Immunological Assessment Pre and Post Transplant

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Disclosures

- I have no financial disclosures

Objectives

- Use clinical cases to:
  - highlight differences between solid phase and cell based assays
  - determine the strength of anti-HLA antibodies
  - understand the concept of auto-antibodies
  - learn about possible interfering factors (immunosuppressive agents) that can affect HLA lab assays

Cell (Donor) based

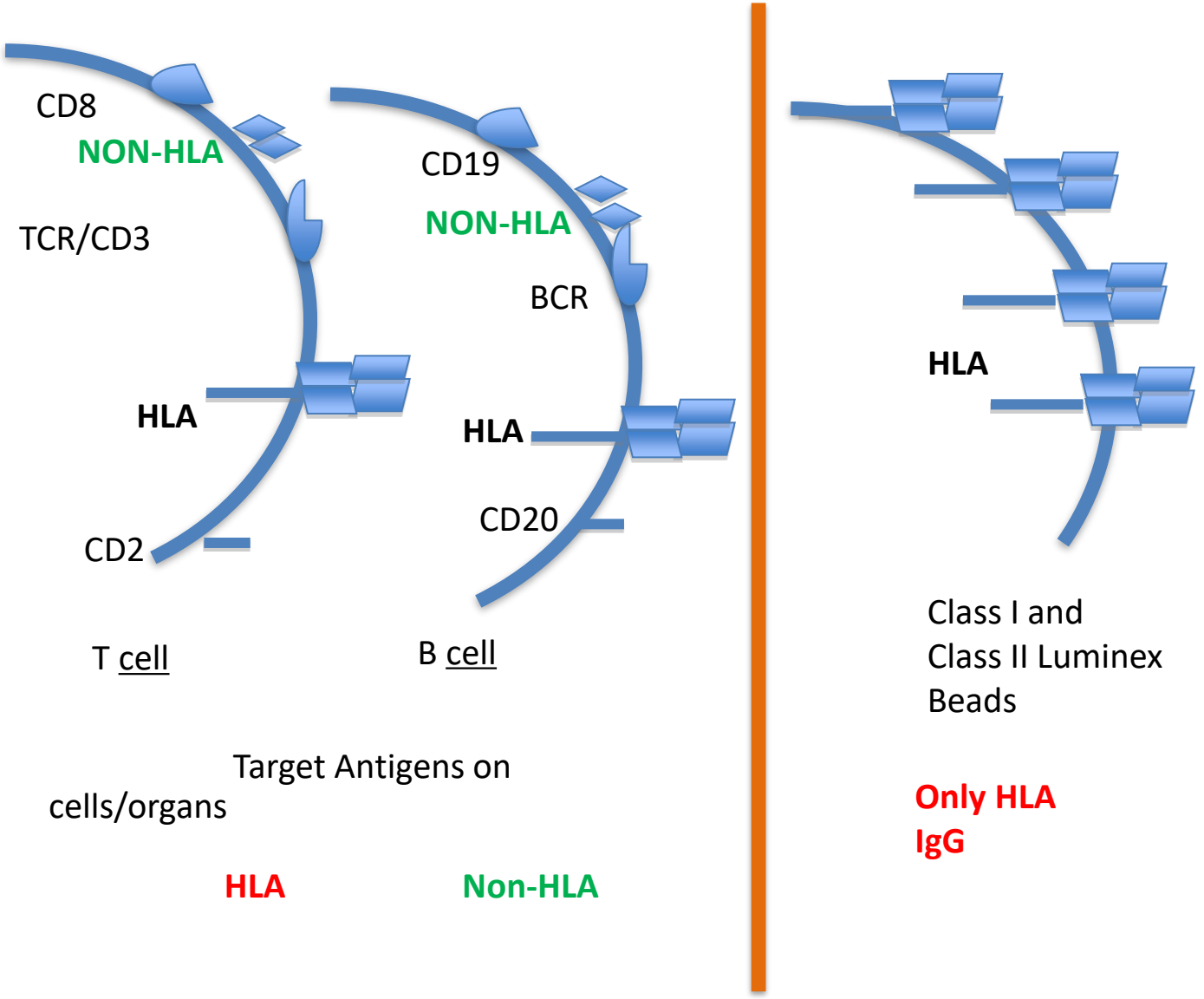
Solid phase Bead based assays

T cells express Class I only

B cells express Class II (&I)

