American Journal of Transplantation 26 (2026) 117–130

Contents lists available at ScienceDirect

American Journal of Transplantation

journal homepage: www.amjtransplant.org

Original Article

Improving the histologic detection of donor-specific antibody-negative antibody-mediated rejection in kidney transplants

Luis G. Hidalgo [1,†], Katelynn S. Madill-Thomsen [2,†], Jeff Reeve [2], Martina Mackova [2], Philippe Gauthier [3], Zachary Demko [3], Adam Prewett [3], Michelle Lee [2], Tarek Alhamad [4], Sanjiv Anand [5], Miha Arnol [6], Rajendra Baliga [7], Mirosław Banasik [8], Christopher D. Blosser [9], Sindhura Bobba [10], Daniel Brennan [11], Jonathan Bromberg [12], Klemens Budde [13], Andrzej Chamienia [14], Kevin Chow [15], Michał Ciszek [16], Nadiesda Costa [12], Dominika Dęborska-Materkowska [13], Alicja Debska-Ślizień [14], Leszek Domański [17], Richard Fatica [18], Iman Francis [19], Justyna Fryc [20], John Gill [21], Jagbir Gill [21], Maciej Glyda [22], Sita Gourishankar [23], Marta Gryczman [17], Gaurav Gupta [10], Petra Hruba [24], Peter Hughes [15], Arskarapurk Jittirat [25], Zeljka Jurekovic [26], Layla Kamal [10], Mahmoud Kamel [7], Sam Kant [11], Nika Kojc [6], Joanna Konopa [14], Dhiren Kumar [10], James Lan [21], David Lowe [27], Joanna Mazurkiewicz [17], Marius Miglinas [28], Irfan Moinuddin [10], Thomas Mueller [29], Marek Myślak [17], Beata Naumnik [20], Leszek Pączek [16], Anita Patel [19], Agnieszka Perkowska-Ptasińska [16], Grzegorz Piecha [30], Emilio Poggio [18], Silvie Rajnochová Bloudíčkova [24], Heinz Regele [31], Thomas Schachtner [29], Soroush Shojai [23], Majid L.N. Sikosana [23], Janka Slatinská [24], Katarzyna Smykal-Jankowiak [22], Željka Veceric Haler [6], Ondrej Viklicky [24], Ksenija Vucur [26], Matthew R. Weir [12], Andrzej Wiecek [30], Ziad Zaky [18], Philip F. Halloran [2,*]

Abbreviations: ABMR; antibody-mediated rejection; ABMRM; ABMR or mixed rejection; dd-cfDNA; donor-derived cell-free DNA; DSA; donor-specific antibody; HLA; human leukocyte antigen; IRB; institutional review board; MMDx; Molecular Microscope Diagnostic System; MFI; mean fluorescence intensity; MVI; microcirculation lesions; NK cells; natural killer cells; NR; no rejection; NRI; net reclassification improvement; OLI; One Lambda Inc; pABMR; possible ABMR; pTCMR; possible TCMR; PRA; panel-reactive antibody; SN; nonspecific reactivities, high risk for DSA-positivity; SOC; standard-of-care; TCMR; T cell-mediated rejection.

* Corresponding author. Alberta Transplant Applied Genomics Centre, #250 Heritage Medical Research Centre, University of Alberta, Edmonton, AB T6G 2S2, Canada.
E-mail address: phallora@ualberta.ca (P.F. Halloran).
† co-first authors

https://doi.org/10.1016/j.ajt.2025.08.029
Received 2 June 2025; Received in revised form 1 August 2025; Accepted 18 August 2025
Available online 23 August 2025

