



Brigham and Women's Hospital

Founding Member, Mass General Brigham

Immunological Assessment Pre and Post Transplant

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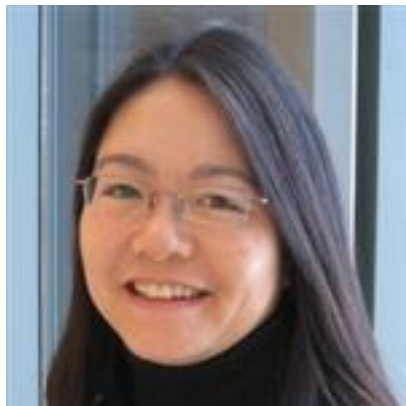
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Clinical focus: kidney transplant, HLA/histocompatibility

Research focus: regulatory B cells, alloantibody

Disclosures

- Consultant, One Lambda Inc.; Alexion Pharmaceuticals Inc.
- Author and Reviewer, UpToDate Inc.
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Objectives

- Review the basics of HLA and histocompatibility
- Discuss the role of histocompatibility testing in determining immunological compatibility between a donor and recipient pair in kidney transplantation

Why do we reject a transplanted organ?

Recognize it as being “foreign”

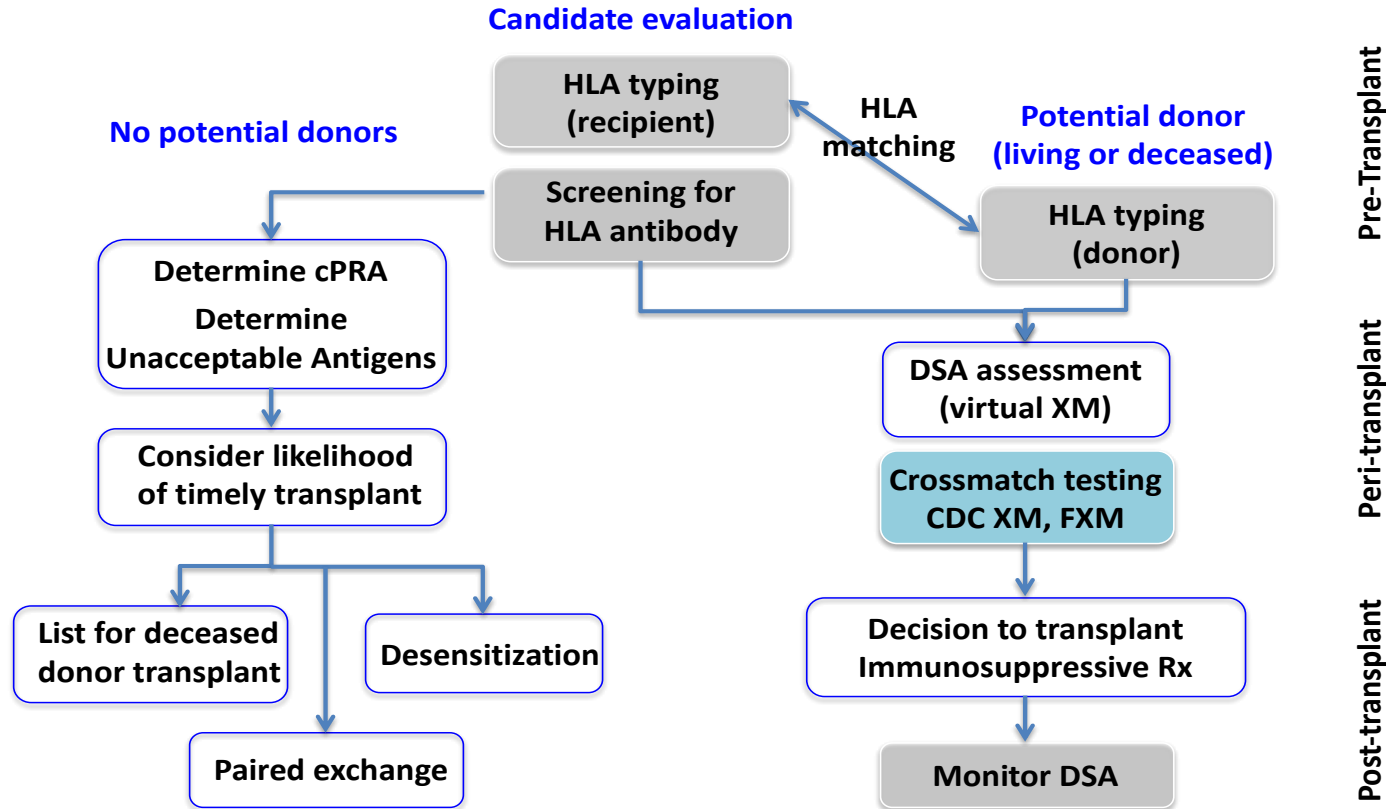
- Any protein that looks different from our own can elicit an immune response
- HLA molecules –very highly polymorphic (>17,000 distinct HLA proteins!)
- ABO blood group antigens
- Other proteins are much more highly conserved

Cellular Rejection

**Antibody-mediated
Rejection**



Role of Histocompatibility Testing



Case Examples: Histocompatibility Considerations

Patient A

35 year-old with ESRD on HD x5 years awaiting
DD kidney transplant. cPRA 32%

Patient B

40 year-old with ESRD on HD x6 years awaiting
DD kidney transplant. cPRA 98.2%

Current clinical practice:

list Unacceptable Antigens (UA) class 1 MFI >3000, class 2 MFI >3000

Local offers: T-CDC XM, FXM

T-CDC: if positive, absolute C/I

FXM: if positive, relative C/I (depends on strength, presence of DSA on SAB screen, donor characteristics, likelihood of another timely offer)

What immunological risks are acceptable for each patient?