T ~ Class I

B ~ Class II



Cell based Assays (Ag-Ab) differ from Single antigen Bead Assays (Ab)

CASE-1: RR

Patient who has a positive cross match (B cell FLOW Xm) with negative result for anti donor specific antibody (Antibody Screening for HLA antigens by Single antigen bead testing)

In the absence of any single antigen bead reactivity in an assay that detects antibodies to HLA class I or class II antigens

-what could be the possible reasons for this positivity?

-is this reactivity relevant to clinical outcomes post transplant (likelihood)?

Crossmatch = Xm

Summary of results

Patient Antigens: A1 A24 B27 B44 Cw7 Cw10 DR15 DR51 DQ6

Donor Antigens: A2 A33 B35 B7 Cw7 DR11 DR52 DQ6

T cell CDC Xm Negative
B cell CDC Xm Negative

T Flow Xm negative
B Flow Xm positive
All testing is valid
Antibody screening: No donor specific anti-HLA antibodies (DSA) detected by single antigen bead assay

Two different assays

Cell based assays (crossmatch assays – B cell Flow Xm in this case) will pick up **Non-HLA antibodies** in addition to HLA antibodies

Other Possible Reasons for Positive B Cell Flow Xm

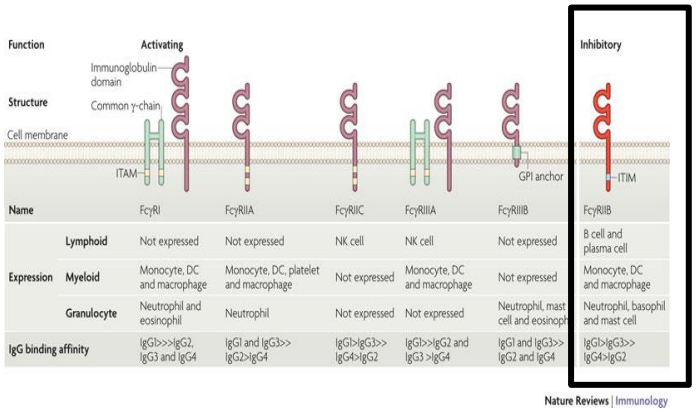
And/Or

Background due to B cells expressing

- Fc receptors

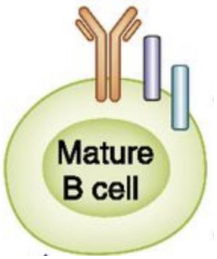
- Surface IgG

- Antibodies can non-specifically bind to these and resulting positivity is Not a contra-indication to transplant



Fc Receptors

Smith and Clatworth Nat Rev Immuno 2010



Surface Immunoglobulins

Post-Transplant Monitoring

Antibody Testing using single antigen beads

- Diagnose AMR
- Determine management of AMR by guiding immunosuppression
- In patients that are at immunological risk (e.g. sensitized, prior episode of Antibody Mediated Rejection) more closely monitoring
- Prognostic information: Persistent or high strength antibodies may predict poor graft outcomes

- **<u>Objective:</u> To determine which Ab tests should be ordered in the setting of AMR and how these results should be followed with treatment**

Need for a way for <u>Quantitation</u>

Post-Transplant Monitoring- Case -2-LK

HLA Typing

Patient Antigens: A3 A30 B35 B45 BW6 Cw4 DR11

Donor Antigens: A2 B44 B70 Bw4 Bw6 Cw5 Cw10 **DQ4** **DQ9** DR7 DR18 DR52

Candidate Antibody Screening (before and at the time of transplant)

