Desensitization Protocols: Expanding Access to Transplantation

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Objectives

- Discuss the biology of HLA
- Describe the impact of HLA antibodies on the availability of suitable donors
- Delineate those patients whom would benefit most from desensitization
- Outline the modalities employed in desensitization, including resources that may be accessed
- Provide examples of desensitization protocols we've utilized and outcomes

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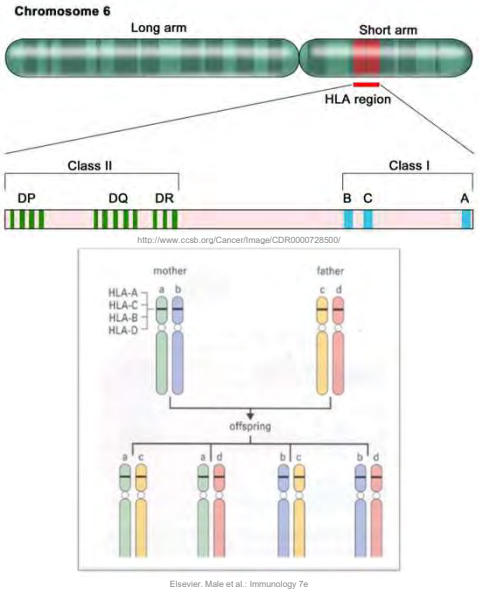
HLA Genes/Antigens

- HLA (human leukocyte antigen) **genes** are located within the major histocompatibility gene **complex** on chromosome 6
- 224 gene loci produce 128 protein products (predicted)
- **2 classes** (I & II) based upon biology
  - Class I: HLA-A, HLA-B, HLA-C
  - Class II: HLA-DR, HLA-DQ, HLA-DP
- **2 copies** of each gene locus (one from Mom, one from Dad) inherited *en bloc* [**haplotype**]
- **Highly polymorphic — Lots of variability within population**



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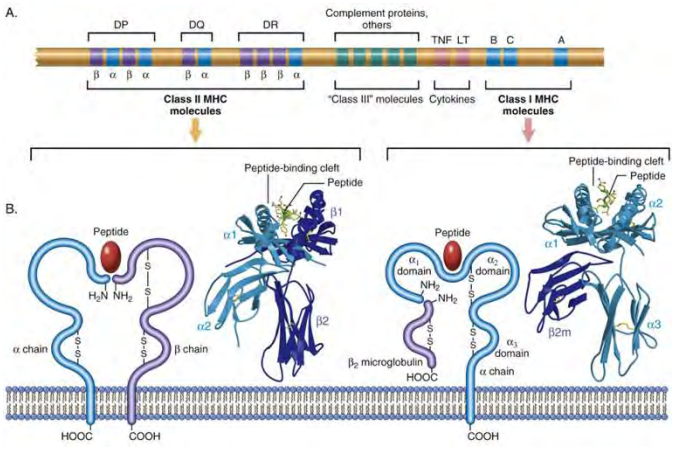
HLA Proteins

- HLA proteins present peptides as part of the **immune response**
- **Co-dominant** expression (contribution from both Mom and Dad)
- **Class I** present **ENDOgenous** peptides (nucleated cells express)
  - Main job is to present viral peptides to mark cell for T-cell mediated kill
- **Class II** present **EXOgenous** peptides (antigen presenting cells express)
  - Main job is to sample peptides in fluid around tissue looking for foreign molecules
  - Leads to production of antibodies…eventually
- **Antigen presenting area is polymorphic, hence differs between individuals, can be recognized as foreign, and is why we care about these proteins in transplant**



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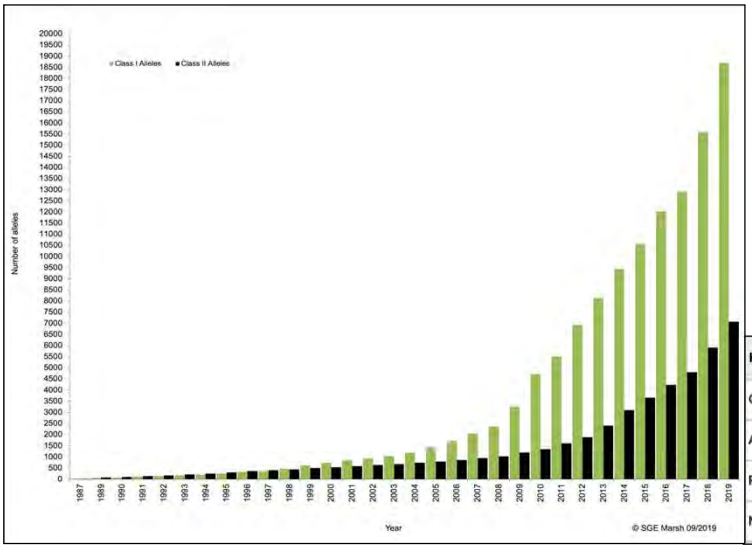
HLA Antigen Diversity



