



David Geffen School of Medicine

Nezar Eltayeb Elsheikh^{1,2}, Michelle Hickey^{1,2}, Nicole Valenzuela^{1,2}, Carrie L. Butler^{1,2}, Rebecca Sosa^{1,2}, Sun-Mi Choi^{1,2} Elaine F. Reed^{1,2}, Qiuheng Zhang^{1,2}

¹Department of Pathology and Laboratory Medicine and ²Immunogenetics Center, University of California, Los Angeles, CA, USA

INTRODUCTION

A pre-transplant crossmatch, either virtual (VXM) or physical (PXM) is required prior to kidney transplantation. Here, we present a near-miss sentinel event involving a discrepant Virtual/Physical crossmatch results in a deceased donor offer for a kidney transplant candidate.

PATIENT CLINICAL HISTORY

A 54-year-old male, highly sensitized (cPRA 99.99%) listed for his third kidney with twelve years of UNOS wait time received an offer from a deceased donor. VXM indicated that the patient displayed two weak donorspecific antibodies (DSA) to Cw10 and DP17, which would result in a negative T and B PXM. However, the PXM results were unexpectedly and strongly positive (T cell = 387 MCS and B cell = 331 MCS).

HLA TYPING

<u>A</u>	B	Bw	<u>C</u>	DR	DRB345	DQB	DQA1	DP	DPA1
2	39	6	7	1		4	01	105	01
30	42	6	17	8		5	04	402	03
2	39	6	12	18	52	4	01	1	02
30	42	6	17	10		5	04	17	02

HLA typing was performed with the solid phase sequence specific oligonucleotide (SSO) method (LabType, One Lambda) Top: Patient typing; Bottom: Donor typing.

HLA ANTIBODY TESTING

HLA antibody testing was performed on a recent serum sample with the solid phase single-antigen bead (SAB) based method (LabScreen, One Lambda). Figure 1 shows SAB testing throughout the year.