Class I: HLA -A66, -A68, -A69 **No Class I** donor specific anti-HLA antibodies

Class II: HLA-DQ2, -DQ5, -DR12 **No Class II** donor specific anti-HLA antibodies

Crossmatch (at the time of transplant)

T CELL CDC Xm Negative

B CELL CDC Xm Negative

Antibody Screening - Post–Transplant

HLA Typing

Patient Antigens: A3 A30 B35 B45 BW6 CW4 DR11

Donor Antigens: A2 B44 B70 Bw4 Bw6 Cw5 Cw10 DQ4 DQ9 DR18 DR52 DR7

Antibodies – post-transplant

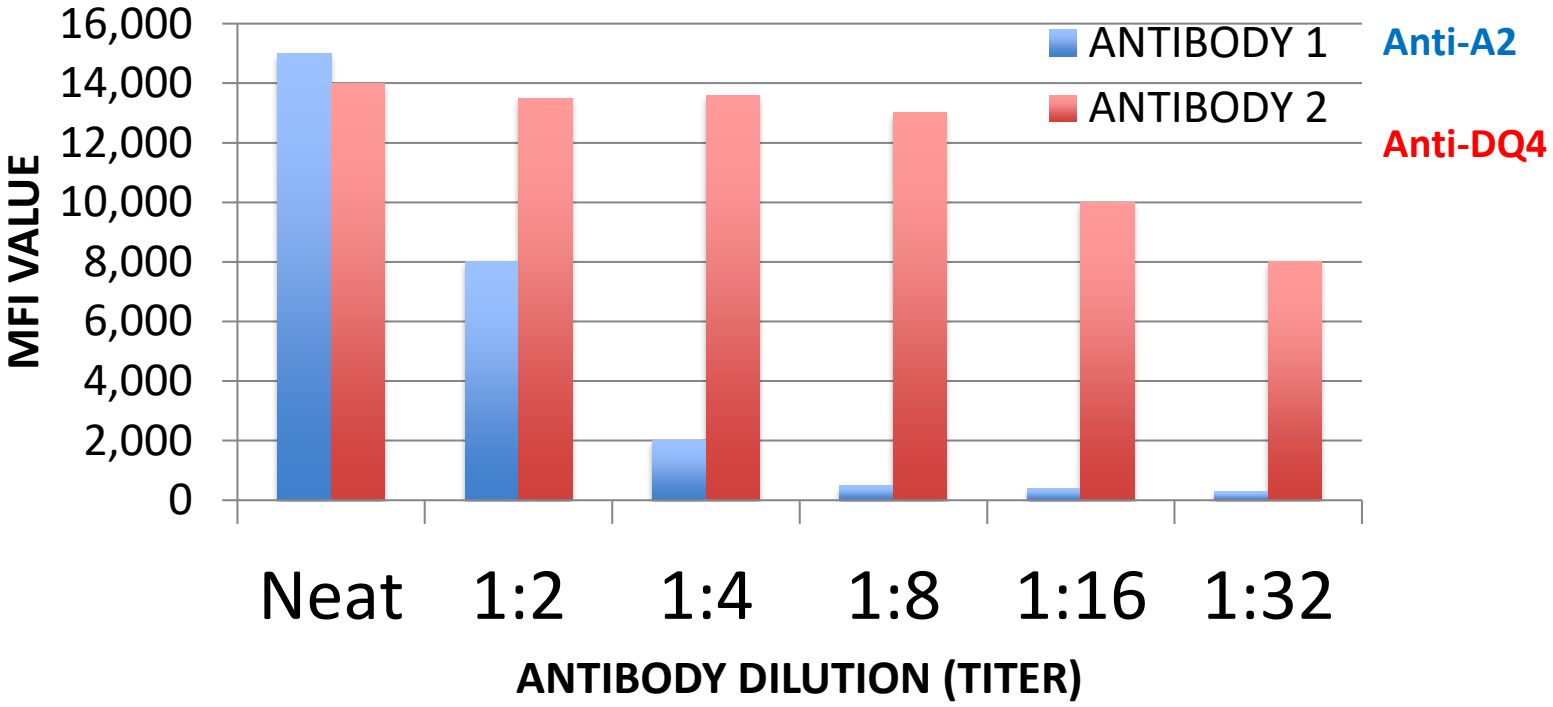
Class I: HLA-**A2**, -A25, -A26, -A66, -A68, -A69 (*de novo* development of antibody to one of the mismatched HLA-A antigen, A2, Pre-transplant no class I antibody but A2 mismatch was there so potential to develop antibodies did exist, with immunosuppression you can minimize but not erase this risk)