HLA Class I			
Gene	*A*	*B*	*C*
Alleles	5,735	7,053	5,653
Proteins	3,629	4,572	3,447
Nulls	300	241	240

HLA Class II									
Gene	*DRA*	*DRB*	*DQA1*	*DQA2*	*DQB1*	*DPA1*	*DPA2*	*DPB1*	*DPB2*
Alleles	29	3,296	216	17	1,771	161	5	1,519	6
Proteins	2	3,158	90	9	1,179	62	0	993	0
Nulls	0	138	6	0	77	2	0	78	0

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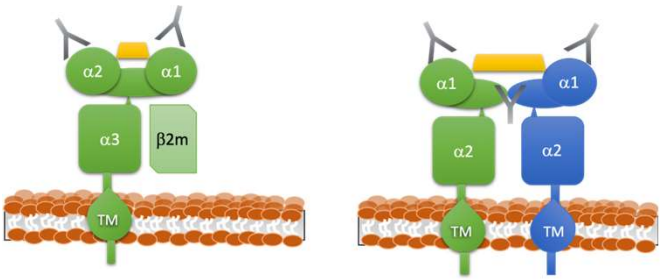
HLA Antibody Production & Rejection

- Rejection is mediated by immunologic reactions, where the recipient's immune system recognizes the donor tissue as foreign
  - Damage to end organs can occur via T-cells (**cellular** rejection) or by binding of **antibodies**
- Antibodies/reactive T-cells form against HLA antigens from two major routes of exposure:
  - **Transfusions** and **Pregnancy**



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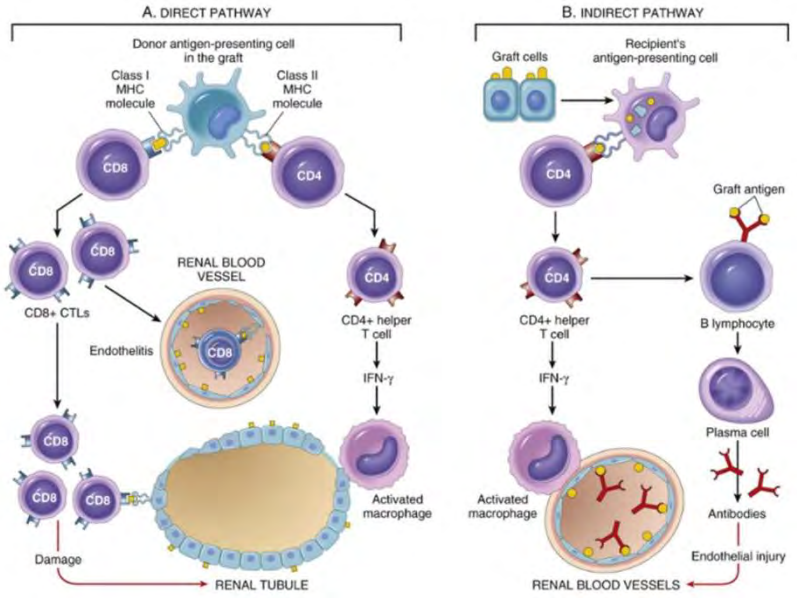
Pathways of Alloantigen Recognition