HLA Expression

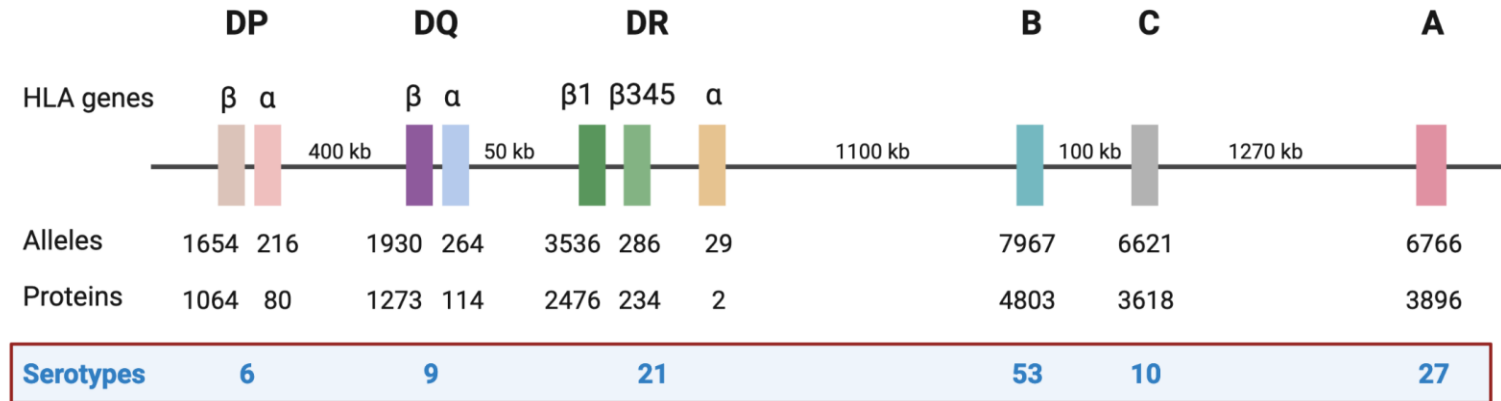
Class I HLA antigen (A, B, C)

- expressed on all nucleated cells

Class II HLA antigen (DP, DQ, DR)

- expressed on antigen presenting cells (DCs, B cells), upregulated on endothelial & epithelial cells in inflammatory conditions
- Co-dominant expression
- Expression levels can modulate immune responses
 - HLA-C and HLA-DP cell surface expression levels are less than other loci

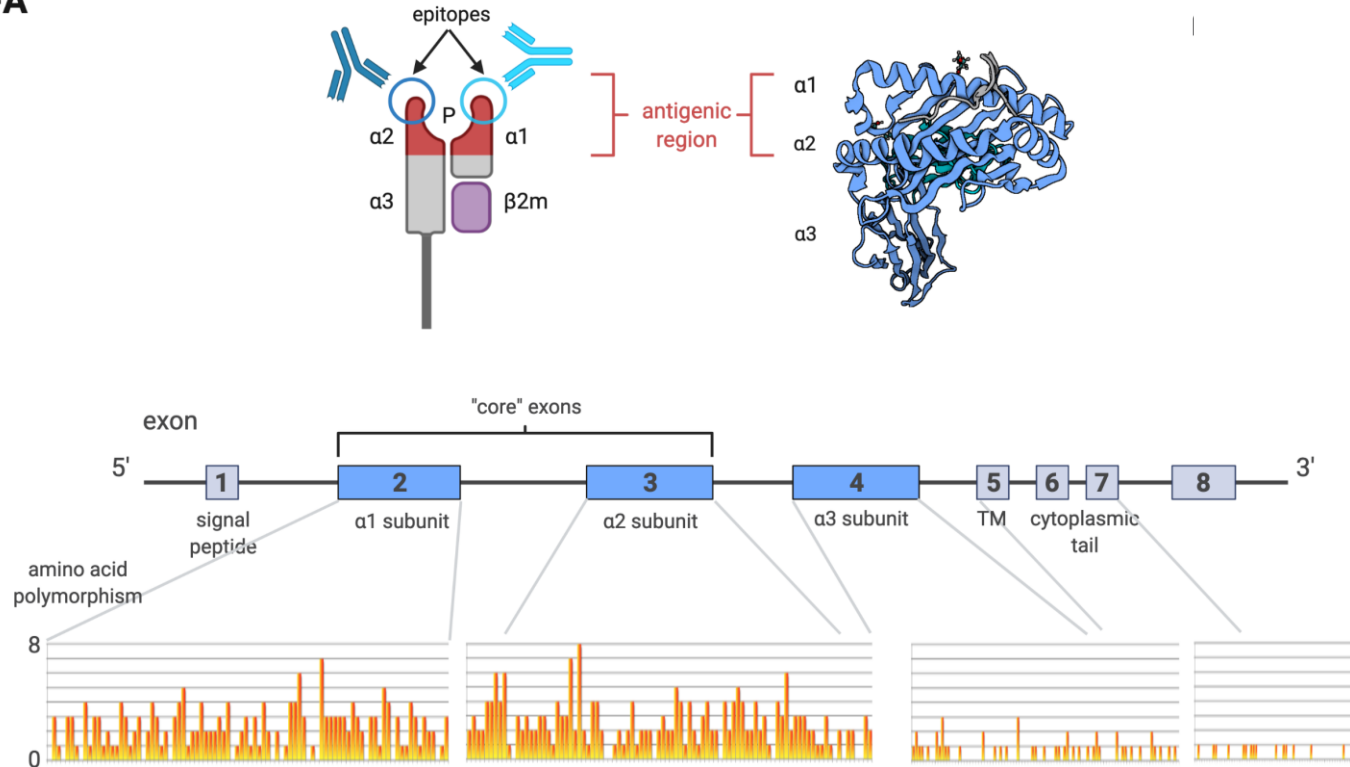
HLA Polymorphism: Over 17,000 distinct HLA proteins!