Class II: HLA - **DQ4**

Donor specific Antibodies: Anti-HLA-A2 (*de novo* development)
and Anti-DQ4 (*de novo* development)

De novo antibodies carry worse prognosis if persistent (hence close **monitoring)**

Titers - Post Transplant monitoring tool - (plasmapheresis/rituximab)
Strong Antibody May Not Dilute in Titer



Titers are performed to measure strength of the antibody
Strong Antibodies DO NOT Dilute out – Antibody 2 in this case

Anti-A2 (Blue bars) the *de novo* antibody was diluting out – thus intervention will be relatively manageable – but antibodies had to be monitored after each intervention

Conclusion = Ab monitoring used to guide treatment of AMR; Class II antibodies are relatively difficult to remove

Case-3: Patient EC – Highly sensitized patient - Correlation Screening vs Xm

Single Antigen Class I Serum Screening Results

B81	B39
B48	B35
B60	B72
B67	B62
B61	B76
B50	B55
B18	B82
B27	B54
B41	B75
B8	B78
B45	B56
B42	B64
B71	B65
B7	B73
B75	B46
B61	

Bw6- public epitope present on
all the B antigens depicted on the left columns)

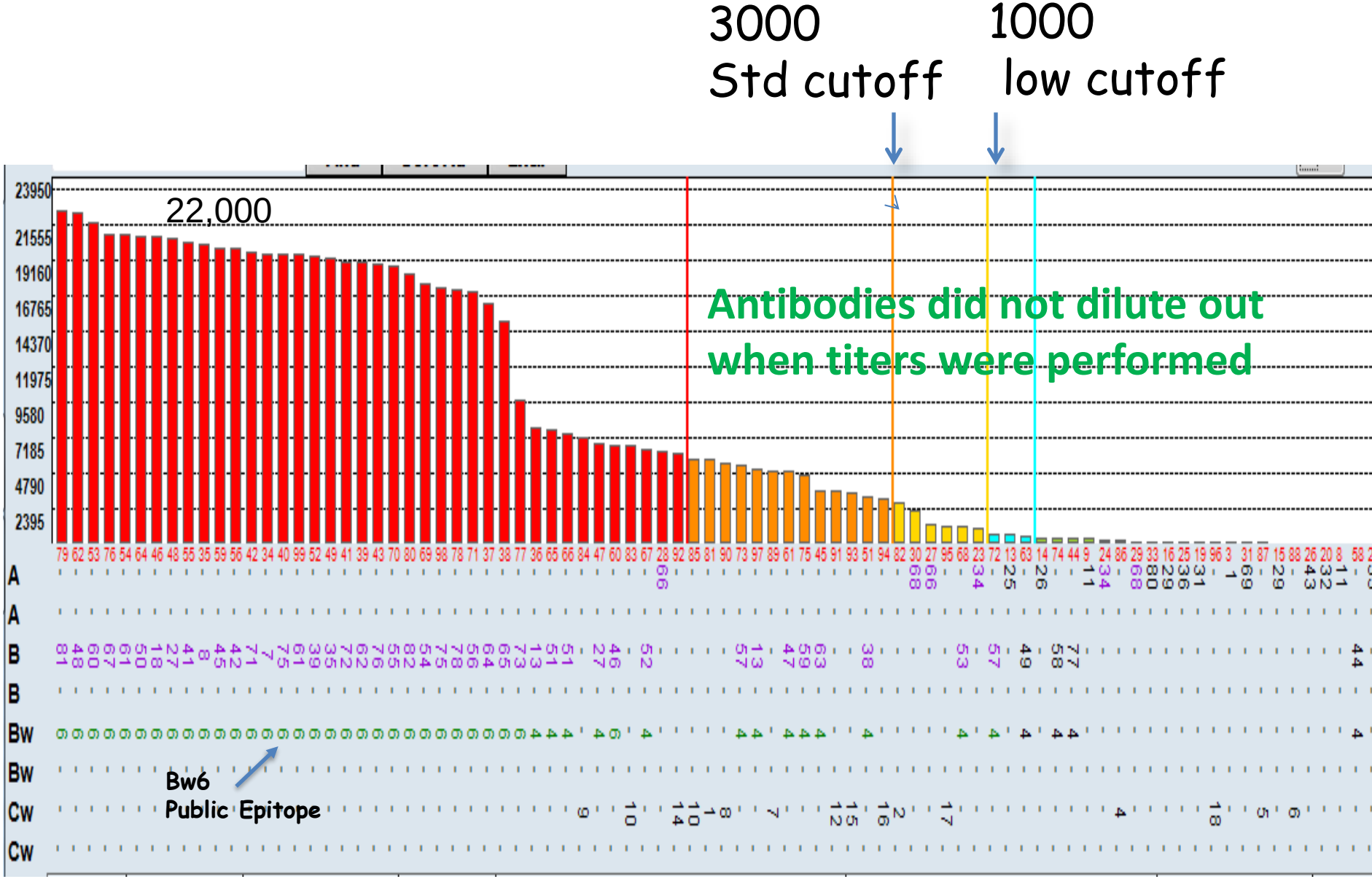
(All These Are “Bw6”)

