Antibody-mediated Rejection Without Detectable Donor-specific Antibody Releases Donor-derived Cell-free DNA: Results From the Trifecta Study

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Table S1. List of abbreviations and their definitions.	
Abbreviation	**Definition**
%dd-cfDNA	Percent donor-derived cell-free DNA as a fraction of total cfDNA
dd-cfDNA quantity	Copies per ml
AMR	antibody-mediated rejection
AIC	Aikeke's information criterion
ATAGC	Alberta Transplant Applied Genomics Centre
AUC	Area under the curve
BK	Polyoma virus nephropathy
cfDNA	Cell-free DNA
CLIA	Clinical Laboratory Improvement Amendments
dd-cfDNA	Donor-derived cell-free DNA
DSA	Donor-specific antibody
EABMR	Early stage AMR
FABMR	Fully-developed AMR
IRB	Institutional review board
LABMR	Late-stage AMR
MMDx	Molecular Microscope Diagnostic System
NK cells	Natural killer cells
NRI	net classification indices
OLI	One Lambda Inc.
PCR	Polymerase chain reaction
PRA	Panel-reactive antibody
PRAHR	PRA – high risk
SNP	Single nucleotide polymorphism
SOC	Standard of care
TCMR	T cell-mediated rejection
TxBx	Time posttransplant

Table S2. Trifecta Study co-authors (N = 280 biopsies): 18 institutions and 41 investigators that have contributed biopsies and cell-free DNA data.

Investigators	Institution	Location
Justyna Fryc	Medical University in Bialystok	Białystok, Poland
Beata Naumnik		
Jonathan Bromberg	University of Maryland School of Medicine	Maryland, Baltimore
Matt Weir		
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Milagros Samaniego-Picota	Henry Ford Transplant Institute	Detroit, Michigan
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Joanna Konopa		
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Andrzej Więcek	Medical University of Silesia	Katowice, Poland
Grzegorz Piecha		
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Miha Arnol		
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Marius Miglinas	Centre of Nephrology, Vilnius University Hospital Santaros Klinikos	Vilnius, Lithuania
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Joanna Mazurkiewicz		
Marta Gryczman		
Leszek Domański	University Hospital n.2, Szczecin	
Rajendra Baliga	Tampa General Hospital	Tampa Bay, Florida
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Dominika Dęborska-Materkowska		
Michał Ciszek		
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Leszek Pączek		
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Mladen Knotek	University Hospital Merkur	Zagreb, Croatia
Ksenija Vucur		
Zeljka Jurekovic		
Thomas Müller	University Hospital Zurich	Zurich, Switzerland
Thomas Schachtner		
Andrew Malone	Washington University at St. Louis	St. Louis, MO, USA
Tarek Alhamad		

Table S3. Demographics and clinical features of the Trifecta Study (N = 280) biopsy cohort.

Biopsy characteristics (N = 280)	
Days to biopsy posttransplant	
Mean	1353
Median (range)	447 (5-11 504)
Days to most recent follow-up after biopsy	
Mean	34
Median (range)	6 (0-308)
Indication for biopsy n (%)	
For cause	261 (94)
Surveillance	16 (6)
Missing	3 (1)
Patient demographics (N = 272)	
Mean patient age (range)	51 (19-77)
Age > 65 years, count	25
Mean donor age (range)	48 (6-81)
Patient sex	
Male, n (%)	173 (64)
Female, n (%)	97 (36)
Not available, n (%)	2 (1)
Donor gender	
Male, n (%)	137 (52)
Female, n (%)	126 (48)
Not available, n (%)	9 (3)
Patient ethnicity, n	
African American	9
Other	262
Not available[a]	1
Donor type n (% deceased donor transplants)	200 (75)
Status at last follow-up, n (%)	
Functioning graft	233 (91)
Graft failure/return to dialysis	21 (8)
Patient death with functioning graft	3 (1)
Primary disease, n	
Diabetic nephropathy (DN)	32
Hypertension / large vessel disease	10
Glomerulonephritis / vasculitis (GN)	102
Interstitial nephritis / pyelonephritis	2
Polycystic kidney disease	0
Others	76
Unknown etiology	50

[a] Some centers preferred not to identify ethnicity.

Table S4. Clinical variables and histologic lesion scores in DSA-negative vs DSA-positive MMDx AMR/mixed biopsies (N = 80).

Variable		Mean value			*P* for DSA-positive vs DSA-negative
		No rejection (N = 164)	DSA-positive AMR[b] (N = 35)	DSA-negative AMR (N = 45)	
Clinical	Median time of biopsy posttransplant, days	935	1564	1608	0.91
	GFR, cc/min	43.34	43.94	42.01	0.72
	Donor age, years	49.70	40.84	44.11	0.34
AMR lesions/ features	g (glomerulitis)	0.22	1.17	1.32	0.56
	ptc (capillaritis)	**0.17**	**1.69**	**1.14**	**0.04**
	cg (double contours)	0.17	1.09	0.95	0.60
	C4d staining[a]	0.13	0.40	0.30	0.33
TCMR lesions	i (interstitial infiltrate)	0.01	0.89	0.93	0.84
	t (tubulitis)	0.51	0.80	0.93	0.58
Rejection lesions	v (vasculitis)	0.08	0.09	0.22	0.20
Atrophy-fibrosis-related	ci (fibrosis)	0.99	1.18	1.14	0.84
	ct (atrophy)	1.08	1.24	1.37	0.47
	cv (fibrous intimal thickening)	1.16	1.00	1.13	0.60
	ah (hyalinosis)	1.26	1.27	1.13	0.60

[a] C4d staining is coded as positive = 1, negative = 0. Therefore, the means for this variable indicates the fraction of biopsies that were positive. Missing values were excluded from the calculations.

[b] Includes PRA high-risk (PRAHR) biopsies. Biopsies from PRA-positive patients with missing/unavailable donor phenotyping to assign DSA status were called PRAHR in this study and were analyzed as DSA positive.

AMR, antibody-mediated rejection; DSA, donor-specific antibody; GFR, glomerular filtration rate; MMDx, Molecular Microscope Diagnostic System; PRA, panel-reactive antibody; TCMR, T cell-mediated rejection.