>30,000 distinct HLA alleles
>18,000 distinct HLA proteins

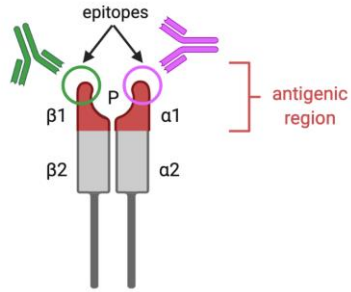
Class I HLA polymorphism

HLA-A

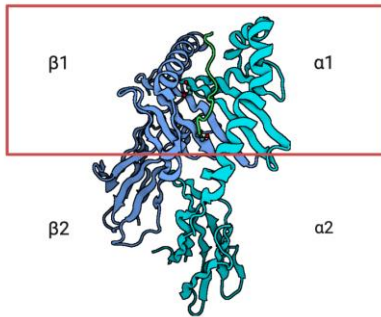


Class II HLA polymorphism

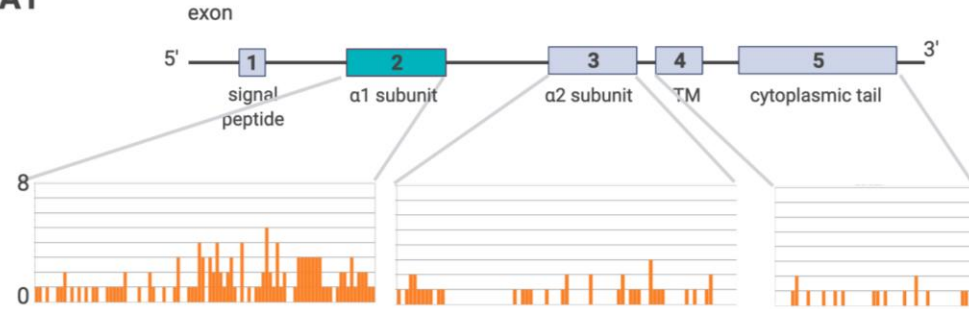
HLA-DQ



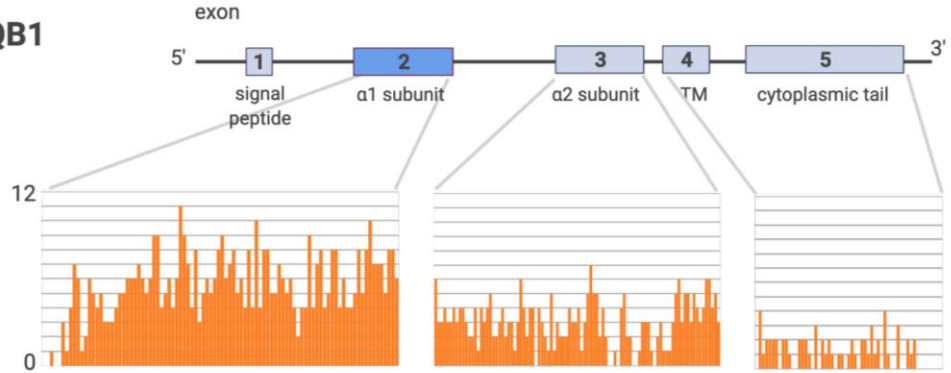
antigenic region



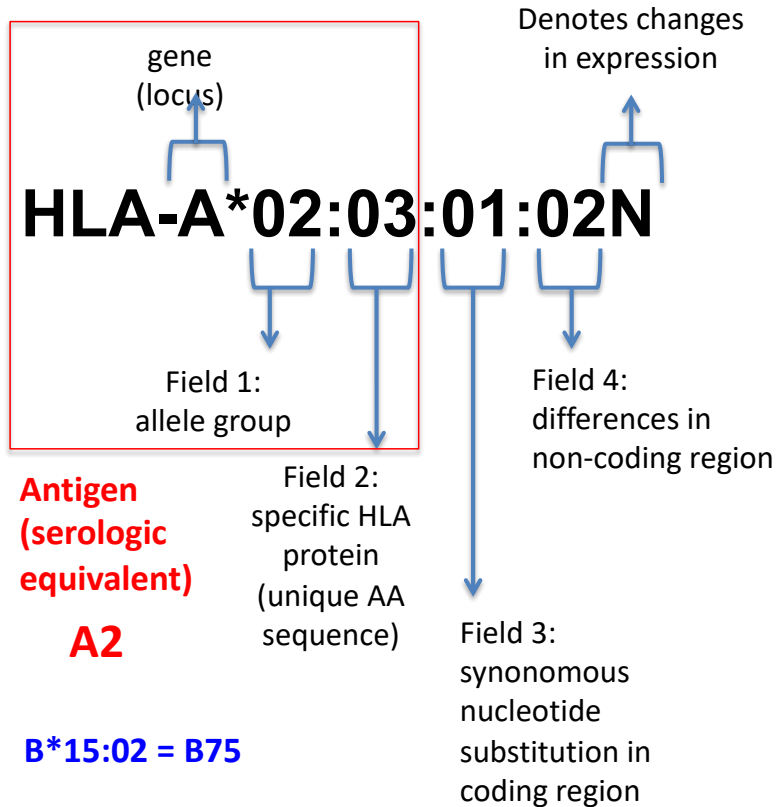
DQA1



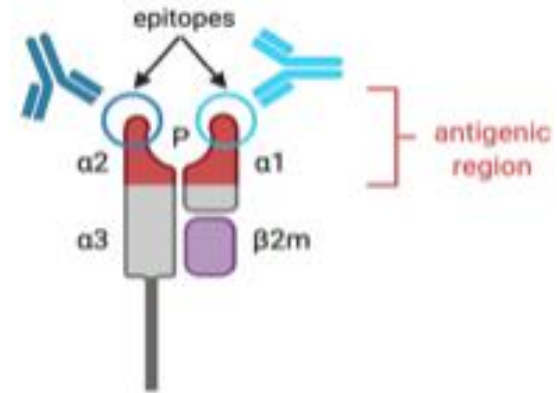
DQB1



HLA Nomenclature



- 4000 unique HLA-A proteins identified
- Named differently at the 2nd field
 - Grouped into serologic Ag if Ab reactivity against them are the same



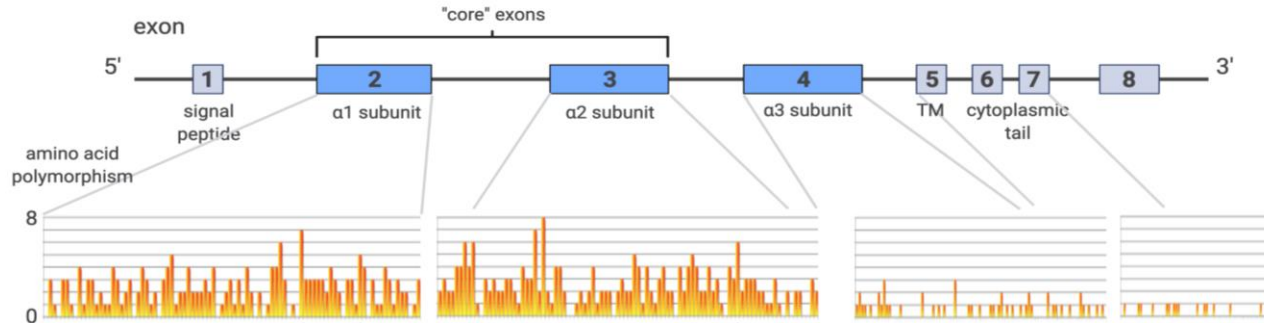
HLA Typing by Molecular Methods

Low/intermed-resolution typing (RT-PCR)

- Focuses on regions that distinguish amongst the epitopes (“core exons”)
- Resolves differences to the antigen/serologic level
- Deceased donors

High-resolution typing (NGS)