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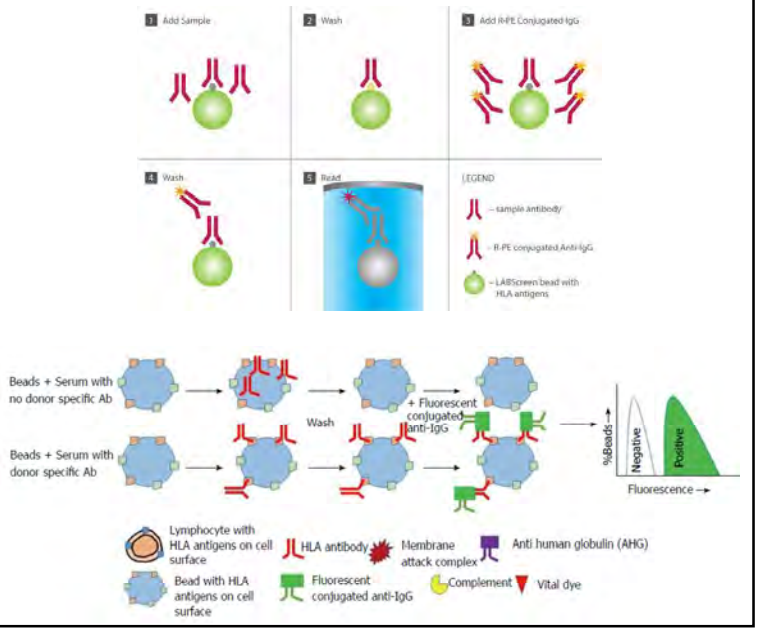
Antibody Testing

- Detection of antibodies is carried out using a flow-cytometry based method in our lab
- Beads are coated with libraries of antigens, incubated with recipient serum, and then secondary detection agents are used to determine whether antibodies have bound
  - Screen with multiple antigens
  - Single antigen with only one antigen type on each bead
- The secondary signal is read and quantified (MFI: mean fluorescence intensity)
- The higher the MFI, the more of an antibody present



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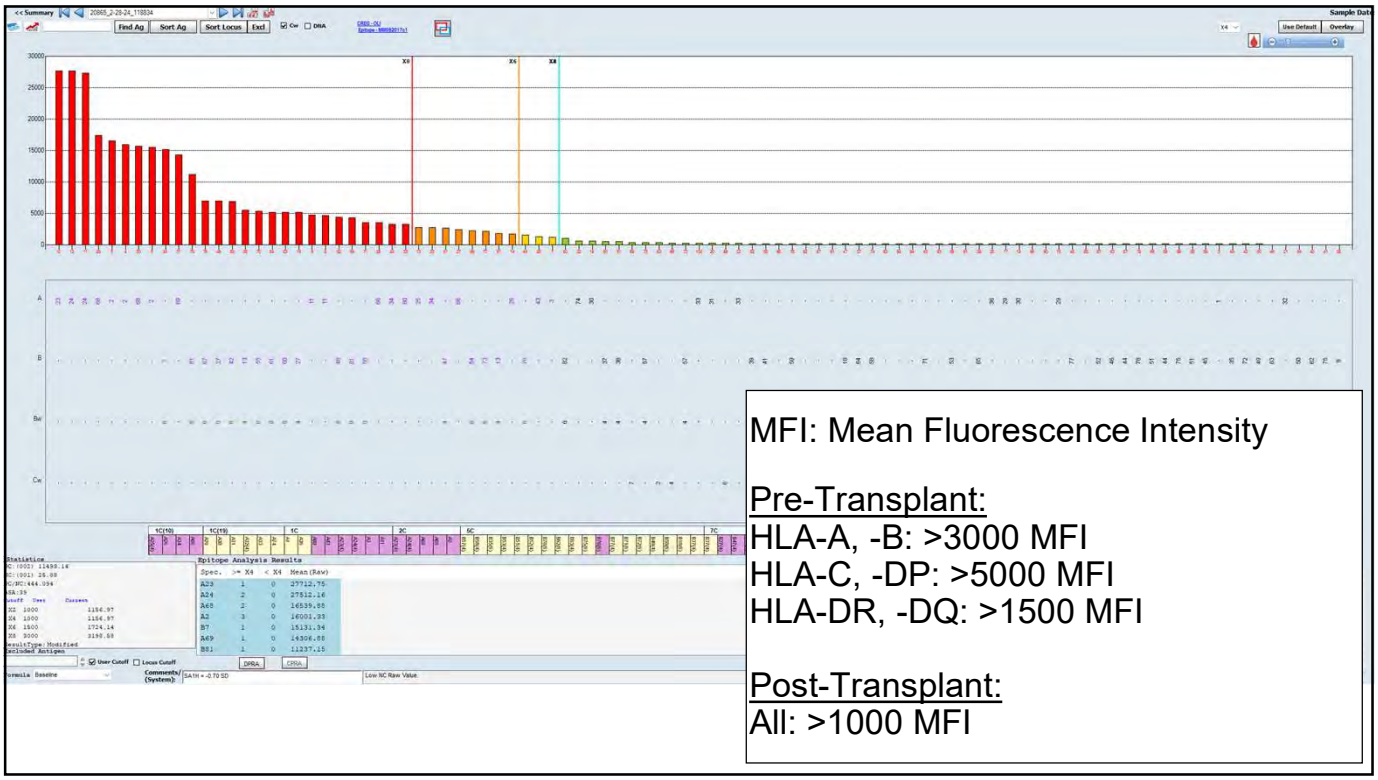

