Antibody Against Epitope 76V+80N Causes Unexpected Positive Crossmatches: A Case for Listing HLA-C as Unacceptable

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#409

Take-home finding: A persistent antibody to the public HLA-C epitope 76V+80N explained five "acceptable" donor offers with unexpected positive flow crossmatches and changed the unacceptable-antigen listing strategy.

Clinical context

- 25-year-old female with ESRD secondary to lupus nephritis; received paternal haploidentical kidney transplant in 2018.
- First transplant mismatch included HLA-A1, B8, Cw7, DQ2, and DR17; pre-transplant DSA, CDC XM, and FCXM were negative.
- Allograft failed in 2022; patient returned to hemodialysis and was relisted for kidney transplant.

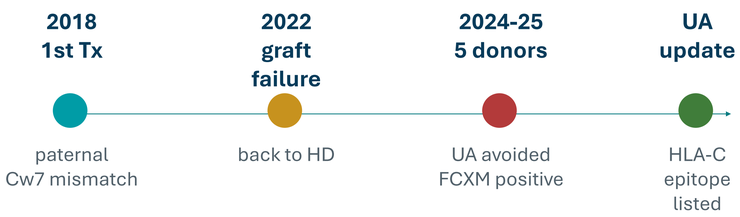
Problem

- After relisting, cPRA remained 98-99% when listing A, B, DR, and DQ unacceptable antigens at a 3000 MFI threshold.
- HLA-C was not listed as UA, reflecting common practice due to lower HLA-C expression and perceived lower crossmatch risk.
- Five deceased-donor offers avoided listed UA, but all produced unexpectedly strong positive T- and B-FCXM, leading to repeated donor decline.

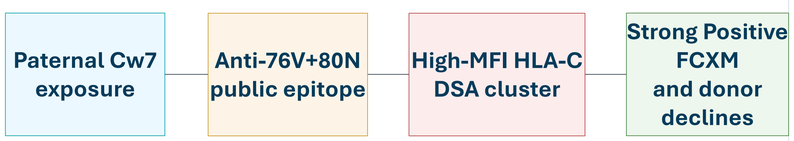
Aim and methods

- Identify the cause of repeated unexpected physical crossmatch positivity despite avoidance of listed unacceptable antigens.
- Reviewed donor HLA typings, CDC XM, FCXM, and single-antigen bead profiles across five declined donors.
- Compared HLA-C SAB MFI patterns by presence or absence of the 76V+80N public epitope.

Case timeline



Working model



- 76V+80N is a BC-loci public epitope on the class I alpha-1 helix.
- Shared-epitope reactivity can make virtual crossmatch assessment based on MFI less reliable.

Key message for practice

Strong, persistent HLA-C antibody should not be dismissed solely because HLA-C expression is lower. In selected highly sensitized patients, listing prior donor epitope-associated antigens as unacceptable may reduce unexpected positive physical crossmatches.

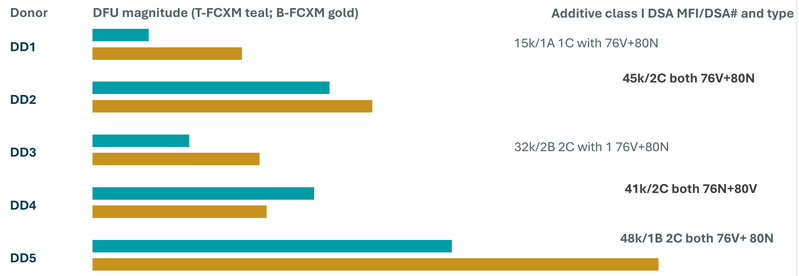
Crossmatch pattern across five deceased donors

Donor	Days Post-Tx	T-FCXM (DFU)	B-FCXM (DFU)	DSA against Epitope 76V+80N	Other Potential DSA
DD1	D+2077	POS (2205)	POS (5865)	Cw7 = 15k	A68 (1k); DR13 (2.5k); DR52 (19k)*; DQ5 (10k)*; DQ6 (5k)*;
DD2	D+2195	POS (9300)	POS (10983)	Cw10 = 24k, 22k (Mean=23k) Cw8 = 22k	DQ5 (9k)*; DQ6 (7k)*
DD3	D+2408	POS (3793)	POS (6553)	Cw8 = 24k	Cw6 (5k); B65 (2k); B37 (1k); DR10 (2k); DR1(1.5k); DQ5 (7k)*
DD4	D+2497	POS (8698)	POS (6835)	Cw10 = 25k, 23k (Mean=24k) Cw7 = 17k	DR15 (1k)
DD5	D+2497	POS (14108)	POS (22216)	Cw12 = 23k Cw9 = 23k	B39 (2k); DR13 (2.5k); DR52 (21k)*; DQ6 (5k)*

Note: *Positivity cutoffs: T-FCXM ≥150 DFU, B-FCXM ≥3,000 DFU. Days post-Tx = days after first transplant at time of serum draw. *Potential allele-specific DSA; imported typing without common allele strings available.*

All donors were CDC T-cell negative.