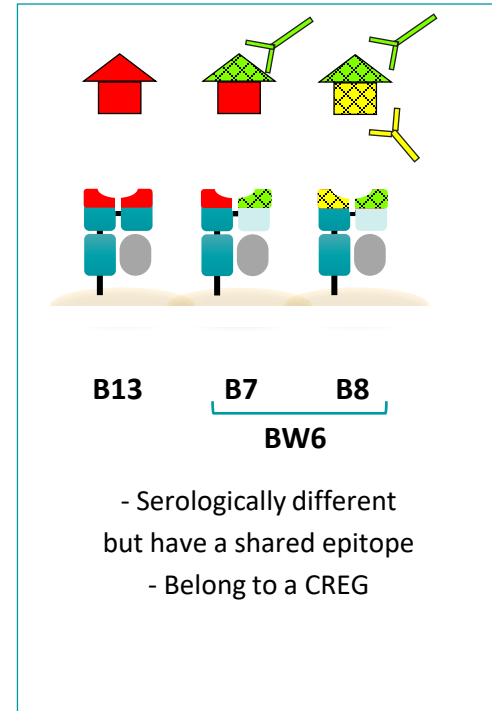
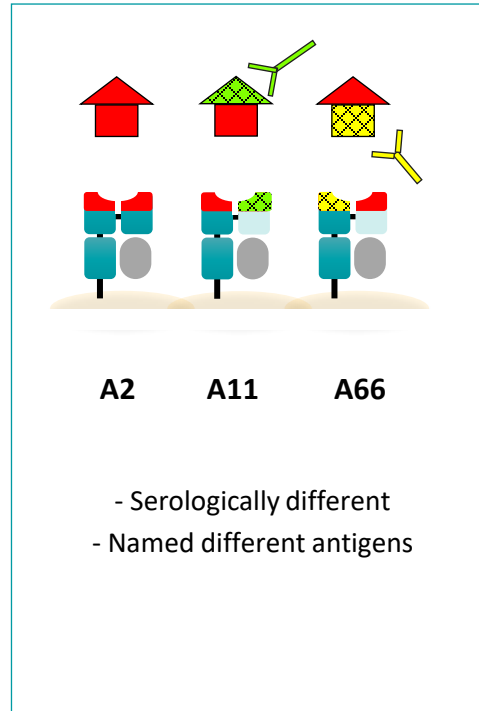
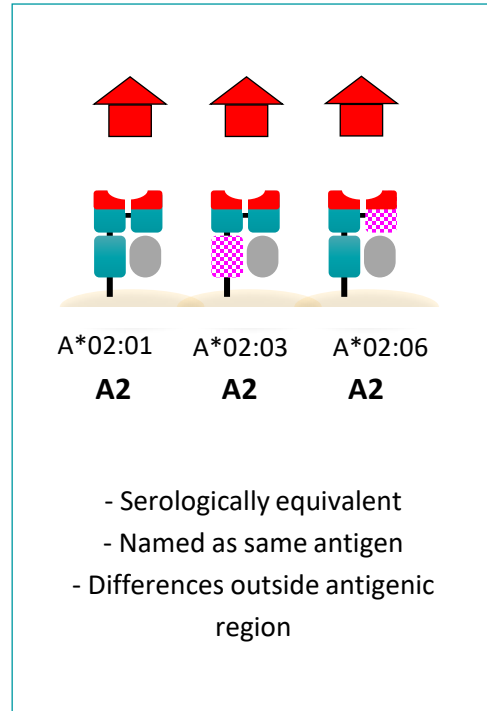
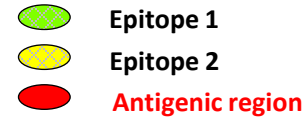
- Can sequence entire HLA gene
- Resolves differences to the nucleotide level
- Living donors, recipients



Heatmap of amino acid variability according to AA position

Serological Antigen Equivalents

What does the immune system “see” ?



HLA matching

- Each individual inherits one **haplotype** (set of HLA genes) from each parent
- **Mismatch**: an HLA antigen found on the cells of the donor (allograft), but not in the recipient
- **6 antigen match (zero mismatch)**: share same HLA-A, HLA-B and HLA-DR antigens, regardless of haplotype
- the greater the disparity between the donor and recipient, the more “foreign” the allograft appears = higher likelihood of developing an immune response
- Why not wait for a “perfect” 12/12 match?
 - Need to consider likelihood of finding such a match
 - impact on ethnic minorities with rarer antigens
 - Points and national sharing are given for zero antigen MM (A/B/DR)

Detection of pre-formed HLA antibodies

Goal:

- To determine the presence of any pre-formed donor-specific HLA antibody (DSA)
- Define its specificity to particular HLA antigen(s)
- Determine their relative amount/strength

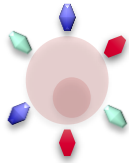
Current methodologies:

- Complement-dependent cytotoxicity crossmatch (CDC XM)
- Flow crossmatch (FXM)
- Solid phase immunoassays (virtual XM)
 - Pooled/phenotype beads
 - Luminex single antigen bead (SAB) assays

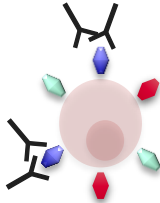
Crossmatch Testing

Complement-dependent cytotoxic crossmatch (CDC XM)