MFI: Mean Fluorescence Intensity

<u>Pre-Transplant:</u>
HLA-A, -B: >3000 MFI
HLA-C, -DP: >5000 MFI
HLA-DR, -DQ: >1500 MFI

<u>Post-Transplant:</u>
All: >1000 MFI

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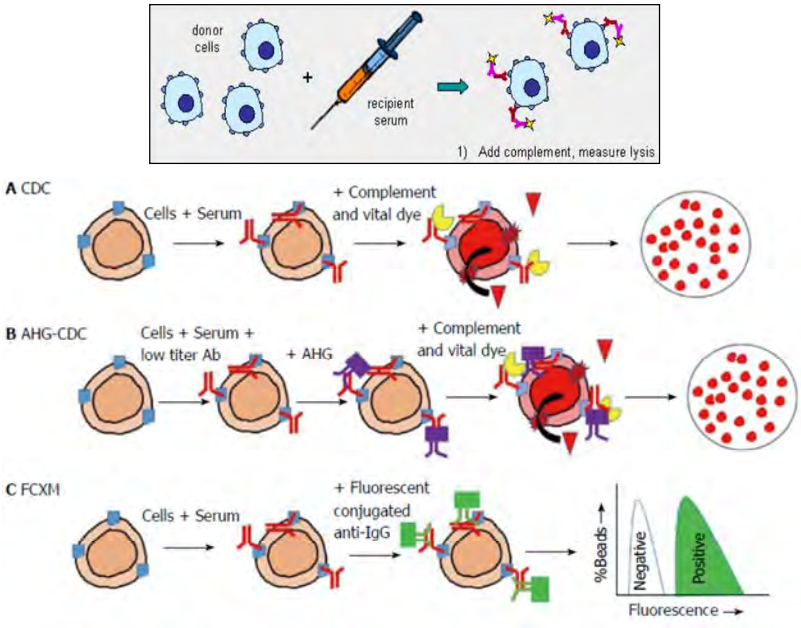
MFI and Clinical / Laboratory Outcomes

- Positivity thresholds are set to roughly correspond with outcome on a physical crossmatch
- Hyperacute rejection: likely at levels >10K
- AHG-CDC likely positive >6000 MFI
- FCXM likely positive >3000 MFI



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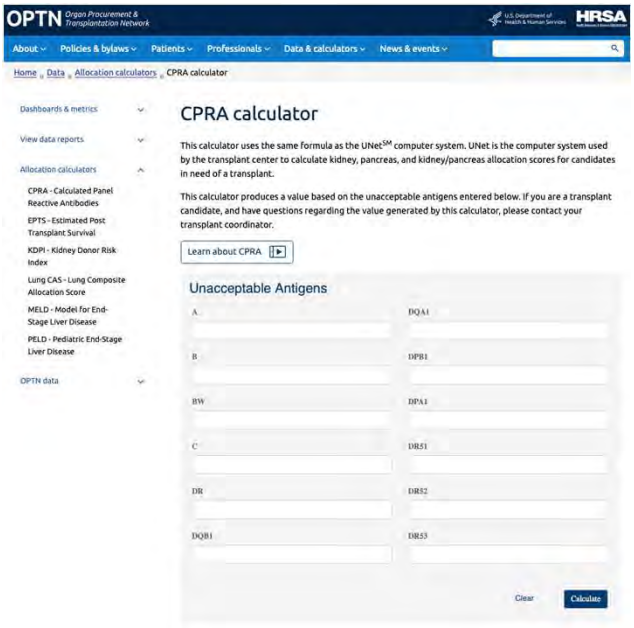
cPRA (Calculated Panel Reactive Antibodies)

- A percentage that reflects the proportion of the population to which one has antibodies
  - Higher the number, more difficult it is to find a donor
- Potential representation of degree of sensitization
- Based upon incidence of HLA antigens within a given, general population
  - HLA-A2 alone: 47%
  - HLA-DR53 alone: 50%



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Who Would Benefit Most?