[1] HLA Laboratory, Division of Transplantation, Department of Surgery, University of Wisconsin, Madison, WI, USA
[2] Alberta Transplant Applied Genomics Centre, Edmonton, AB, Canada
[3] Natera, Inc., San Carlos, CA, USA
[4] Division of Nephrology, Department of Internal Medicine, Washington University at St. Louis, St. Louis, MO, USA
[5] Intermountain Transplant Services, Murray, UT, USA
[6] Department of Nephrology, University of Ljubljana, Ljubljana, Slovenia
[7] Tampa General Hospital, Tampa, FL, USA
[8] Department of Nephrology and Transplantation Medicine, Medical University of Wrocław, Wrocław. Poland
[9] University of Washington, Seattle, WA, USA
[10] Division of Nephrology, Department of Internal Medicine, Virginia Commonwealth University, Richmond, VA, USA
[11] Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA
[12] Department of Surgery, University of Maryland, Baltimore, MD, USA
[13] Department of Nephrology, Charite-Medical University of Berlin, Berlin, Germany
[14] Department of Nephrology, Transplantology and Internal Diseases, Medical University of Gdańsk, Gdańsk. Poland
[15] Department of Nephrology, The Royal Melbourne Hospital, Parkville, Australia
[16] Department of Immunology, Transplantology and Internal Diseases, Warsaw Medical University, Warsaw, Poland
[17] Department of Nephrology, Transplantology and Internal Medicine, Pomeranian Medical University, Szczecin, Poland
[18] Department of Kidney Medicine, Cleveland Clinic Foundation, Cleveland, OH, USA
[19] Henry Ford Transplant Institute, Detroit, MI, USA
[20] 1st Department of Nephrology and Transplantation With Dialysis Unit, Medical University in Bialystok, Białystok, Poland
[21] St. Paul's Hospital, Vancouver, BC, Canada
[22] Wojewodzki Hospital, Poznan, Poland
[23] Department of Medicine, University of Alberta, Edmonton, AB, Canada
[24] Department of Nephrology, Transplant Center, Institute for Experimental and Clinical Medicine, Prague, Czech Republic
[25] University Hospital Cleveland Medical Center, Cleveland, OH, USA
[26] Renal Replacement Therapy, Department of Nephrology, University Hospital Merkur, Zagreb, Croatia
[27] One Lambda Inc., West Hills, CA, USA
[28] Nephrology and Kidney Transplantation Unit, Nephrology Center, Vilnius University Hospital Santaros Klinikos, Vilnius, Lithuania
[29] Department of Surgery and Transplantation, University Hospital Zurich, Zurich, Switzerland
[30] Department of Nephrology, Transplantation and Internal Medicine, Silesian Medical University, Katowice, Poland
[31] Department of Clinical Pathology, Medical University of Vienna, Vienna, Austria

ARTICLE INFO

Key words:
donor-derived cell-free DNA
kidney biopsy
donor-specific antibody
microarrays
antibody-mediated rejection
kidney transplant rejection

ABSTRACT

Emerging treatments for antibody-mediated rejection (ABMR, NEJM391 (2):122-132) have increased the importance of ABMR detection when donor-specific antibody (DSA) is negative. We addressed this issue in the Trifecta-Kidney study (ClinicalTrials.gov #NCT04239703) using 3 centralized tests in 690 kidney transplant biopsies: DSA (One Lambda Inc), blood donor-derived cell-free DNA (dd-cfDNA, Prospera™ test, Natera, Inc), and molecular biopsy assessment (MMDx). We used an "AutoBanff 2022" algorithm to model the impact of alternative DSA interpretations on the histologic diagnosis of DSA-negative ABMR following Banff guidelines, including agreement with dd-cfDNA and molecular ABMR. Lowering MFI cutoffs for DSA positivity did not improve the detection of DSA-negative ABMR. However, simply calling all DSA as positive allowed the Banff 2022 guidelines to identify 46% more ABMR cases with no measurable conventional DSA, and per net reclassification improvement increased agreement between histologic diagnoses and both dd-cfDNA ($P = 7.72E{-}7$) and molecular ABMR ($P = 7.69E{-}7$). New ABMR cases were as strongly positive for dd-cfDNA and molecular ABMR as those found using the conventional DSA interpretation. A validation set analysis using INTERCOMEX study data (ClinicalTrials.gov NCT#01299168) confirmed these findings and found that the new DSA-negative ABMR cases identified by calling all DSA-positive had the same risk for graft loss as those found with conventional DSA interpretation.

