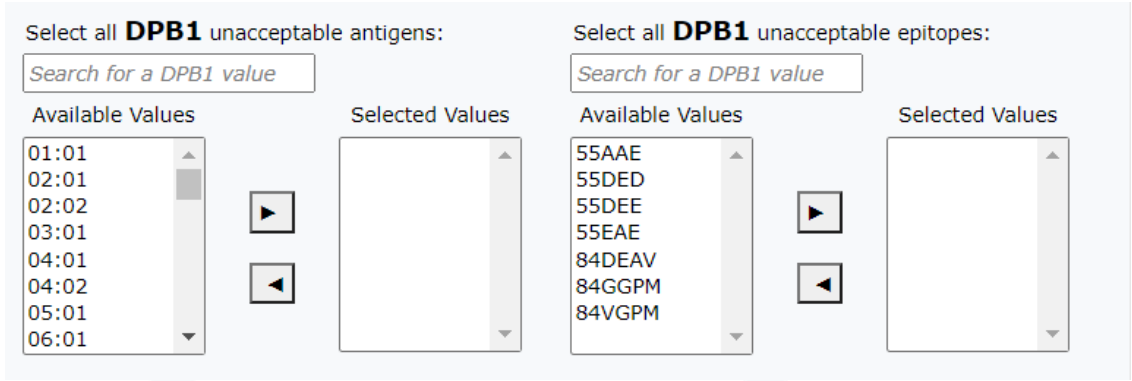
→ Better chance at finding a good “match”
→ Lower risk of dnDSA
→ Better graft survival
→ Lower breadth of sensitization if graft fails

Practical Applications of HLA Eplet Mismatching

14th Annual Living Donation Conference
Presented by the American Foundation for Donation and Transplantation

Epitopes for Antibody Analysis: Unacceptable Antigen Entry and VXM

Serologic group	P 55-57	84-87
DP4	AAE	GGPM
	DEE	GGPM
DP2	DEE(EAE)	GGPM
	DEE	VGPM
	DEE	VGPM
	DED	DEAV
	DED	DEAV
DP3	DED	DEAV
	DED	DEAV
	DED	DEAV
	AAE	VGPM
DP4	AAE	GGPM
	AAE	DEAV
DP1	AAE	DEAV
	EAE	DEAV
DP3	DEE	DEAV
	EAE	DEAV
	AAE	DEAV



Donor: DPB1*105:01

Recipient antibodies: DPB1*02:01, 04:01, 04:02

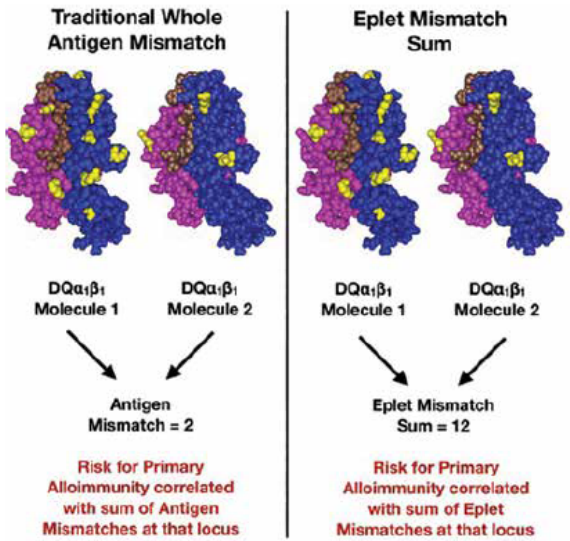
Predict DSA to DPB1*105:01 based on crossreactive epitopes

Eplets for Allocation?

