

Unexpected Bw4 Alloantibody Reactivity in a Bw4+ Patient: The Contribution of Non-canonical Amino Acid Polymorphism Beyond Residues 77-83

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AIM

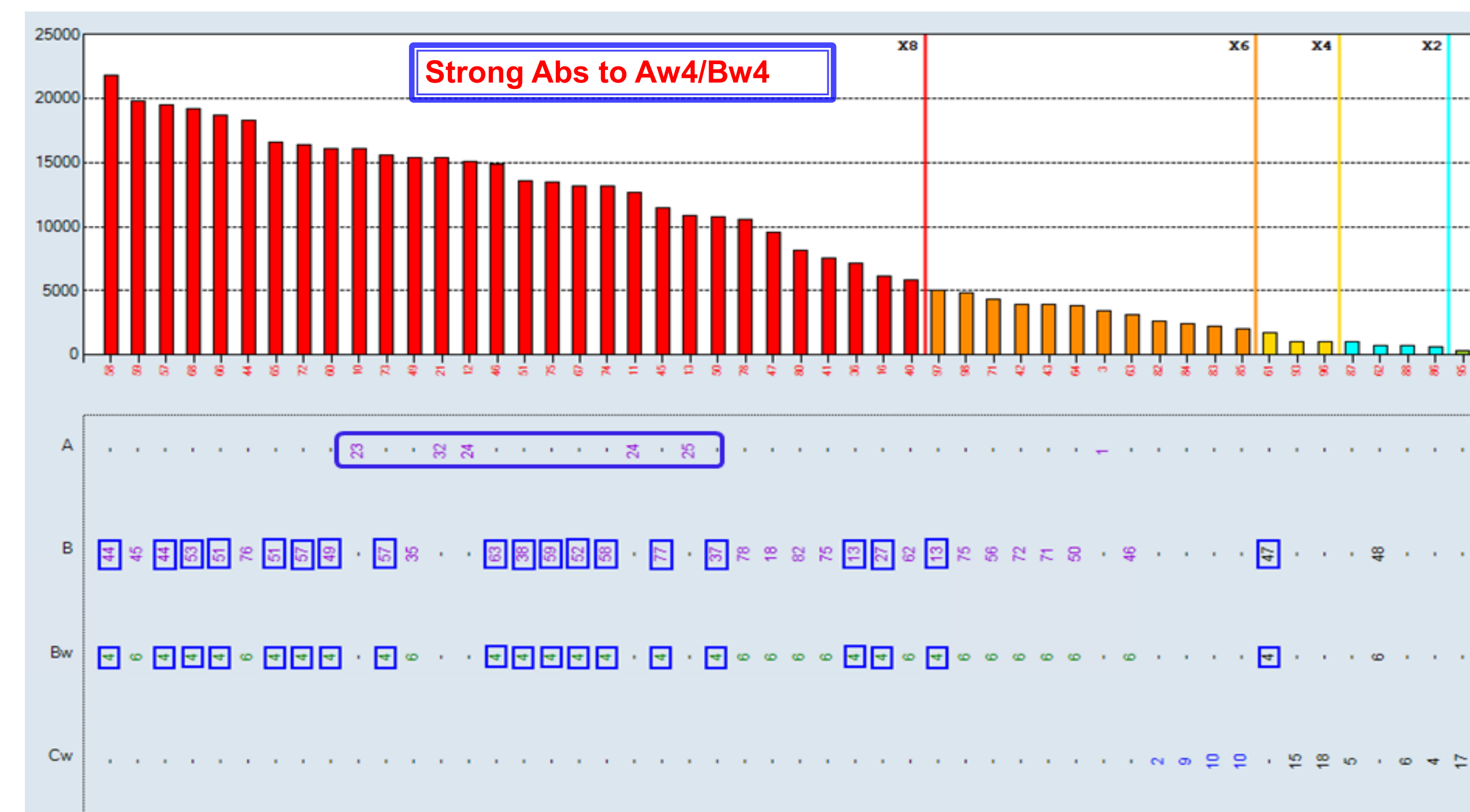
This study present a unique case of alloantibody to Bw4+ alleles and investigates the complexity of determining Bw4 antibody specificity in a highly sensitized patient.