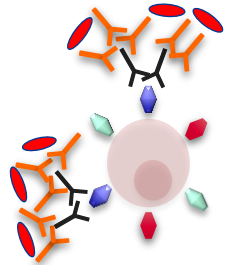
Donor T/B cell



Candidate sera



Detection reagents



(AHG) + complement

Readout

IgM/IgG DSA



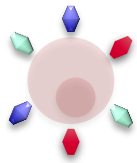
Cell lysis

- Strong predictor of hyperacute rejection
- Detects both IgM and IgG anti-HLA antibodies
- T-CDC: class I HLA antibodies
- B-CDC: class I and II HLA Ab
- Also detects Ab directed against other non-HLA antigens present on T/B cells

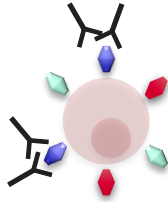
Crossmatch Testing

Flow cytometric crossmatch (Flow XM)

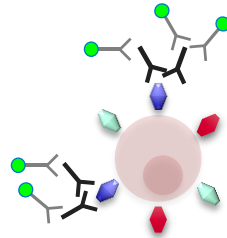
Donor T/B cell



Candidate sera

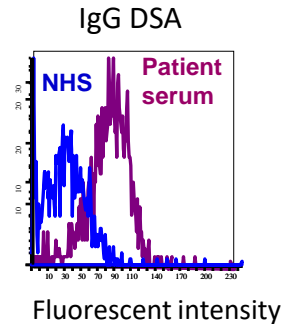


Detection reagents



Fluorochrome
conjugated Ab

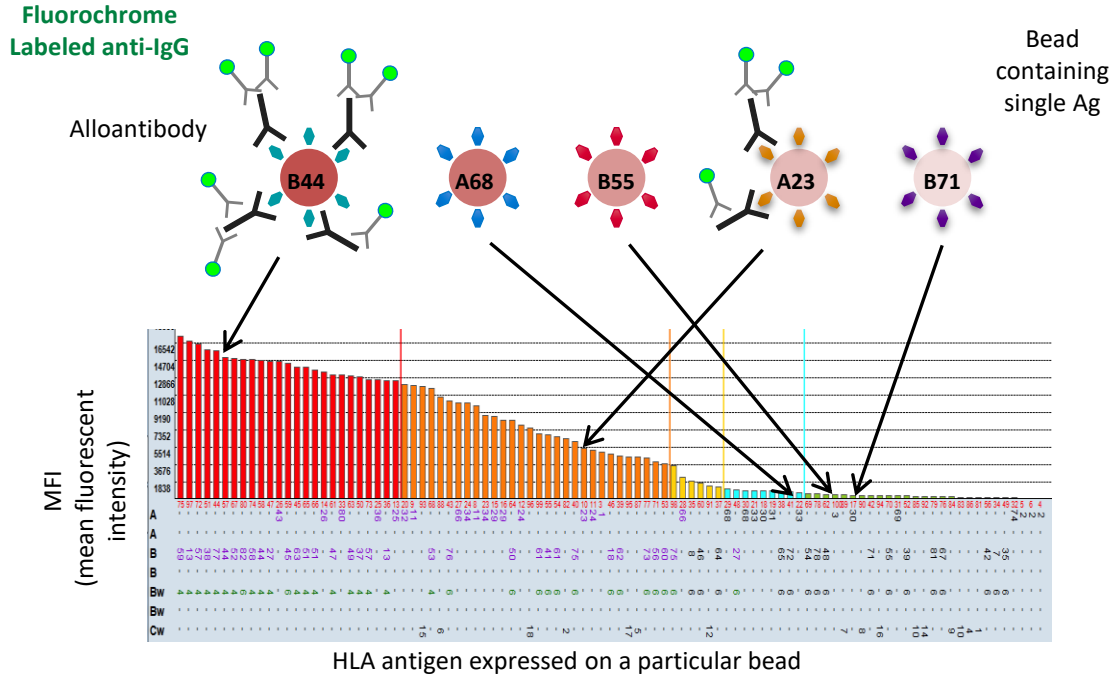
Readout



- Detects IgG anti-HLA antibodies
- Also detects Ab directed against other non-HLA antigens present on T/B cells
- Results expressed as a difference between fluorescent intensity shift of patient serum compared with normal human serum
 - Mean channel shift (MCS)
 - Direct fluorescent units (DFU)
- Threshold for positivity is set by lab
 - Lower threshold = more sensitive, higher false + rate
 - Higher threshold = less sensitive, higher false - rate