NATIONAL KIDNEY REGISTRY®
FACILITATING LIVING DONOR TRANSPLANTS

Precise Technology to Find You the Best Match

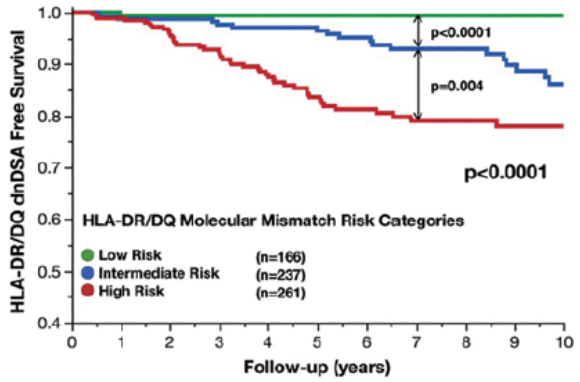
Traditionally, transplant matches were measured by an HLA match score from 0-6 (6 being the best). HLA scores are generally based on A, B and DR antigens. An antigen mismatch is where rejection often starts.



Wiebe C, Kosmoliaptsis V, Pochinco D, et al. HLA-DR/DQ molecular mismatch: A prognostic biomarker for primary

By minimizing eplet mismatches and understanding the match they receive, a recipient can potentially:

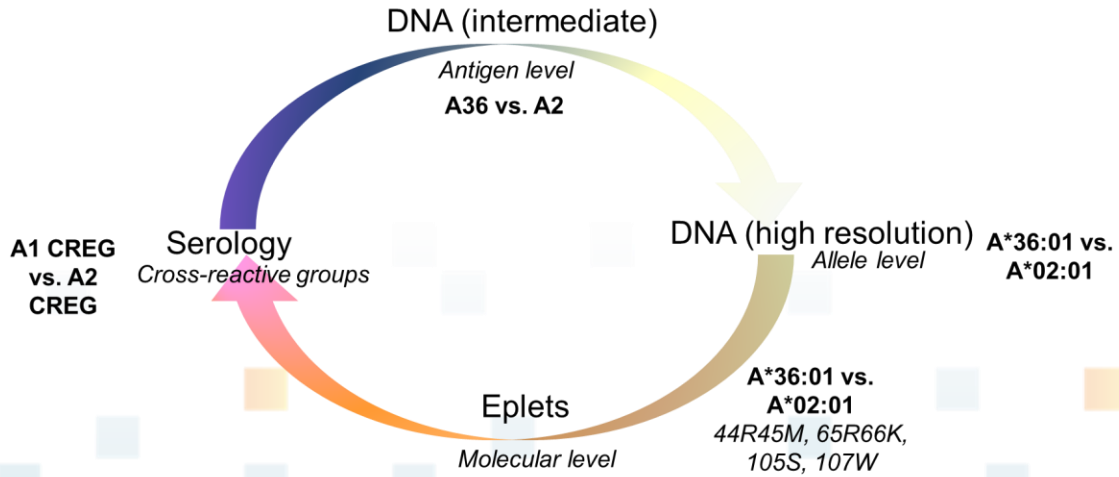
a) reduce the risk of de novo DSA formation
b) lower the probability of rejection
c) lower the probability of graft failure
d) lower their immune-suppression dosage



Wiebe C, Kosmoliaptsis V, Pochinco D, et al. HLA-DR/DQ molecular mismatch: A prognostic biomarker for primary alloimmunity. Am J Transplant. 2019;19:1708–1719.

NKR is currently employing high-resolution genotyping to improve molecular matching and blood typing (for A subtyping), and these efforts may be particularly important for compatible pairs entering paired exchange.

Current Consensus on HLA eplet mismatching



1. Approaches need to be optimized and algorithms standardized and locked before implementation in clinical practice.

2.Thresholds for risk categories need to be established and the impact of other factors on these thresholds need to be accounted for. Formal evaluation, in prospective clinical trials, should be performed.

3.Tools to prospectively determine donor/recipient HLA specific **immunogenicity** beyond the mismatch load (given that DSA can be developed in some patients with low HLA mMM score) should be developed.

4.This is essential before considering evaluation and implementation of immunogenicity analysis as a guide to organ allocation schemes.