cPRA = 97%
Highly sensitized)

Other non-Bw6 antibodies present

A66, A68, A34, B27, B51, B52, B13, B47, B59, B63, B38

Patient EC Class I Antibody Profile



Patient EC - Correlation Screening vs Xm

- cPRA by Single Antigen = 97% - **Screening assay**

- 41/47 T cell Cytotoxic **Crossmatches** (T CDC) against deceased donors were positive = 87%

Good Correlation between screening and Xm result

Case 4: Patient AB
Correlation Screening vs Xm

Single Antigen Class I Serum Screening Results

A43
A11
A26
A68
B8
B13
B45
B76
B13
B44
B82

cPRA = 62%
Even though high enough PRA

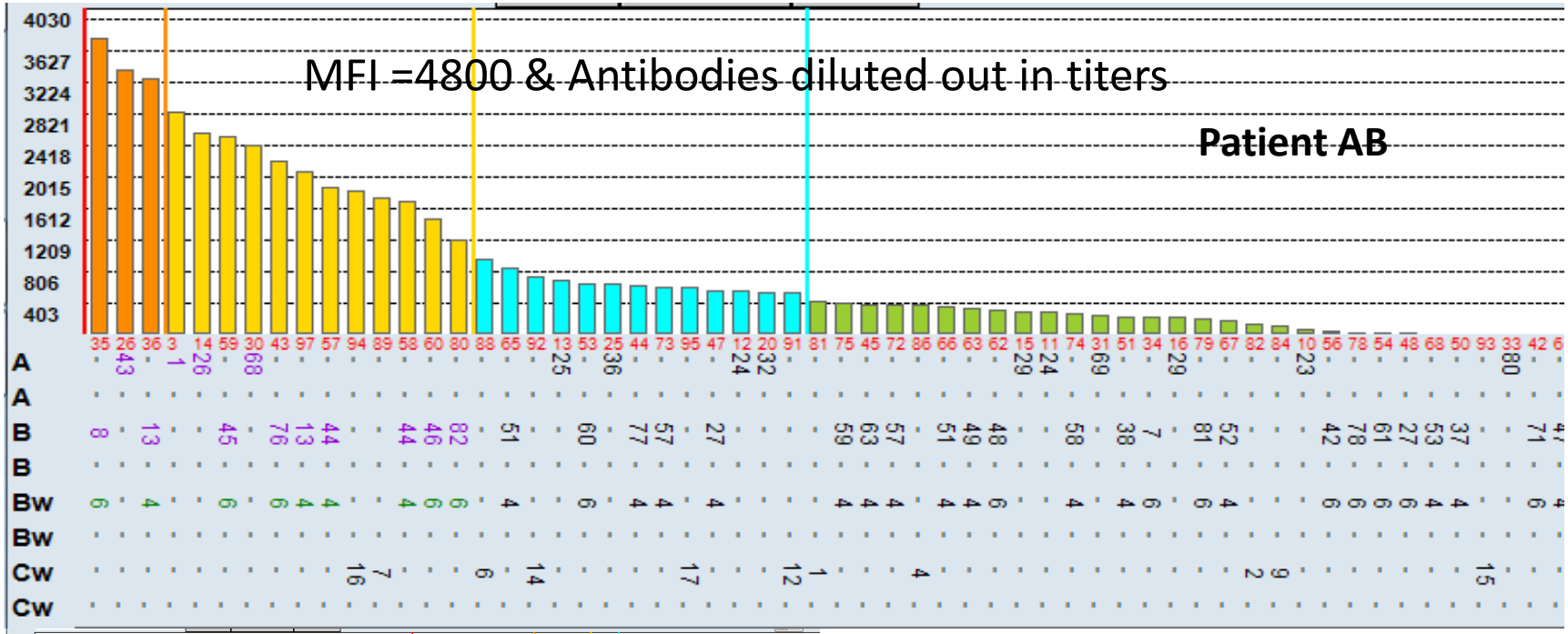
Only 1/31 T cell cytotoxic crossmatches (T CDC Xm) against deceased donors were positive = 3%

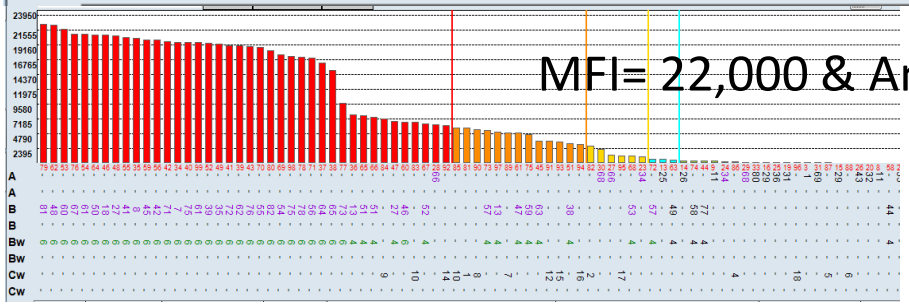
Lack of Correlation between screening and Xm result