CASE DESCRIPTION

Patient demographics

Characteristic	Value
Age	36 years
Sex	Female
Race/Ethnicity	African American
Diagnosis	Nephrotic Syndrome
Sensitizing Event	History of Multigravida
HLA typing	
A*02:01	A*29:02
B*08:01(Bw6)	B*47:03(Bw4)
C*07:01	C*07:18
DRB1*04:04	DRB1*08:04
DRB4*01:03	
DQA1*03:01	DQA1*04:01
DQB1*03:02	DQB1*04:02
DPA1*01:03	DPA1*03:01
DPB1*03:01,	DPB1*105:01

Antibody profile of a B*47:03 (Bw4)+ renal patient



Surrogate flow crossmatches against multiple Bw4+ donors with T & B cell

Crossmatch (XM)	Median Channel Shift (MCS)		HLA Class I					
	T Cell	B Cell						
Auto XM	Neg (-22)	Neg (-14)	A*02:01	A*29:02	B*08:01(Bw6)	B*47:03 (Bw4)	C*07:01	C*07:18
Surrogate XM 1	Pos (308)	Pos (259)	A2		B57 (Bw4)		Cw6	
Surrogate XM 2	Pos (345)	Pos (296)	A2	A25 (Aw4)	B39(Bw6)	B44 (Bw4)	Cw5	Cw12
Surrogate XM 3	Pos (323)	Pos (356)	A3	A30	B55 (Bw6)	B57 (Bw4)	Cw10	Cw18
Surrogate XM 4	Pos (292)	Pos (346)	A1	A2	B8 (Bw6)	B27 (Bw4)	Cw2	Cw7
Surrogate XM 5	Pos (375)	Pos (398)	A2	A29	B27 (Bw4)	B58 (Bw4)	Cw1	Cw7
Surrogate XM 6	Pos (256)	Pos (313)	A3	A30	B65 (Bw6)	B44 (Bw4)	Cw9	Cw8
Surrogate XM 7	Pos (253)	Pos (266)	A1	A32 (Aw4)	B7(Bw6)	B65 (Bw6)	Cw7	Cw8

Positive crossmatch cutoff values: T cell MCS >50, B cell MCS >100

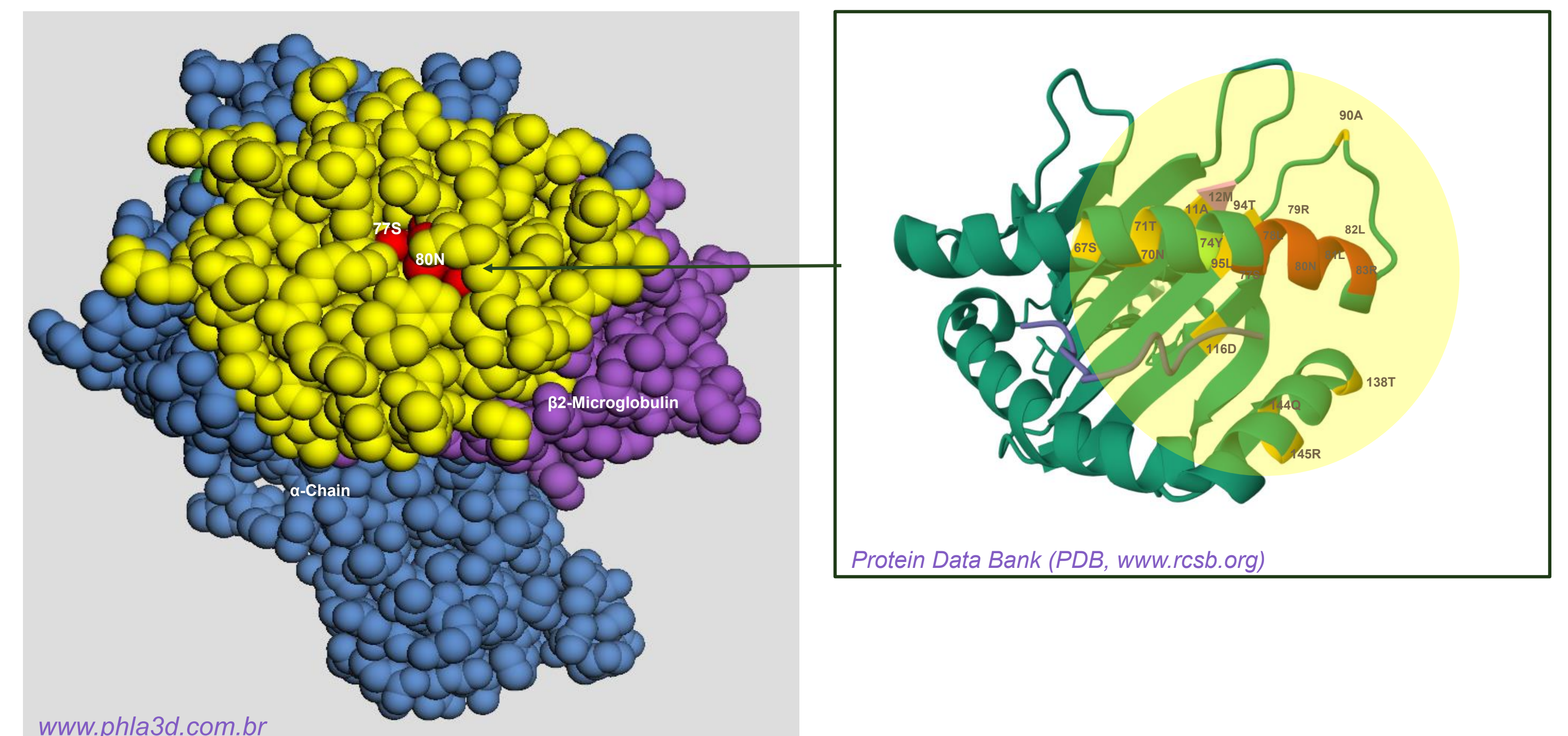
Amino acid sequence alignment of the Bw4+ alleles in the SAB panel showing polymorphic residues (highlighted in blue) that are within a 15Å radius of residue 77S and 80N of B*47:03

