Reduced ambiguity in sera profiling for highly sensitized HLA-B46 Homozygous Renal Candidates using MagSort beads

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Abstract

HLA-B46 homozygous (B46+/+) candidates for kidney transplantation commonly display highly saturated single antigen bead (SAB) profiles for HLA Class I antigens, making risk assessment for potentially crossing weaker specificities of donor-specific antibodies (DSA) challenging. We aimed to characterize three complex sera samples obtained from highly sensitized HLA-B46 homozygous candidates using HLA-specific MagSort beads to test B locus epitope reactivity to Bw4/Bw6 public epitopes or CREG serological reactivities suggested by clinical single antigen-bead (SAB) testing.

Study Desing: Materials & Methods

SAB I/II (One Lambda) with titration (1:5, 1:10, 1:20, 1:40, 1:100) was performed to determine the breadth and strength of sensitization. A panel of HLA-B locus specific MagSort beads (B*07:02, B*08:01, B*15:01, B*53:01, B*57:01, B*67:01) were used to identify reactivity patterns in sera from three highly sensitized HLA-B46 homozygous renal candidates (n=3) to determine the number and strength of HLA antibodies driving their 100% cPRA Proteomic analysis was conducted by R software (version 4.4.1, (C) 2024 and visualized with the package igraph (version 2.1.4). Three-dimensional Eplet analysis with Fusion 3D.

Fig. 1A.

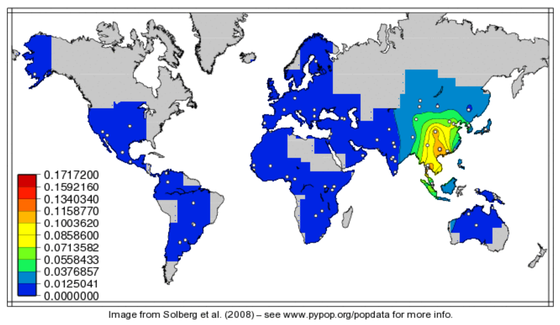
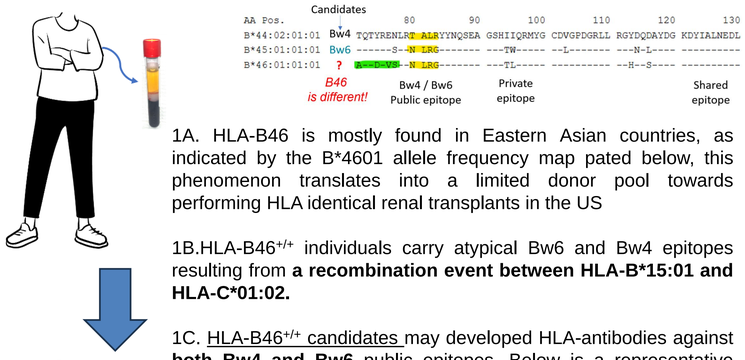


Fig. 1B.



1A. HLA-B46 is mostly found in Eastern Asian countries, as indicated by the B*4601 allele frequency map pated below, this phenomenon translates into a limited donor pool towards performing HLA identical renal transplants in the US

1B.HLA-B46$^{+/+}$ individuals carry atypical Bw6 and Bw4 epitopes resulting from **a recombination event between HLA-B*15:01 and HLA-C*01:02.**

1C. HLA-B46$^{+/+}$ candidates may developed HLA-antibodies against **both Bw4 and Bw6** public epitopes. Below is a representative SABI histogram from a highly sensitized HLA-B46$^{+/+}$ candidate.

Fig. 1C.

Results

HLA-coated MagSorts Beads are Cross-reacted with sera from highly sensitized HLA-B46 homozygous candidates. Eluted HLA specificities are analyzed by Solid-Phase platform (SABI).

Fig. 2A.