Patient AB Class I profile compared to that of Patient EC

3000
Std cutoff

1000
low cutoff



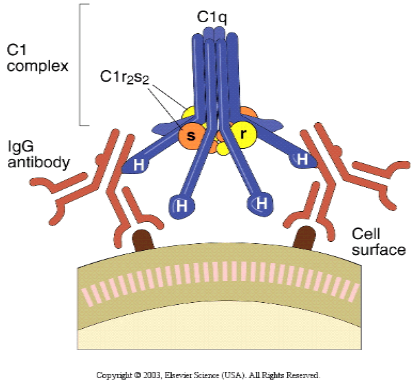


MFI= 22,000 & Antibodies **did not** dilute out in titers

Patient EC

Patient AB – Lack of Correlation between screening and Xm result

- Even though the cPRA is high, class I antibodies are Not strong enough to cause a positive **cytotoxic crossmatch**



<u>Complement binding antibody</u>:
Antibody strong antibody/more molar amount
~ complement binding

Case-5- EM
Auto-antibodies

0/6 antigen (HLA-A, -B, -DR) match but **no donor specific antibody** to mismatched antigens

CDC T and **CDC** B cell **Allo XM**: **Both** positive
Flow T and Flow B cell Allo Xm: Negative
What does this tell us about characteristic of this antibody?