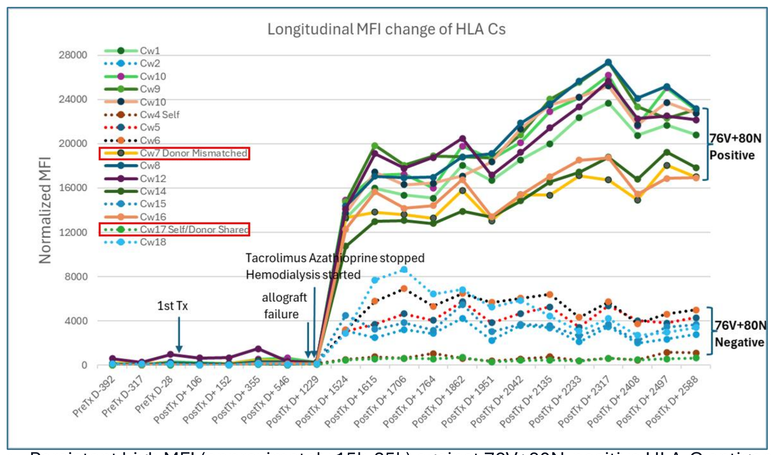
Class I MFI and T-FCXM



All results exceeded positivity cutoffs (T ≥150; B ≥3000).

- MFI is semiquantitative, is not an antibody titer, and should not be interpreted in isolation.
- DSA MFI values should not simply be added, but the combined pattern is less favorable than a single weak DSA
- **CREGs and public epitopes explain cross-reactive patterns but do not necessarily make every antigen within a CREG clinically donor-specific.**
- The cumulative ("stacking") effect of antibodies to shared epitopes may not be predictable from SAB alone and may be influenced by donor-cell HLA expression.

HLA-C SAB pattern reveals epitope-specific clustering



- Persistent high MFI (approximately 15k-25k) against 76V+80N-positive HLA-C antigens, with clear separation from epitope-negative beads.
- A single public epitope positive aligned with the sensitization by mismatched prior donor antigen.
- Epitope verification requires absorption and elution testing with cells or single-antigen C beads.

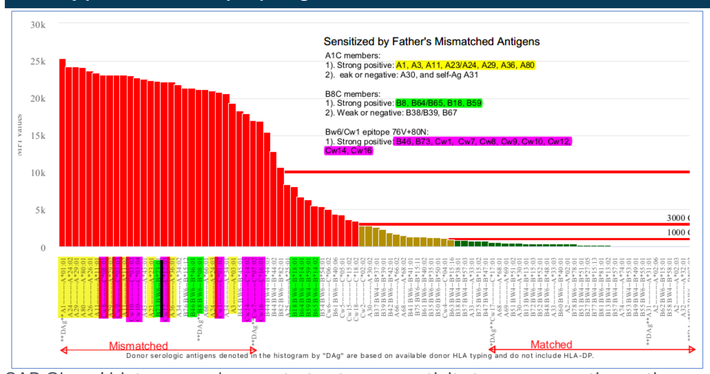
This pattern aligned with failed FCXM despite avoidance of listed UA.

Evidence Summary

- All five donors carried one or two HLA-C antigens sharing 76V+80N.
- Patient developed high-MFI HLA-C DSA against the shared epitope-bearing antigen(s) for these donors.
- Low-level A/B/DR signals were insufficient to explain the strong T-FCXM pattern; class II allele-specific reactivity may contribute to B-FCXM.

Primary driver: high-MFI HLA-C antibody to a shared public epitope

Antibody profile: shared-epitope signal



SAB Class I histograms demonstrate strong reactivity to cross-reactive antigens associated with the father's mismatched HLA antigens A1, B8, and C07.

Interpretation

- The patient was likely sensitized by paternal Cw7, which carries 76V+80N.
- The antibody recognized a shared public epitope across Cw1, Cw7, Cw8, Cw9, Cw10, Cw12, Cw14, and Cw16.
- High antibody strength overcame the usual lower HLA-C surface expression and produced clinically meaningful FCXM positivity.

Clinical action

List 76V+80N-positive HLA-C antigens as unacceptable for this candidate.

cPRA 98.50% regional priority → **cPRA 99.77% national priority**

Goal: reduce avoidable donor declines and better align allocation priority with the candidate's true immunologic barrier.

Conclusion

- HLA-C DSA can be clinically significant when the antibody is strong and epitope-focused. Analysis of our historical data showed that HLA-C DSA >5000 MFI was associated with a positive T-cell FCXM.
- A single public epitope sensitized by **a prior failed donor** explained the repeated unexpected positive physical crossmatches despite virtual UA avoidance.
- Strong HLA-C UA listing can improve compatibility screening for select highly sensitized candidates.

Identifying and precluding this immune barrier may help transplant teams avoid incompatible offers, reduce organ allocation delays, and improve access to a successful kidney transplant for this specific patient

Verification and limitations

- Epitope verification requires absorption and elution testing with cells or single-antigen C beads.
- Potential allele-specific DR52/DQ5/DQ6 DSA may contribute to B-FCXM and needs surrogate/phenotype bead confirmation.
- High-resolution donor typing can help prevent over- or under-restriction when allele-specific class II reactivity is suspected.

Disclosures: No. ASHI 52nd Annual meeting Poster ID: 409.

Selected references: El-Awar et al., J Immunol Res 2017; Garcia-Sanchez et al., Clin Transplant 2020; Maldonado et al., Clin Transplant 2023; Bachelet et al., Transplantation 2016.