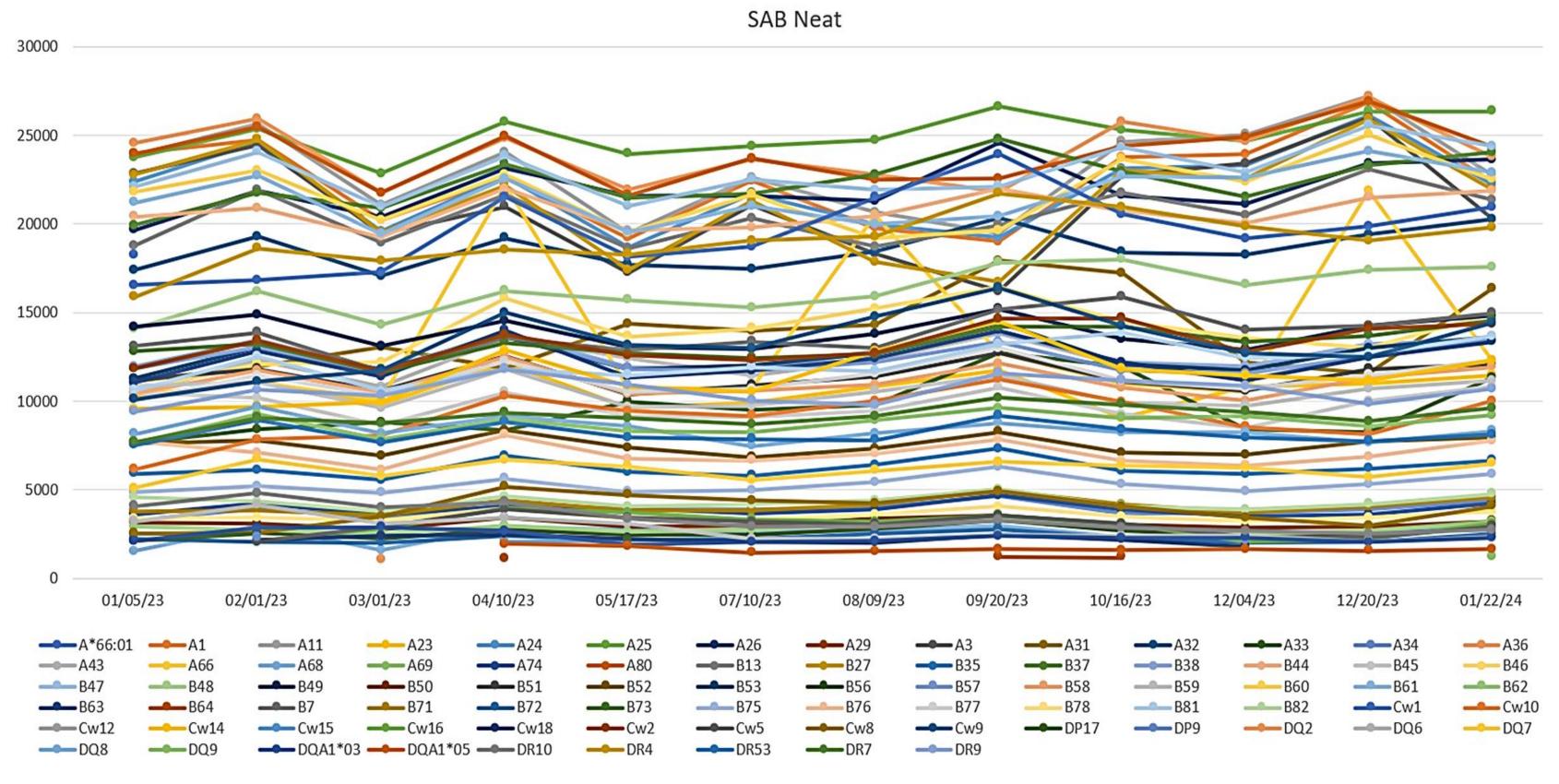


Figure 1: SAB testing Jan 2023 – Jan 2024

IDENTIFICATION OF A NEAR-MISS SENTINEL EVENT IN A DISEASED DONOR KIDNEY TRANSPLANT CANDIDATE

VIRTUALCROSSMATCH ASSESSMENT

Date of most recent serum tested for HLA antibodies by solid phase assay: 12/20/2023. The patient displays DSA to DR10 (2531 MFI) in the current 12/20/2023 serum. The patient displays historic DSA to DP17 (2280 MFI) seen last in the 12/04/2023 serum. DSA to DR10 is not a repeat mismatch from previous transplants. DP typing is not available for both previous donors to determine mismatch.

Current and historic donor-specific antibodies (DSA) were identified in the virtual crossmatch (VXM). Previous donors were not typed for HLA-DP locus.

PHYSICAL CROSSMATCH RESULTS

PXM results were unexpectedly strong positive for both T- and B-cells. PXM is discordant with VXM.

Serum Date	<u>Treatment</u>	Cell	Method	MCS*	Result**
01/22/2024	Pronase	T	FLOW	387	Pos
01/22/2024	Pronase	B	FLOW	331	Pos

INVESTIGATIONS

Repeated PXM results were still inconsistent with VXM findings. Communications with the transplant center ruled out possible interferences observed in some cases such as autoimmune diseases.

Coincidentally, the patient received a second offer from a deceased donor (donor 2) with identical HLA typing from the same Organ Procurement Organization (OPO) on the same day. Further information on the ethnicity of the two donors revealed that donor 1 was of African American (AFA) origin, while donor 2 was a Caucasian (CAU). Haplostats analysis indicated that donor 1 has an AFA haplotype (Figure 2). Offers from donor 1 were declined, and investigations were ongoing. HLA typing for donor 1 was conducted in two separate laboratories, resulting in completely different typing. The VXM and PXM results for