False positive (technical error)
Or Positive due to auto-antibodies, clinically irrelevant antibodies

Other clinical history: Lupus

Is it safe to proceed? What additional information is needed for **full** immunological assessment?

CDC XM picks up IgM antibodies

Crossmatch with Dithiothreitol (DTT) a reducing agent can reduce the disulfide bonds in IgM

DTT added (to cleave IgM)

Original CDC Positive Xm turned Negative following DTT treatment (below), suggesting presence of IgM antibodies ; autoantibodies are usually IgM type

DTT – CDC T-cell Xm -ve

DTT – CDC B-cell Xm -ve

Autoantibodies are usually IgM type - however

To establish if autoantibodies are responsible for the result an auto-crossmatch should be performed.

Auto-crossmatch (Recipient lymphocytes used rather than Donor lymphocytes)

Auto-Xm was positive that also turned negative following DTT treatment

All data point towards there being IgM autoimmune antibodies

IgM (Auto) antibodies are generally regarded as having no pathological significance in transplantation – **AlloAbs – IgM**

Case 6 – BC

Non-specifically Unexpected Positive Crossmatch OR Invalid Crossmatch results

Auto-crossmatches are an important tool to help explain such results
Example: Interference from Immunosuppressive agents

Autologous Crossmatch Example: B cell Depeltion

Auto- T cell Flow Xm - Negative (Valid result)

Auto- B cell Flow Xm (Patient cells with patient serum)

- Invalid result- no B cells were isolated as patient B cells (Recipient cells in auto-Xm) depleted and hence autologous Xm is affected

Could be because of depleting antibody being used for immunosuppression Rituximab (anti-CD20 antibody) in the serum of recipient can deplete B cells (in addition to other mechanisms of action of Rituxan)

T cells will be unaffected

B cell Auto-Xm (patient cells affected – no cells) could not be performed

Allo Crossmatch Example: Interference from Immunosuppressive agents

B cell allo Xm IF performed would be invalid as B cells express CD20 (Donor cells in allo-Xm) on their surface and Rituximab which is anti-CD20 will be present in patient's serum and will bind to donor cells expressing CD20 resulting in positive Xm non-specifically in the absence of any DSA.

Question 1

Donor Antigens: A2 B44 B70 Bw4 Bw6 Cw5 Cw10 DQ4 DQ9 DR18 DR52 DR7

Patient/candidate antibody profile:

Class I: HLA -A66, -A68, -A69 **No Class I** donor specific anti-HLA antibodies

Class II: HLA-DQ2, -DQ4 -DQ7, -DQ8, -DQ9

Patient has Class II DSA : anti-DQ4 and -DQ9 antibodies

Circle the right answer

a) Class II DSA can result in a positive T cell crossmatch
b) Class II DSA can result in a positive B cell crossmatch
c) Crossmatch results due to Class II DSA's will always be negative
d) Class II DSA can result in both T and B cell positive crossmatches

Question - 2

A living donor transplant candidate has the following assay results

Positive T and B cell CDC Xm, a Negative Flow Xm T and B cell results

No DSA (0% PRA) by antibody Screening for HLA antigens by Single antigen Luminex bead testing

What will you do next?

a) Perform DTT treatment to determine if the antibodies are IgM isotype and determine if a negative Xm result is obtained post treatment with reducing agent DTT

b) Perform auto-crossmatches to determine the contribution of autoantibodies to these positive crossmatches

c) Enquire about autoimmune disease history if any on the patient

d) All of the above

e) None of the above

Summary

CDC Xm picks up both IgM and IgG, Flow Xm and single antigen bead assay picks up IgG

Cell based assays pick up non-HLA antibodies in addition to HLA antibodies

Strength of antibody can be tested by performing titers of the sera

Lack of correlation between antibody screening and crossmatch could be a function of strength (and isotype) of antibody