Patient's B*47:03 differs from B*47:01 at residues 77 and 80 within the canonical Bw4 motif (77-83) (SLRNLLR and DLRTLLR, respectively). Notably, residues 77S80N of B*47:03 are typically found in Bw6+ alleles, whereas 82L83R are present in all Bw4+ alleles.

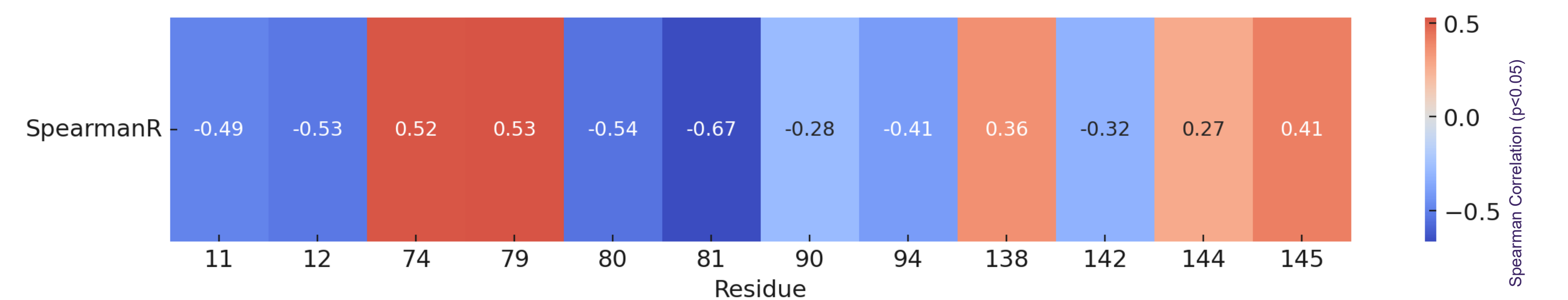
Despite sharing the exact Bw4 motif with B*47:01, B*27:05 and B*37:01 exhibited significantly stronger Ab reactivity likely due to nearby structural differences.