Trifecta-Kidney Study ClinicalTrials.gov #NCT04239703

1. Introduction

In kidney transplantation, antibody-mediated rejection (ABMR) is a major cause of functional deterioration and graft loss. Fortunately, new treatments such as anti-CD38 monoclonals are emerging in clinical trials,[1,2] but these will demand accurate assignment of histologic diagnosis, as well as effective monitoring of treatment efficacy and relapse. The key features of ABMR assessment[3-12] are microcirculation lesions and donor-specific human leukocyte antigen (HLA) antibody (DSA), as well as elevated plasma donor-derived cell-free DNA (dd-cfDNA)[13,14] and molecular features (Molecular Microscope Diagnostic System [MMDx]).[15,16] Histologic diagnosis of ABMR has been based on microvascular inflammation (MVI), glomerular cg lesions, C4d deposition, and positive DSA, but this has been complicated by the recognition that cases with no measurable DSA can have typical ABMR-related histologic, molecular, and dd-cfDNA findings.[15,17-21] Possible explanations proposed for DSA-negative ABMR include natural killer (NK) cell recognition of missing-self,[22-24] complete absorption of DSA by the kidney tissue,[25] undetected alloantibodies against HLA or non-HLA polymorphic proteins, and autoantibodies.[26] The Banff system has introduced changes to address the histologic diagnoses of ABMR when DSA is negative.[12]

The issue of DSA-negative ABMR was central to the launch of the Trifecta-Kidney study (ClinicalTrials.gov #NCT04239703). This study aimed to clarify the relationships between local standard-of-care (SOC) histology at the time of indication biopsy and 3 tests measured centrally to ensure standardization: DSA, dd-cfDNA, and molecular biopsy assessment. The local SOC diagnosis was assigned by local pathologists following their interpretation of the current Banff guidelines as known at the time of biopsy, but also reflects their "gestalt" assessment considering locally available information and clinical suspicions, and can sometimes differ from the rigorous application of the guidelines. Because the Trifecta study aimed to reflect the SOC diagnosis that will guide therapy, no attempt was made to reinterpret the calls recorded by the local pathologists using central review. Dd-cfDNA was measured using the Prospera™ test (Natera, Inc), DSA was measured by One Lambda and interpreted by LGH, and the molecular phenotype of the biopsy was assessed by MMDx.[27,28] The Trifecta-Kidney study previously found that dd-cfDNA was strongly related to molecular ABMR activity as defined by increased expression of NK cell-expressed and *IFNG*-inducible genes,[27,29,30] but was also increased in T cell-mediated rejection (TCMR) and AKI[13]; was more strongly related to molecular than histologic rejection[27,31]; and was as elevated in DSA-negative ABMR as in DSA-positive ABMR.[14]

The present analysis examined options for improving the histologic diagnosis of ABMR using the Banff guidelines when DSA is negative, and tested agreement between these alternatives and both dd-cfDNA and molecular ABMR findings. Our hypothesis was that current DSA measurements may be missing DSA in some cases with typical ABMR microvascular lesions. We developed an "AutoBanff 2022" algorithm to model the impact of alternative DSA interpretation on histologic SOC ABMR diagnosis by local pathologists following the existing Banff guidelines.[12]

The study workflow is summarized in Figure 1A, B; abbreviations used are listed in Supplementary Table S1.

2. Materials and methods

2.1. Population and demographics

Trifecta-Kidney (ClinicalTrials.gov #NCT04239703) is a prospective multicenter study of consenting patients, currently involving 64 investigators from 28 transplant institutions under local institutional review board-approved protocols as previously described.[27] Of 821 biopsies collected, 39 were excluded because DSA was not analyzable, and a further 92 cases were removed due to missing histology lesion or diagnosis data, leaving 690 for these analyses.