- Algorithms in organ matching try to prioritize those patients with high cPRAs
- This effect has its limits though, especially with regard to patients with cPRAs >99.9%
- Desensitization looks to decrease cPRA below levels where transplant rate begins to increase

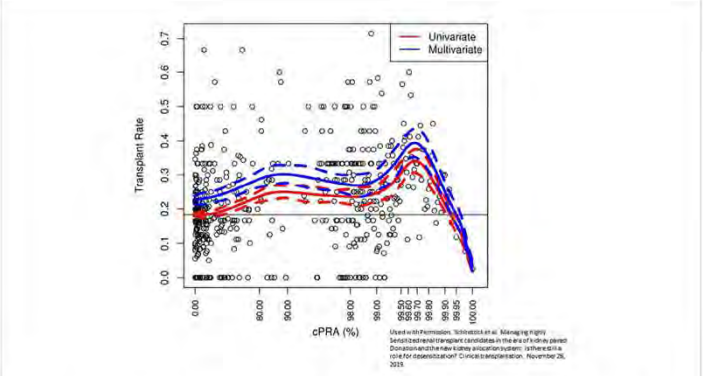

FIGURE 1 | Reduced transplantation rate among patients with cPRA > 99.9%. Multivariate fit of transplantation rate versus calculated panel reactive antibody (CPRA). A fit of transplant rate versus cPRA using a restricted cubic spline with 95% confidence interval controlling for time on the waitlist, age, gender, blood type, waitlist region, and ethnicity. The red line is univariate, while the blue line indicates the multivariate fit corresponding to a candidate with male gender, blood type A, waitlist region 5, Caucasian ethnicity, and waiting time of 2.5 y. Markers represent the observed transplant rate within each window of CPRA of width 0.01%. This prevalent cohort was active as of June 1, 2016, and followed for change in status through June 1, 2017. Used with permission. Schinstock et al. Managing highly sensitized renal transplant candidates in the era of kidney paired donation and the new kidney allocation system: is there still a role for desensitization? Clinical transplantation. November 26, 2019 (19).

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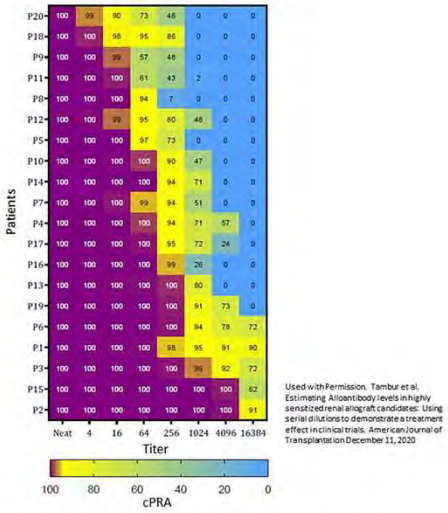
How High is High?

- Antibody titers may help determine which patients may show an appreciable decrease in antibody levels with desensitization
- Patients with broad, though 'low-level' antibody burden may benefit from removal of circulating antibodies through PLEX (will discuss in a couple slides)
- Antibodies that reduce to 'negative' levels at 1:16 may respond best to PLEX



Used with Permission. Tambur et al. Estimating Alloantibody levels in highly sensitized renal allograft candidates: Using serial dilutions to demonstrate a treatment effect in clinical trials. American Journal of Transplantation December 11, 2020

FIGURE 2 | Using titer to stratify patients with cPRA > 99.9%. This stratification was based on the first replicate from the baseline sample. The heat map shows the cPRA obtained from positive antibody specificities per titer for each of the 20 patients. The patients were ordered to show patients who would be the most likely to respond to desensitization (top) to least likely to respond to desensitization (bottom). For example, P2 continued to have a 100% cPRA when serum was diluted 1:4096, and thus it may be extremely difficult to remove enough antibodies to render this patient transplantable. Used with Permission. Tambur et al. Estimating Alloantibody levels in highly sensitized renal allograft candidates: Using serial dilutions to demonstrate a treatment effect in clinical trials. American Journal of Transplantation December 11, 2020 (55).