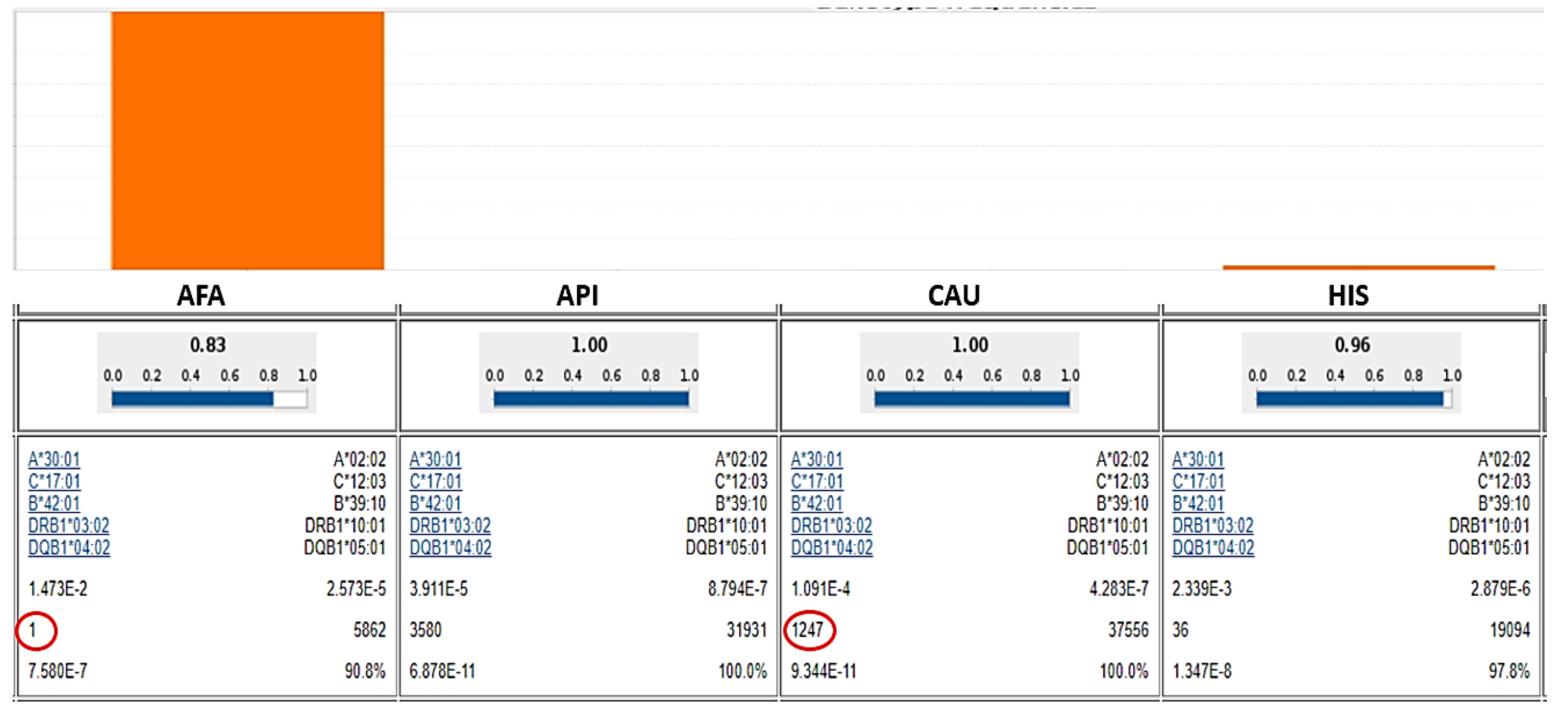


Figure 2: Haplostats Analysis shows the patient has one common haplotype in AFA and very rare in CAU

the patient donor1 and donor 2 are illustrated in Table 1

	- VXM														PXM				
		4		3	В	w	C			DR	DRB3		B345 DQB		DQA		DPB		
Patient	2	30	39	42	6	6	7	17	1	8			4	5	1	4	105	42	
Donor 1 (Original) CAU	2	30	39	42	6	6	12	17	18	10		52	4	5	1	4	1	17	T=387 MCS B=331 MCS
DSA MFI to Donor 1 (Original)										2762								2358	
Donor 2 AFA	2	30	39	42	6	6	12	17	18	10		52	4	5	1	4	1	17	NI/A
DSA MFI to Donor 2										2762								2358	N/A
						U	odated	VXM	l with	n correc	ct don	or ty	pin	g					
A			B Bw		C		DR	DRB345		DQB		DQA		DPB					
Patient	2	30	39	42	6	6	7	17	1	8			4	5	1	4	105	42	
Donor 1 (corrected) CAU	1	33	8	65	6	6	7	8	17	12	52	52	2	7	5	5	3	401	
DSA MFI to Donor 1 (Corrected)	22350	11345					4099							6509	1686	1686			

DISCUSSION

Investigations into the discrepancy have eliminated the possibility of new sensitization and prozone, as multiple single antigen bead (SAB) tests with dilutions in the patient's history have been conducted. Repeated PXM testing has consistently shown results that align with the initial findings, ruling out any potential sample swap of the donor cells and/or patient serum internally. Auto-immune diseases or HIV which could potentially cause false positive PXM results have also been ruled out.

Serendipitously, the patient was presented with a second deceased donor offer (Donor 2) on the same day, with identical HLA typing as the first donor (donor 1), both from the same Organ Procurement Organization. Further investigation into the ethnicity of the two donors revealed that Donor 1 was of African American (AFA) origin, while Donor 2 was a Caucasian (CAU). Based on HLA disequilibrium, it is highly unlikely for the two donors to carry the same HLA typing, because A30-B42-DR18-DQ4 haplotype is predominant in AFA populations and rare in CAU populations. Upon repeated HLA typing of Donor 1, it was discovered that there were discrepancies from the initial HLA typing provided for VXM. The updated VXM with the correct typing revealed strong DSA to A1, A33, Cw8, DQ7, and DQA1*05, which correlated with the strongly positive PXM result.

The deceased donor sample swap could have led to a hyper acute rejection and potential harm to recipients of other allocated organs. In conclusion, thorough investigation and clear communication are essential for resolving discrepancies in VXM/PXM results. Donor's ethnicity is very helpful in resolving such cases. PXM plays a vital role in ensuring the safety of highly sensitized and regrafted patients.

UCLA Health Immunogenetics Center

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