Screening for anti-HLA antibodies

Single antigen bead assay



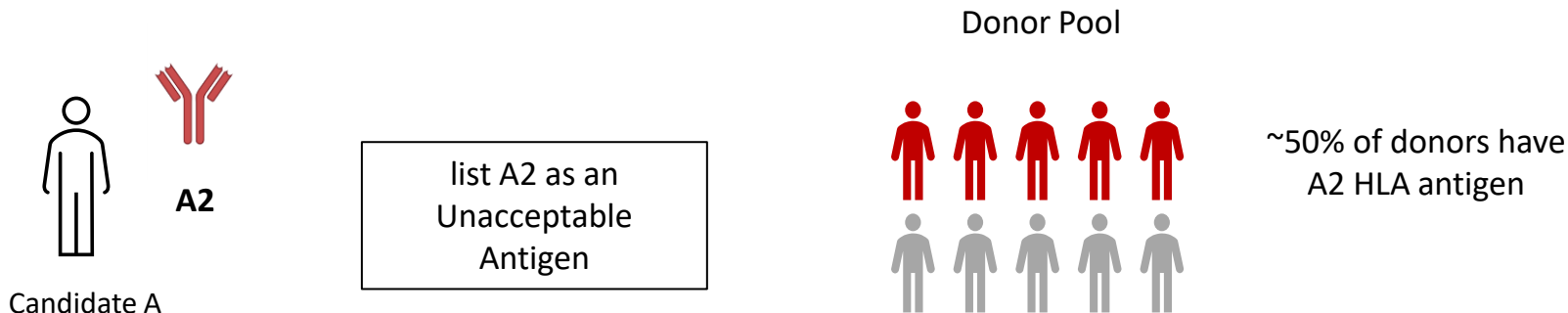
1. Single antigen bead mix –each bead identified by different shade of fluorescent color
2. Add patient sera –if antibody is present, it will bind to the specific bead
3. Add a fluorochrome-labeled anti-IgG antibody
4. Detect by flow cytometric methods (“Luminex”) which beads are coated with antibody, and to what degree (semi-quantitative)

cPRA (calculated Panel of Reactive Antibody)

- **Calculates likelihood of transplant by:**
 - comparing the specificity of the anti-HLA antibodies present in the potential recipient to...
 - the phenotypic prevalence of the particular HLA antigen in the donor population
- **a high cPRA means a lower chance for a transplant**
 - a cPRA of 80% means the patient will be ineligible (on the basis of having DSA) for 80% of deceased donor kidneys
- **Used in UNOS/OPTN kidney allocation system**
 - to create greater parity between waitlisted patients, regardless of their degree of sensitization by giving “priority points” based on increasing cPRA levels