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How to Reduce Antibody Burden

- Plasma exchange
  - Physically remove circulating anti-HLA antibodies
- IVIG
  - Incorporated for its immunomodulatory effects
  - Prevent hypogammaglobulinemia
- Pharmacologic Interventions
  - Anti-CD20 (e.g. Rituximab)
  - Proteosome Inhibitors (e.g. Bortezomib - may reduce plasma cell populations; tolerance is a challenge)
  - Anti-CD38 (e.g. Daratumumab - may reduce DSA but shown to lead to rebound ABMR and T-cell mediated rejection)
  - IL-6 Blockade (e.g. Tocilizumab - may inhibit effectiveness of plasma cells reducing MFI)
  - Cysteine Proteases (cleave circulating antibodies, leads to rapid rebound on cessation)
  - Complement Inhibition (e.g. Eculizumab - may minimize immediate effects of DSA on allograft [early active ABMR])

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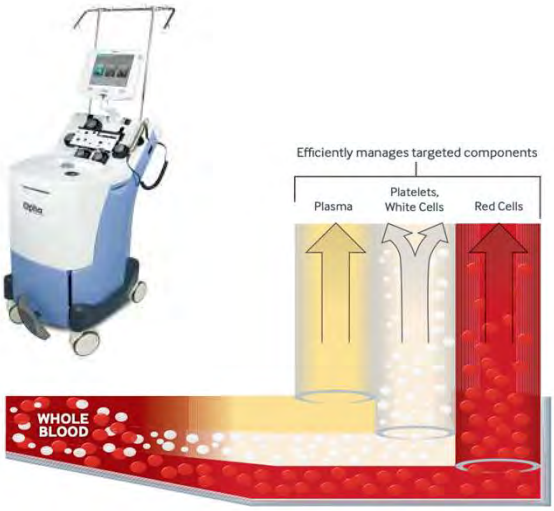
How to Lower Antibody Burden via PLEX

- ASFA Guidelines outline the effectiveness of PLEX in various medical conditions and diagnoses
- 1 to 1.5 plasma volumes is exchanged with albumin and/or fresh frozen plasma
  - Use of plasma dependent upon coagulation factor status as coagulation factors are removed along with antibodies in plasma
- Timing is typically every other day
- PLEX removes proportion of antibody in **circulation**
  - **Antibody levels are reduced by ~65% with each procedure**
  - Pause between exchanges allows for **extravascular antibody to redistribute into circulation**
- Administration of anti B-cell/plasma cell therapies (e.g. rituximab) , along with IVIG **(after PLEX)** works to suppress replenishment of removed antibodies



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Guidelines on the Use of Therapeutic Apheresis in Clinical Practice – Evidence-Based Approach from the Writing Committee of the American Society for Apheresis: The Ninth Special Issue

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Transplantation, heart	Cellular rejection	ECP	II	1B	249
	Recurrent rejection	ECP	II	1B	
	Rejection prophylaxis	ECP	II	2A	
	Desensitization	TPE	II	1C	
	Rejection prophylaxis[a]	TPE	II	1C	
	Antibody mediated rejection	TPE	III	2C	
Transplantation, hemapoietic stem cell, ABO incompatible	Major ABO incompatible, HPC(M)	TPE	II	1B	251
	Major ABO incompatible, HPC(A)	TPE	II	2B	
	Minor ABO incompatible, HPC(A)	RBC exchange	III	2C	
	Pure red cell aplasia	TPE	III	2C	
Transplantation, hematopoietic stem cell, HLA desensitization		TPE	III	2C	253
Transplantation, intestine[a]	Antibody mediated rejection	TPE	III	2C	255
	Desensitization	TPE	III	2C	
Transplantation, kidney, ABO compatible	Antibody-mediated rejection	TPE/IA	I	1B	257
	Desensitization/prophylaxis, living donor	TPE/IA	I	1B	
Transplantation, kidney, ABO incompatible	Desensitization, living donor	TPE/IA	I	1B	259
	Antibody mediated rejection	TPE/IA	II	1B	
Transplantation, liver	Desensitization, ABOi, living donor	TPE	I	1C	261
	Desensitization, ABOi, deceased donor	TPE	III	2C	
	Antibody mediated rejection	TPE	III	2C	
	Antibody mediated rejection	ECP	III	2B	
	Immune suppression withdrawal	ECP	III	2B	
	Desensitization, ABOi	ECP	III	2C	
Transplantation, lung	Chronic lung allograft dysfunction[a]	ECP	II	1C	263
	Bronchiolitis obliterans syndrome	ECP	II	1C	
	Antibody mediated rejection	TPE	III	2C	
	Desensitization	TPE	III	2C	