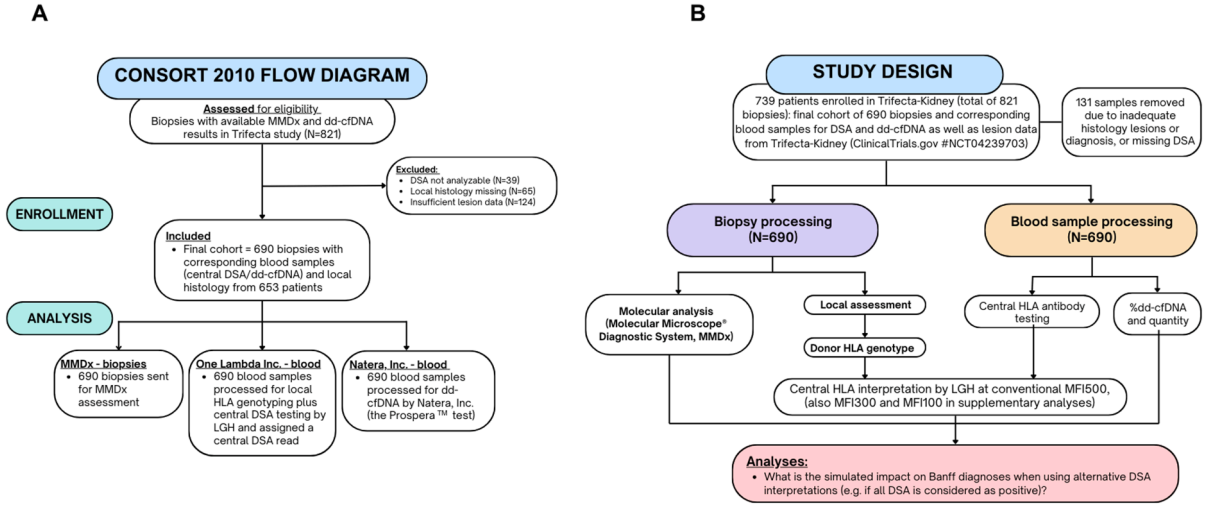

Figure 1. Sample inclusion diagram and study workflow for this Trifecta-Kidney N782 study. (A) CONSORT showing sample inclusion and exclusion, as well as sample processing methodology for this study. (B) Study design showing analytical workflow for the biopsy and blood-based assessments. ABMRM, antibody-mediated rejection plus mixed rejection; CONSORT, consolidated standards of reporting trials.

Selected analyses were repeated or supplemented with data from the International Collaborative Microarray Study Extension (INTERCOMEX) study (ClinicalTrials.gov #NCT01299168), where a subset of 1709 biopsies (from the published N = 5086 dataset[28]) had the recorded histology lesions and clinical data necessary for these analyses.

2.2. Sample collection and data processing

The study CONSORT and workflow are outlined in Figure 1A, B. Centrally tested HLA antibodies and dd-cfDNA (both quantity and percent of total) were measured in blood drawn per established protocols (blood for dd-cfDNA was taken immediately before the biopsy to avoid detecting dd-cfDNA released by the biopsy procedure).

2.3. Microarray analysis

The molecular biopsy phenotype was assessed by MMDx. A portion of one core of each biopsy (mean length 3 mm)[32] was immediately stabilized in RNA*later* and shipped to the Alberta Transplant Applied Genomics Centre (Edmonton, Canada, http://atagc.med.ualberta.ca) for RNA extraction and processing per established protocols[32] using GeneChip PrimeView 219 Human Gene Expression Arrays (Applied Biosystems). CEL files are available on the Gene Expression Omnibus website (GSE303670).

2.4. Biopsy sampling, data collection, and histologic diagnoses

Molecular analyses were completed and diagnoses sent to participating centers usually within 2 working days of receiving the biopsy, and were made without knowledge of the biopsy's corresponding histology, clinical data, HLA antibody status, or dd-cfDNA results.