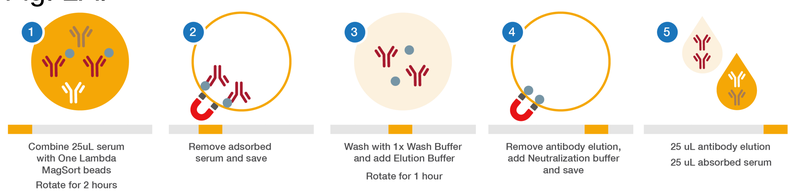
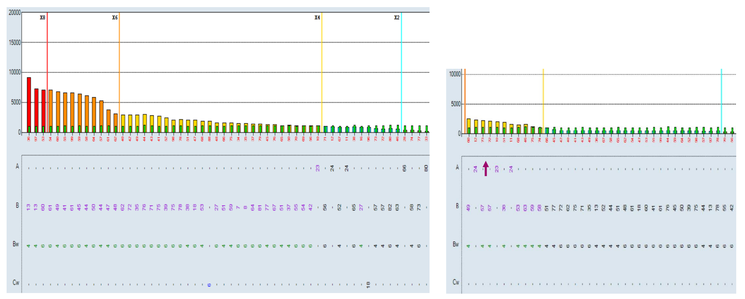


Fig. 2B.

Fig. 2C.



Right. HLA-B57 MagSort beads were cross-reacted serum from a highly sensitized HLA-B46$^{+/+}$ candidate. Unbound HLA specificities were analyzed with the Single Antigen Bead assay for Class I specificities. *Left*. SAB-I analysis confirmed eluted HLA-B57 specificites along with eight other specificities sharing the Bw4 public epitope, A23, A24, B49, B36, B63, B59 and B58. Indicating an unidentified Epitope or HLA-Eplet among these eluted specificities.

Fig. 2D. Proteomic analysis for Eluted HLA-B57 Specificities

HLA class I antibody and specificity network analysis using the **HLA-B57 MagSort**. Blue circles indicate MFI > 1000, MFI. Network exclusion consider very low MFIs. Network inclusion cut off is 800 MFI. The HLA-B57 MagSort bead isolated the HLA-B57 antibody and other related specificities.

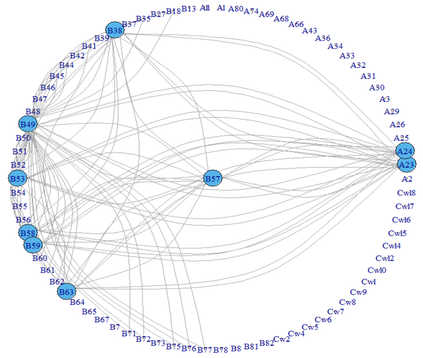


Table 1. Eplets Identified in Eluted fractions following adsorption with MagSort HLA-Specific Beads

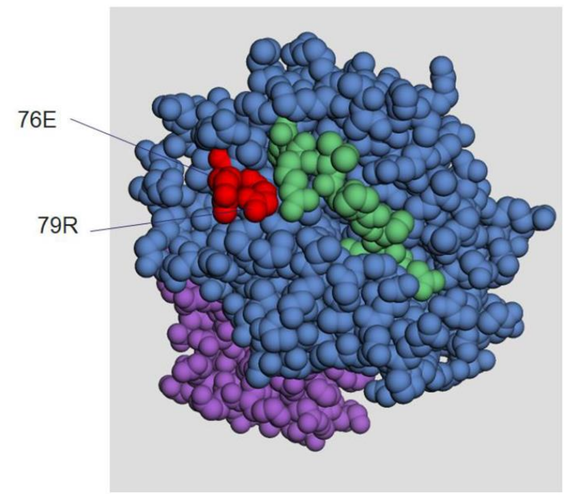
	MagSort Bead HLA Specificity	Eluted Eplets (Strongest to Weakest Reactivity)
Patient 1	B*07:02	76E79R, 66I, 163EW
	B*08:01	76E79R, 66I
	B*15:01	76E79R, 66I
	B*53:01	76E79R, 66I
	B*57:01	76E79R
	B*67:01	76E79R, 66I
Patient 2	B*07:02	76E79R, 66I, 163EW
	B*08:01	76E79R, 66I
	B*15:01	76E79R, 66I
	B*53:01	76E79R, 66I
	B*57:01	76E79R
	B*67:01	76E79R, 66I
Patient 3	B*07:02	43P76E, 66I
	B*08:01	43P76E, 66I
	B*15:01	43P76E, 66I
	B*53:01	43P76E, 66I, 144Q83R
	B*57:01	43P76E, 144Q83R
	B*67:01	144Q152V