Amino Acid Residue	11	12	67	70	71	74	77	78	79	80	81	82	83	90	94	95	116	138	144	145		
Patient B*47:03	A	M	S	N	T	Y	S	L	R	N	L	L	R	A	T	L	D	T	Q	R		
Distance from Residue 77/80 (Å)	13.1	13.4	14.6	14.7	14.1	9.4	4.9	5.4	3.8	4.9	3.8	5.4	5.3	14.2	12.3	11.1	13.8	13.8	13.4	14.3		
Bw4 Allele	MFI	No. of Different Residues from B*47:03																				
B*44:03	21784	5	A	M	S	N	T	Y	N	L	R	T	A	L	R	A	I	I	D	T	Q	R
B*44:02	19482	5	A	M	S	N	T	Y	N	L	R	T	A	L	R	A	I	I	D	T	Q	R
B*53:01	19248	7	A	M	F	N	T	Y	N	L	R	I	A	L	R	A	I	I	S	T	Q	R
B*51:02	18733	6	A	M	F	N	T	Y	N	L	R	I	A	L	R	A	T	W	Y	T	Q	R
B*51:01	16601	6	A	M	F	N	T	Y	N	L	R	I	A	L	R	A	T	W	Y	T	Q	R
B*57:01	16425	9	A	M	M	S	A	Y	N	L	R	I	A	L	R	A	I	I	S	T	Q	R
B*49:01	16109	5	A	M	S	N	T	Y	N	L	R	I	A	L	R	A	T	W	L	T	Q	R
A*23:01	16042	11	S	V	V	H	S	D	N	L	R	I	A	L	R	A	T	L	Y	M	Q	R
B*53:03	15544	9	A	M	M	S	A	Y	N	L	R	I	A	L	R	A	I	I	Y	T	Q	R
A*32:01	15375	10	S	V	V	H	S	D	S	L	R	I	A	L	R	A	T	I	D	M	Q	R
A*24:03	15090	12	S	V	V	H	S	D	N	L	R	I	A	L	R	A	T	L	Y	M	K	R
B*15:16	14911	8	A	M	M	S	A	Y	N	L	R	I	A	L	R	A	T	W	S	T	Q	R
B*38:01	13554	7	S	V	C	N	T	Y	N	L	R	I	A	L	R	A	T	L	F	T	Q	R
B*59:01	13484	6	A	M	F	N	T	Y	N	L	R	I	A	L	R	A	T	W	L	T	Q	R
B*52:01	13212	5	A	M	S	N	T	Y	N	L	R	I	A	L	R	A	T	W	Y	T	Q	R
B*58:01	13139	9	A	M	M	S	A	Y	N	L	R	I	A	L	R	A	I	I	S	T	Q	R
A*24:02	12678	12	S	V	V	H	S	D	N	L	R	I	A	L	R	A	T	L	Y	M	K	R
B*15:13	11500	6	A	M	S	N	T	Y	N	L	R	I	A	L	R	A	I	I	S	T	Q	R
A*25:01	10854	11	S	V	V	H	S	D	S	L	R	I	A	L	R	D	T	I	D	M	Q	R
B*37:01	10799	6	S	V	S	N	T	Y	D	L	R	T	L	L	R	A	T	I	F	T	Q	R
B*13:02	7127	6	A	M	S	N	T	Y	N	L	R	T	A	L	R	A	T	W	L	T	Q	L
B*27:05	6118	8	S	V	C	K	A	D	D	L	R	T	L	L	R	A	T	L	D	T	Q	R
B*13:01	4983	7	A	M	S	N	T	Y	N	L	R	T	A	L	R	A	I	I	L	T	Q	L
B*47:01	1690	2	A	M	S	N	T	Y	D	L	R	T	L	L	R	A	T	L	D	T	Q	R

Molecular modeling of B*47:03 showing surface-exposed residues surrounding 77S and 80N within a 15Å radius [in yellow] and location of residues in α-chain [yellow circle].



Heatmap of Spearman correlation between amino acid residues and MFI



Spearman's rank correlation analysis revealed significant association between residues 11, 12, and 74 and Ab reactivity (Spearman Rank Coefficient, $r_s = -0.49, -0.53, \text{ and } 0.52$, respectively; $p < 0.0001$). Among all the polymorphic residues within 15Å of the Bw4 motif 77-83 of all Bw4 alleles, seven were associated with MFI variability, with residues 74 and 12, respectively, showing the strongest positive and negative correlation.

CONCLUSION

Determining the specificity and clinical relevance of HLA antibody (Ab) remains challenging- particularly in highly sensitized patients. This case highlights the complexity of determining Ab specificity, demonstrating that alloantibodies can recognize conformational epitopes formed by multiple eplets, including non-linear and adjacent polymorphic residues outside the conventional epitope. Understanding these structural nuances is crucial for accurately assessing their validity and clinical relevance.