Histologic and clinical data, DSA testing results, and local histology diagnoses (based on the local opinion following Banff guidelines) were collected at each center per SOC as approved by institutional review boards and submitted to the study as available. As detailed in the earlier report,[33] histology diagnoses were interpreted as "No rejection," "ABMR," "possible ABMR" ("pABMR", equivalent to "ABMR suspicious"), "TCMR," "possible TCMR" ("pTCMR", equivalent to "Borderline"), and "Mixed rejection", to facilitate direct comparison with MMDx (with no knowledge of MMDx results) and the AutoBanff simulated histology output (see section below in "Methods"). Central histology review was not specified in the protocol because it was not SOC.

2.5. Central HLA antibody measurements and DSA interpretation

Serum samples for HLA antibody testing were collected at the time of biopsy and shipped per standard protocols to One Lambda Inc (OLI) for centralized testing. HLA antibodies were tested using LABScreen Single Antigen beads (OLI, Canoga Park, California) according to the manufacturer's recommendations. HLA typing at each center was done per SOC and was judged by an expert (LGH) as moderate resolution. Donor and recipient HLA genotypes provided by each center were analyzed along with HLA antibody test results and interpreted as DSA by a single expert (LGH), blinded to histology, molecular diagnoses, and dd-cfDNA results.

Although HLA antibody specificities were interpreted as positive using a conventional MFI threshold of 500, and alternate thresholds MFI300 or MFI100, we emphasize that DSA assignment did not only depend solely on MFI cutoffs but also on patterns of epitope reactivity and avoidance of nonspecific bead reactivities. DSA was generally identified when the beads corresponding to the donor HLA type was $\geq$500 MFI (ie, conventional DSA), $\geq$300 MFI, or $\geq$100 MFI. Samples negative for all HLA antibodies were labeled panel-reactive antibody (PRA)-negative (and by definition DSA-negative); samples positive for HLA antibodies but negative for DSA were labeled as DSA-negative, PRA-positive. Biopsies from PRA-positive patients with missing/unavailable donor typing were called PRA-High Risk and were analyzed here as DSA-positive unless otherwise noted to avoid under-calling DSA positivity, as done in previous Trifecta studies.[14] Analyzed HLA antibody results were labeled as "suspected non-specific" (SN) when the reactivity pattern followed that of well-recognized nonspecific reactivities (also known as natural HLA antibodies[34]) or reactivities that do not align with a given epitope, and analyzed here as DSA-negative unless otherwise noted, similar to previous analyses.[14] In some analyses, PRA-High Risk and SN samples were left out to assess only clear DSA-positive versus DSA-negative status.

2.6. Statistics

All analyses used version 4.2.2 of *R*.[35] Net reclassification improvement (NRI) used the "improveProb" function in the Hmisc package.[36]

2.7. AutoBanff model

The effect of calling all DSA as positive or changing DSA cutoffs on histology calls following the Banff 2022 guidelines was estimated using the previously described "AutoBanff" algorithm[37] updated to simulate the 2022 guidelines.[12] The algorithm was reviewed by 2 Banff-participating pathologists (Heinz Regele and Agnieszka Perkowska-Ptasińska) to be certain it represented Banff 2022 guidelines as accurately as possible. (AutoBanff is a research tool and is not intended to be used clinically, but could be made available if pathologists and clinicians wish to use it.) AutoBanff, outlined in Figure 2, follows through panels A-D in order, using the locally reported rejection-associated histology lesions (i-, t-, v-, g-, ptc-, and cg-lesions) as well as DSA and C4d status (+/−) to produce an ABMR call (no ABMR, possible ABMR "pABMR," microvascular inflammation "MVI," or ABMR) and a TCMR call (no TCMR, possible TCMR "pTCMR," TCMR). These calls and the MMDx signouts were both condensed to a set of 6 classes in Supplementary Table S2 to permit direct comparison of histology and MMDx signouts. The pABMR category (ie, possible