Sensitization in transplantation: Assessment of risk (STAR) 2019 Working Group Meeting Report

Takeaways

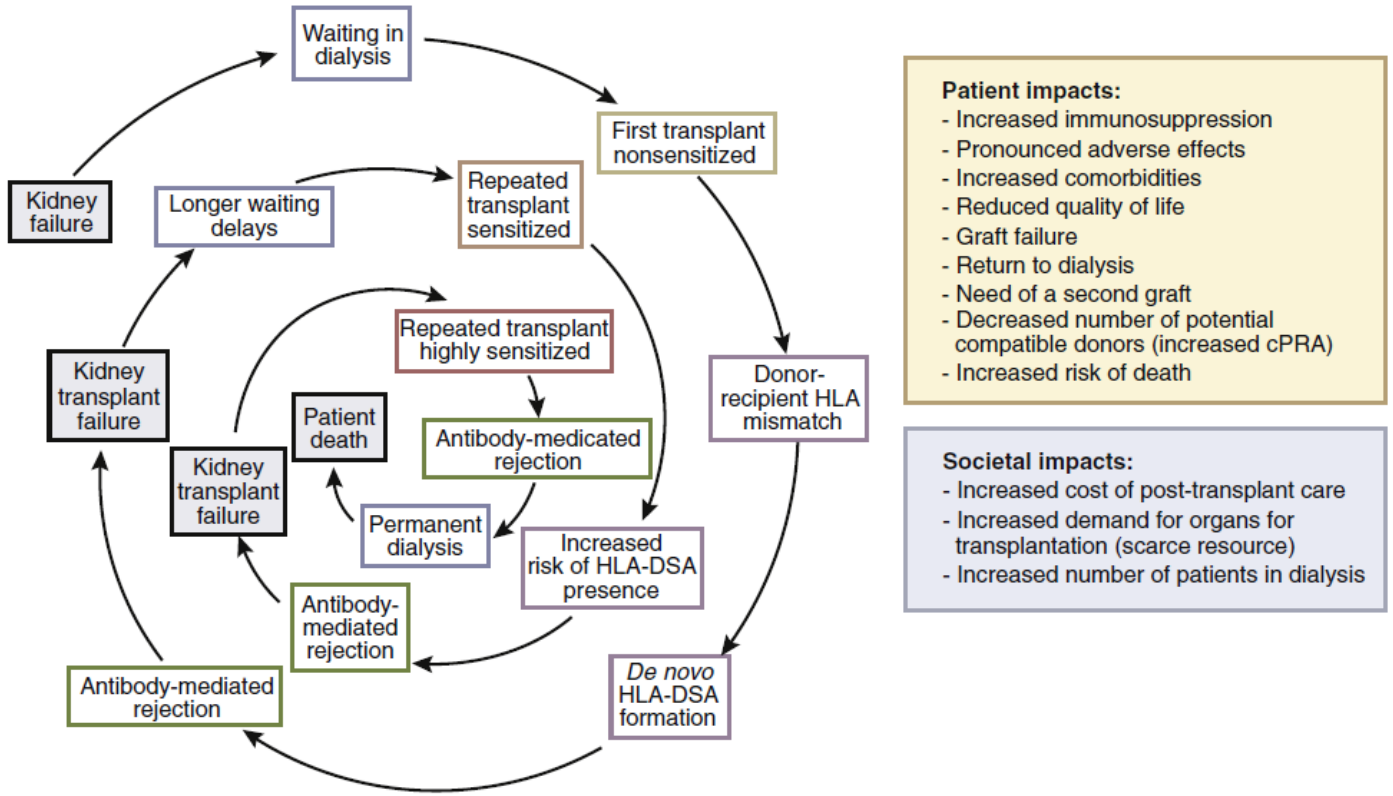
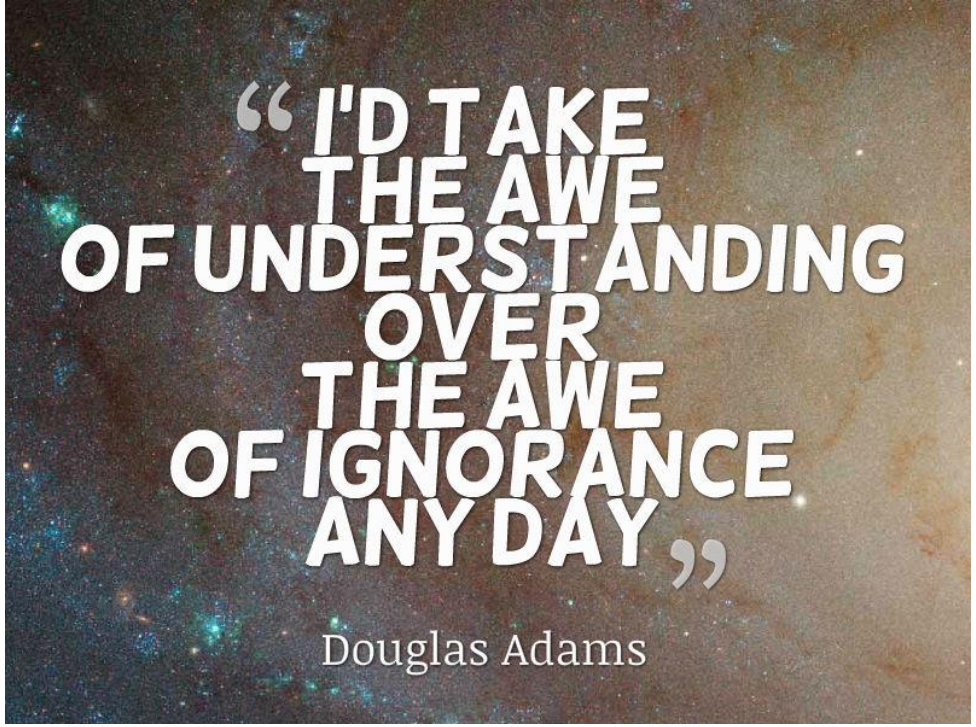