cPRA: calculated Panel of Reactive Antibody

Tells you about the likelihood of an HLA-compatible transplant

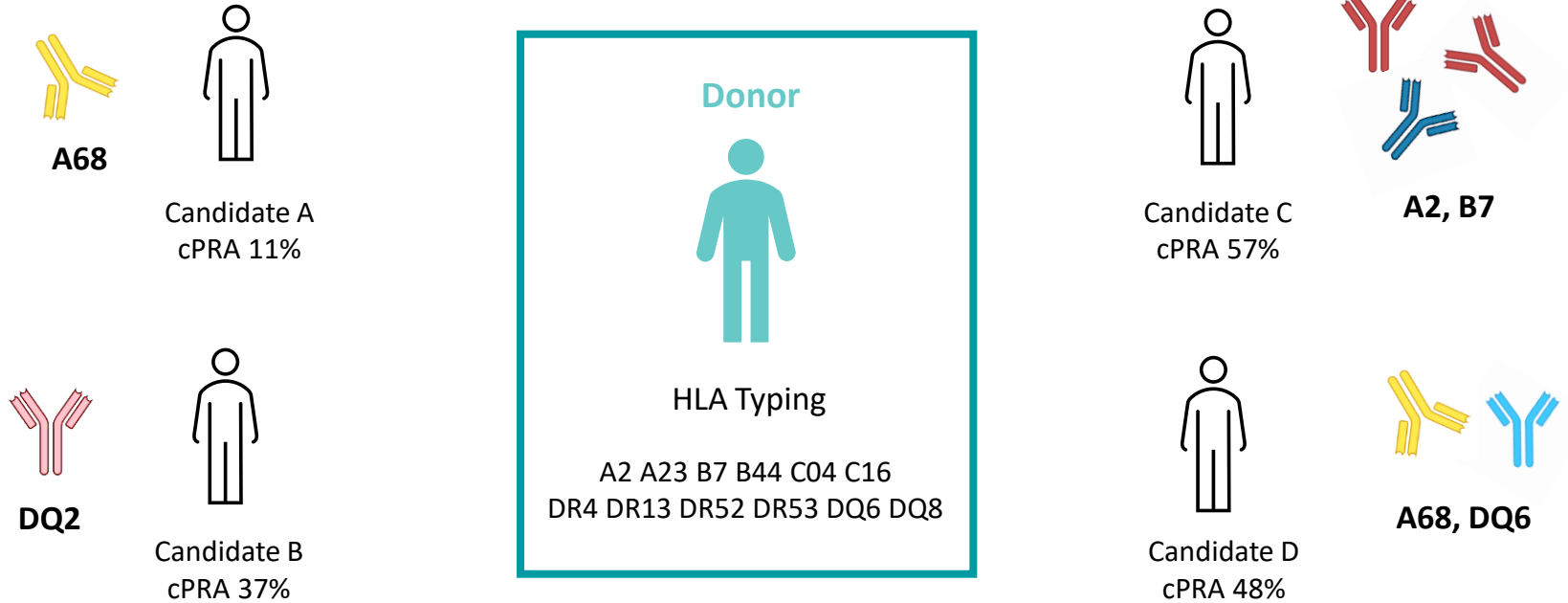


Candidate has a cPRA of 50%

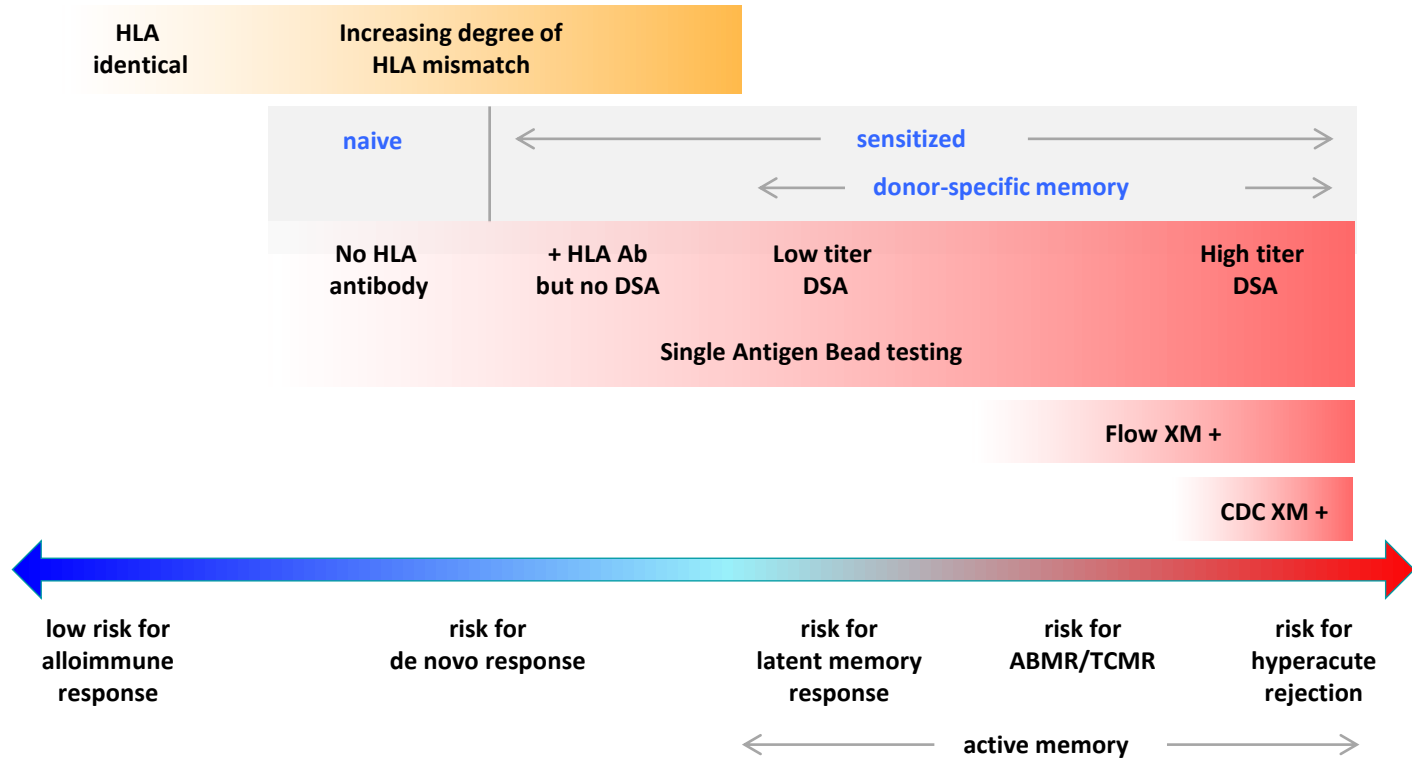
Incompatible and ineligible for half the organs that become available

Performing a “virtual” crossmatch (DSA assessment)

Compare donor HLA typing to see if candidates have donor-specific antibody



Immunological Risk Assessment



Comparison between tests

| Single Antigen Bead Testing | Flow XM | CDC XM |
|--|--|--|
| Pre-tx risk assessment Post-tx monitoring | At time of transplant | At time of transplant |
| Beads | Donor cells | Donor cells |
| IgG | IgG | IgG and IgM |
| Anti-HLA (denatured HLA Ag) | Anti-HLA Non-HLA | Anti-HLA Non-HLA |
| More consistent than Cell-based assays | Detects Ab that can bind to the donor cells | Detects Ab that can bind donor cells and fix complement |
| Highest sensitivity | High sensitivity | Lowest sensitivity |
| Clinical significance of low-level Ab unclear | False positive with poor quality cells | False positive with poor quality cells |

Take Home Messages

- Current histocompatibility testing assesses for the presence/absence of pre-formed donor specific antibody (DSA)
- The various histocompatibility assays should be used in totality to provide a comprehensive assessment of immunological risk
- When relying on a virtual crossmatch alone, be cognizant that limitations in testing methodologies may make it difficult to conclude presence/absence of pre-formed donor specific antibody

References

- Yeung MY. Overview of HLA sensitization and crossmatch testing in renal transplantation. In: UpToDate, Basow DS (Ed), UpToDate, Waltham, MA.