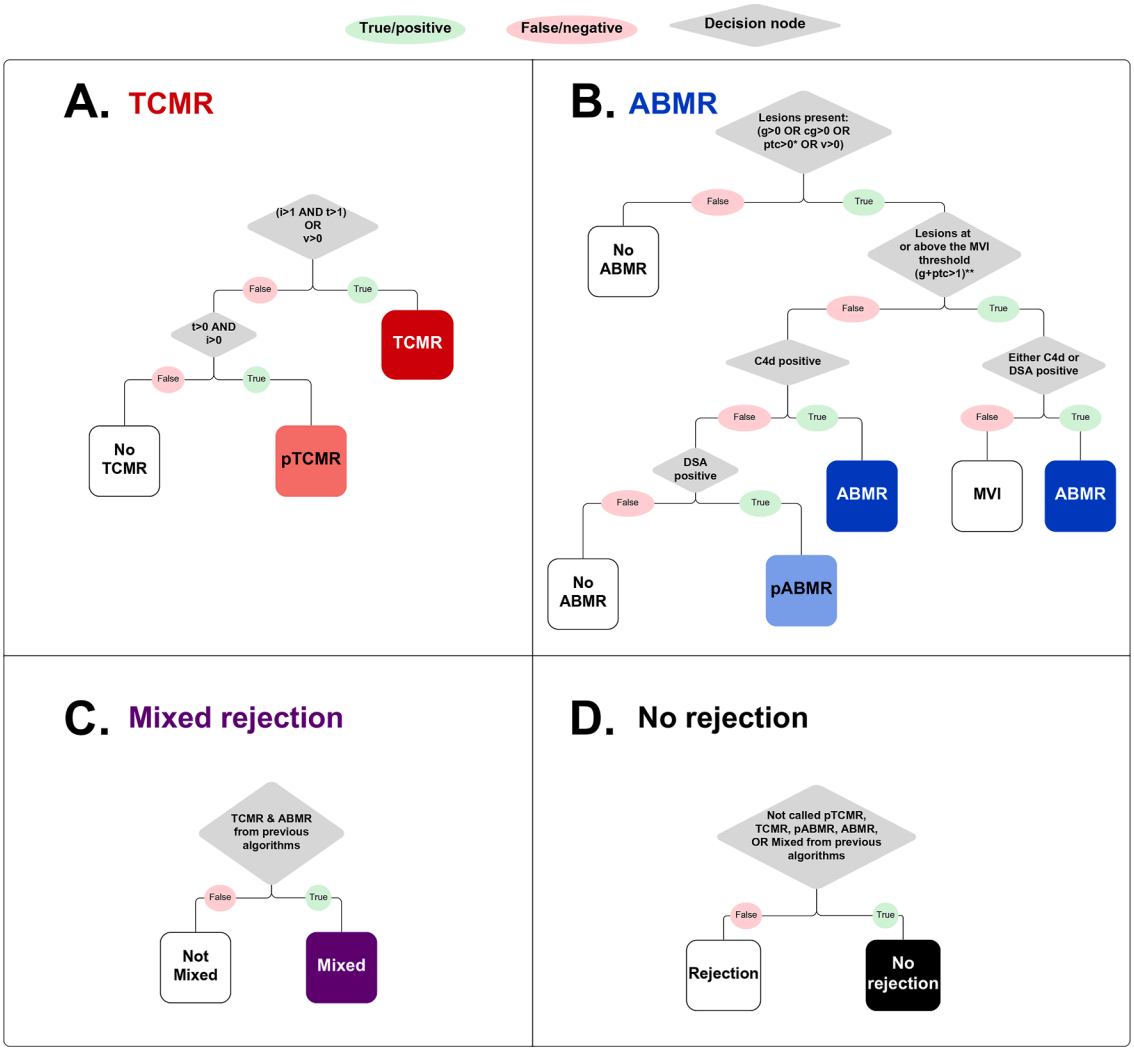

Figure 2. AutoBanff algorithm schematic (reviewed by 2 pathologists—AP and HR), showing the decision tree algorithm for each of (A) TCMR, (B) ABMR, (C) Mixed rejection, and (D) No rejection (in this order). AutoBanff uses only rejection-associated lesions and clinical features: g-, ptc-, cg-, v-, i-, and t-lesions, as well as C4d and DSA status (positive or negative). If any given condition is false (not met), the path follows through red nodes, and if the condition is true (is met successfully), the path follows through green nodes. Diagnostic calls are shown at terminal nodes and consist of 7 possible outcomes: pABMR (equivalent to "ABMR suspicious"), MVI, ABMR, pTCMR (equivalent to "borderline"), TCMR, Mixed rejection "Mixed", and No rejection "NR." For further analyses in this study, pABMR and MVI were collapsed into a single pABMR/MVI group. In situations where missing data could not impact the final diagnosis, the missing data were ignored; however, in scenarios where missing data would not permit a final call on either ABMR or TCMR (ie, missing lesions), the sample was labeled as "inadequate" and left out of further analyses in this study. *If there is no TCMR, then ptc $>$ 0; however, if there is TCMR/pTCMR present, ptc $>$ 0 alone is not sufficient, and you must also have g $>$ 0. **This is only valid if there is no TCMR/pTCMR present; otherwise, if TCMR/pTCMR are present ptc $>$ 0 alone is not sufficient, and you must have g $>$ 0.

ABMR or suspicious for ABMR) was combined with MVI into a single pABMR/MVI category to compare with MMDx pABMR. AutoBanff diagnosed no MVI cases when all DSA was considered as positive because this converted the MVI criteria to full ABMR.

3. Results

3.1. Study population and demographics

Characteristics of the 690 biopsy population (Table 1) were similar to previous Trifecta-Kidney analyses.[14] Of the 690 biopsies, 634 (92%) were recorded as being for indications. The mean time of biopsy posttransplant was 1301 days (approximately 3.6 years), the mean patient age was 50 years, 38% of patients were female, and 75% of kidneys were from deceased donors.