Figure 3 | The vicious cycle of human leukocyte antigen (HLA) mismatched transplantation and antibody-mediated rejection (ABMR). Ignoring HLA mismatching may expedite receiving the first kidney transplantation, but also puts patients at an increased risk of developing *de novo* donor-specific antibody (DSA), episodes of ABMR, adverse effects from intensified immunosuppression, graft failure, return to dialysis, and prolonged wait time as sensitized patients. Consequences on both the patient and society are listed. cPRA, calculation panel-reactive antibody.

Future Applications:

- Individualized immunologic risk assessment in subpopulations to achieve maximum survival benefit
  - Highly sensitized
  - Pediatric/allograft longevity
- Equitable allocation
- Desensitization efficacy
- Optimization of immunosuppression for retransplant candidates



Thank you

Questions?

Extra Information

Excellent Reviews

- https://www.sciencedirect.com/science/article/pii/S0198885921002925

Evidence for Clinical Utility

Application	Correlation	Reference
Predict dnDNA risk	DR or DQ eplet mismatch was significantly correlated with production of dnDSA to that locus (OR 2.50 and 2.00 per 10MM) There was no threshold below which the risk of dnDSA occurrence was absent	Wiebe JASN 2017 Senev JASN 2020
Identify compatible donors for highly sensitized candidates	Reduced transplant wait time by 50% with comparable outcomes as non-sensitized patients	Eurotransplant; Lemieux Int Immuno 2021; Heidt Transpl 2019
Predict graft loss	Graft survival comparable 0-2 triplet mismatch recipient and 0 AB antigen mismatches 1.23-1.41 HR per 10 MM	Eurotransplant Sapir-Pichhadze Kid Int 2020
Predict rejection risk	primarily eplet mismatch load on the DQ molecule (OR 1.06) discriminative performance for graft failure was low	Senev JASN 2020
Risk of rejection after dnDSA formed?	once a patient has developed de novo DSA, eplet mismatches did not correlate with ABMR or death-censored allograft loss	Wen Hum Immun 2021
Breadth of sensitization after graft failure	HLA eplet mismatch burden associated with higher risk of higher cPRA after renal allograft failure	Kosmoliaptsis AJT 2016 Singh Clin Transpl 2016
Risk stratification and/or biomarker in clinical trials	FDA agreed to evaluate DR-DQ eplet mMM score for enrichment or risk stratification in phase 2 and 3 transplant clinical trials and as a prognostic biomarker for de novo DSA, graft rejection, and graft failure.	Wiebe AJT 2019

Session Survey

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April 20th 8:45 AM-9:30 AM