Patients 1 and 2 showed bispecific reactivity to Bw4/Bw6 public epitopes based on SAB date. MagSort beads specific for B*07:02, B*08:01, B*15:01, B*53:01, B*57:01, B*67:01 showed nearly identical eluted fractions from both sera, with high antibody titers against all antigens carrying the 76E79R eplet, suggesting a strong monoclonal specificity. **Patient 3** showed broad sensitization to multiple specificities at various strengths in SAB. Ranking of eluted HLA antibodies reduced ambiguity and improved interpretation for crossing potential DSA.

Patient 3 showed broad sensitization to multiple specificities at various strengths in SAB. Ranking of eluted HLA antibodies reduced ambiguity and improved interpretation for crossing potential DSA (**Table 1**). MagSort beads revealed distinct reactivity against Bw4/Bw6 public epitopes or CREGs, enhancing SAB testing by differentiating antibody patterns for eplets of 43P76E, 66I, and others reacting with HLA-A and B alleles. Patient 3 is typed as A11/A24, with their prior donor being A29, further supporting their unique reactivity. Importantly, only 66I is listed in the HLA eplet registry, revealing the presence of previously undescribed eplets.

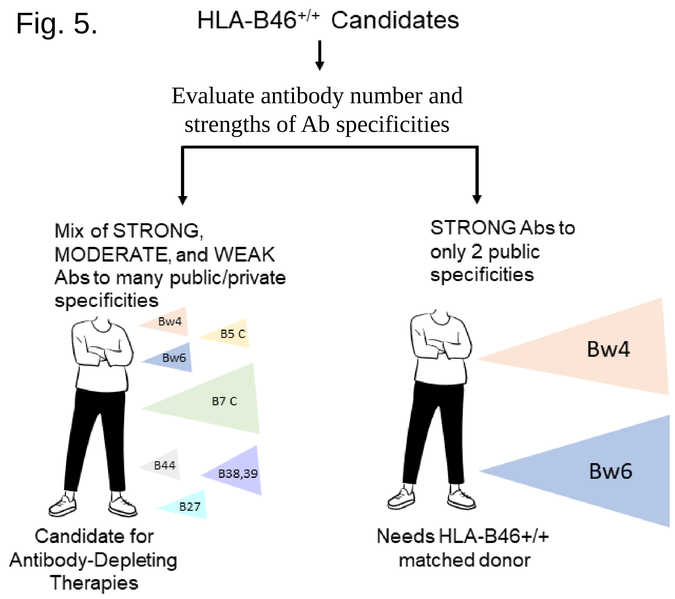
Monoclonal Specificity against HLA-Eplet 76E79R identification with the MagSort Beads

Fig. 4. 76E79R – Immunogenic region



HLA Eplet analysis for the 76E79R immunogenic region revealed a highly accessible Eplet shared among B46-homozygous Patient 2 and 3's eluted sera. Strong HLA-B antibody against only the 76E79R immunogenic region is predicted to result in a 100% cPRA and exclude most donors.

Fig. 5.



Assessment of patient antibody reactivity to public vs private epitopes can inform likely success of antibody depleting therapies. Patients who have a mix of antibody strengths and specificities may potentially be able to cross antibodies that become weaker through desensitization. Patients who make only strong antibodies to the two Bw4/6 public epitopes require an HLAB-46$^{+/+}$ matched donor.

Summary

- HLA-specific MagSort beads can clarify broad-specificity sera from patients with high cPRAs.
- This patient-specific virtual desensitization protocol could guide clinical by identifying expected antigen specificities able to be crossed following initial versus multiple rounds of antibody-depleting therapies.