3.2. The effect of calling all DSA-positive on histologic recognition of ABMR and mixed rejection

We used an updated AutoBanff algorithm[37,38] (see Methods) representing the Banff 2022 guidelines[12] to model the impact of alternative DSA interpretations on the SOC histologic diagnoses of ABMR and mixed rejection (ABMRM), as well as examine agreement between local histology, molecular ABMRM diagnoses and dd-cfDNA.

Table 1
Demographics and clinical features of the Trifecta-Kidney study (N = 690) biopsy cohort.

Biopsy characteristics (N = 690)	
Days to biopsy posttransplant	
Mean	1301
Median (range)	419 (1-13,441)
Days to the most recent follow-up after biopsy	
Mean	26
Median (range)	6 (0-308)
Indication for biopsy (% of total)	
For cause	634 (92%)
Surveillance	52 (8%)
Missing	4 (1%)
Patient demographics (N = 653)	
Mean patient age (range)	50 (18-81)
Age >65 y (count)	79
Mean donor age (range)	45 (5-81)
Patient gender	
Male (% of known)	401 (62%)
Female (% of known)	250 (38%)
Not available	2 (0%)
Donor gender	
Male (% of known)	339 (57%)
Female (% of known)	260 (43%)
Not available	54 (8%)
Patient ethnicity	
African American	100 (15%)
Other	548 (85%)
Not available[a]	5 (1%)
Donor type (% deceased donor transplants)	477 (75%)
Status at last follow-up	
Functioning graft	572 (95%)
Graft failure/return to dialysis	29 (5%)
Patient death with functioning graft	3 (<1%)
Primary disease	
Diabetic nephropathy (DN)	113 (17%)
Hypertension/large vessel disease	50 (8%)
Glomerulonephritis/vasculitis (GN)	221 (34%)
Interstitial nephritis/pyelonephritis	5 (1%)
Polycystic kidney disease	67 (10%)
Others	124 (19%)
Unknown etiology	73 (11%)
Histology rejection diagnoses (% of known)	
No rejection (NR)	341 (49%)
Possible ABMR (pABMR)	63 (9%)
ABMR	104 (15%)
Possible TCMR (pTCMR)	55 (8%)
TCMR	95 (14%)
Mixed rejection	32 (5%)
Not available	-

[a] Some centers preferred not to identify ethnicity.