TABLE 2 Category definitions for therapeutic apheresis

Category	Description
I	Disorders for which apheresis is accepted as first-line therapy, either as a primary standalone treatment or in conjunction with other modes of treatment.
II	Disorders for which apheresis is accepted as second-line therapy, either as a standalone treatment or in conjunction with other modes of treatment.
III	Optimum role of apheresis therapy is not established. Decision-making should be individualized.
IV	Disorders in which published evidence demonstrates or suggests apheresis to be ineffective or harmful. IRB/Ethics Committee approval is desirable if apheresis treatment is undertaken in these circumstances.

Abbreviation: IRB, Institutional Review Board.

TABLE 3 Grading recommendations, strength and quality of evidence

Recommendation	Description	Methodological quality of supporting evidence	Implications
Grade 1A	Strong recommendation, high-quality evidence	RCTs without important limitations or overwhelming evidence from observational studies	Strong recommendation, can apply to most patients in most circumstances without reservation
Grade 1B	Strong recommendation, moderate quality evidence	RCTs with important limitations (inconsistent results, methodological flaws, indirect, or imprecise) or exceptionally strong evidence from observational studies	Strong recommendation, can apply to most patients in most circumstances without reservation
Grade 1C	Strong recommendation, low-quality or very low-quality evidence	Observational studies or case series	Strong recommendation but may change when higher-quality evidence becomes available
Grade 2A	Weak recommendation, high-quality evidence	RCTs without important limitations or overwhelming evidence from observational studies	Weak recommendation, best action may differ depending on circumstances or patients' or societal values
Grade 2B	Weak recommendation, moderate-quality evidence	RCTs with important limitations (inconsistent results, methodological flaws, indirect, or imprecise) or exceptionally strong evidence from observational studies	Weak recommendation, best action may differ depending on circumstances or patients' or societal values
Grade 2C	Weak recommendation, low-quality or very low-quality evidence	Observational studies or case series	Very weak recommendations; other alternatives may be equally reasonable

Abbreviation: RCT, randomized controlled trial.
Source: Adopted from References 1 and 2.

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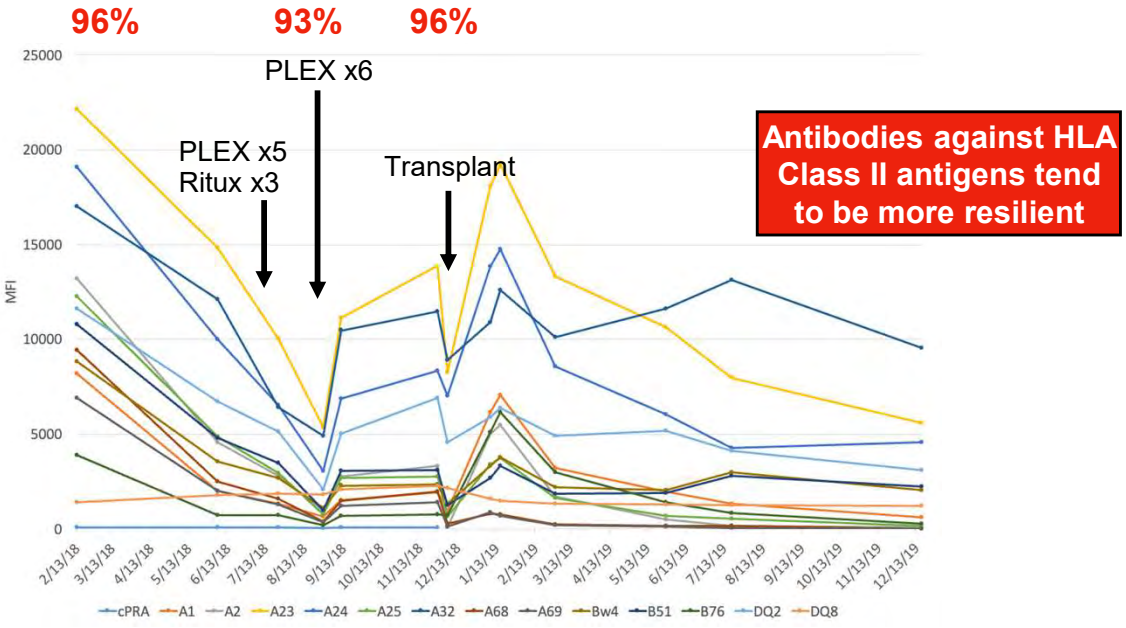
Examples of Impact on Antibody Levels



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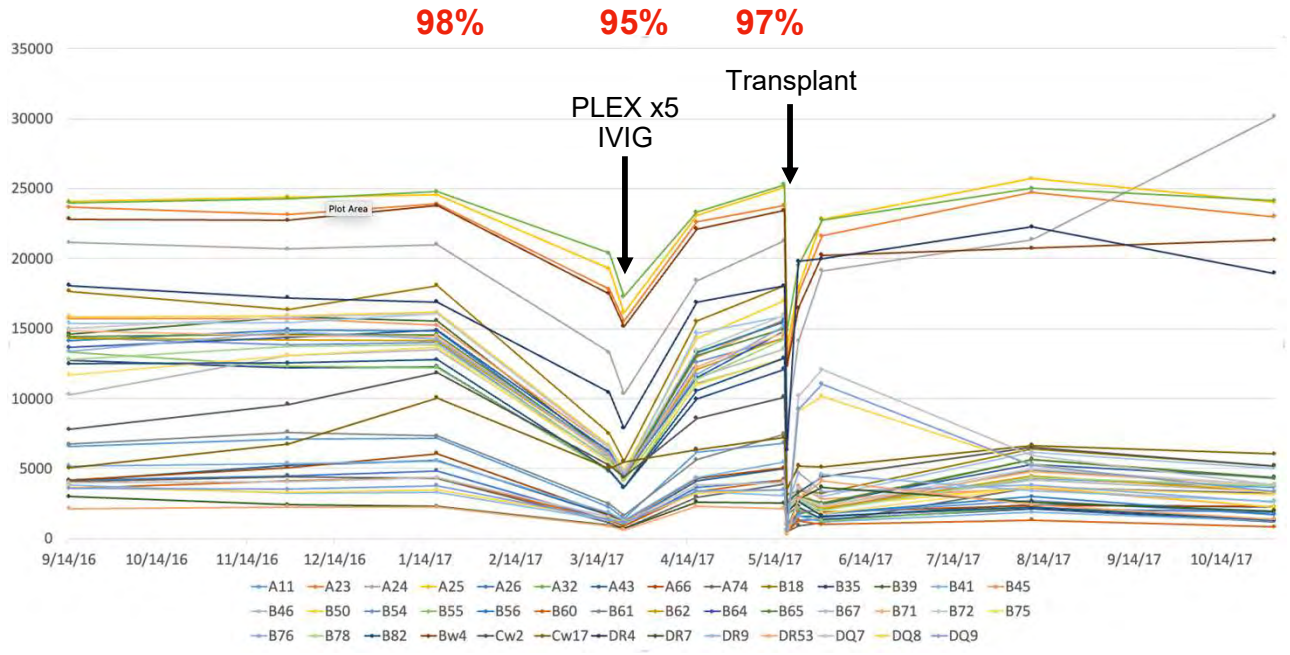

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Conclusions

- The polymorphic nature of HLA antigens makes them prime drivers of DSA development
- Levels seen in *in vitro* assays have clinical corollaries with regard to rejection
- cPRA may provide a broad reflection regarding the degree of sensitization
- Desensitization seeks to reduce antibody levels increase the chance of transplantation (goal should be to drop cPRA levels below 99%)
  - Rebound is seen in antibody levels following desensitization interventions
  - Should be paired with an increase in recipient status level to enable transplantation within reduced antibody window
- ASFA guidelines may help to establish appropriate protocols for desensitization and for post-transplant DSA management

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Thank you!

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