AutoBanff using current DSA assessments diagnosed 149 histologic ABMRM and 541 no ABMRM (Table 2). To our surprise, simply calling all DSA as positive allowed AutoBanff to identify 68 new ABMRM cases, an increase of 46%, all with no measurable conventional DSA.

We examined the details of these 68 newly recognized histologic ABMR cases (Table 3, Fig. 3). Fifty cases had previously been called suspicious for ABMR or MVI (ie, pABMR/MVI) using the conventional DSA interpretation, indicating typical ABMR lesions. In addition, 17 cases previously called TCMR were reclassified as Mixed rejection. One case previously called NR with conventional DSA was reclassified as histologic ABMR.

3.3. Molecular ABMR and dd-cfDNA findings in the additional ABMRM cases identified when all DSA was considered positive

A key issue in these analyses was whether considering all DSA to be positive improved the agreement between histologic ABMR diagnoses and both dd-cfDNA and molecular ABMR assessments (Table 4, comparing ABMRM to No ABMRM). Using NRI, calling all DSA as positive in the AutoBanff model significantly improved agreement with dd-cfDNA (NRI, $P = 7.72E{-}7$) and molecular ABMR (NRI, $P = 7.69E{-}7$), and even with local histology diagnoses (NRI, $P = 0.003$).

These findings were validated in 1709 biopsies from the independent INTERCOMEX study with available histology lesions and clinical features required for AutoBanff assessment. Considering all DSA as positive significantly improved the agreement between AutoBanff ABMRM and both local histology (NRI, $P = 9.6E{-}6$) and molecular ABMR (NRI, $P = 6.1E{-}11$) compared with using conventional DSA (Supplementary Table S3).

We specifically compared the 68 new AutoBanff ABMR diagnoses identified when all DSA was considered positive to the 149 cases found by AutoBanff using conventional DSA interpretation (Table 5, showing all 6 diagnostic categories, the 68 new cases and 149 conventional cases do not overlap). The 68 newly found DSA-negative ABMR cases were as likely to have high dd-cfDNA (71% vs 66%) and molecular ABMRM (63% vs 66%) as the 149 cases identified using conventional DSA

Table 2
Using simulated histology diagnoses per rejection calls assigned by the AutoBanff 2022 algorithm to examine the effect of calling all DSA as positive compared with conventional DSA-positivity (N = 690).

		Simulated histology diagnoses using conventional DSA-positivity cutoff		Row totals
		ABMRM[a]	No ABMRM[b]	
Simulated histology diagnoses when calling all DSA as positive	ABMRM[a]	149	68 (+46%)	*217*
	No ABMRM[b]	0	473	*473*
Column totals		*149*	*541*	*690*

Gray shading indicates agreement.

Italics denote rows or columns with sum totals.

Samples with insufficient lesions/features for a Banff rejection assessment, ie, missing some/all of the i-, t-, v-, ptc-, cg-, g-lesion scores, DSA status (unknown or ambiguous), or C4d status, were called "inadequate" and were left out of this table (N = 85 samples removed).

ABMRM, antibody-mediated rejection plus mixed rejection; DSA, donor-specific antibody.

[a]ABMRM includes clear cases of ABMR or mixed rejection only.

[b]No ABMRM included TCMR, pTCMR, pABMR, and NR calls.

Table 3
Simulated histology diagnosis categories assigned by the AutoBanff 2022 algorithm with central DSA status determined bycalling all DSA as positive (rows) versus using the conventional threshold (excluding all "inadequate",[a] N = 690 [columns]).

		Simulated histology diagnoses using the conventional DSA threshold						
		ABMR	Mixed	NR	pABMR/MVI	pTCMR	TCMR	Row totals
Simulated histology diagnoses when calling all DSA as positive	ABMR	120	0	**1**	**50**	0	0	*171*
	Mixed	0	29	0	0	0	**17**	*46*
	NR	0	0	263	0	0	0	*263*
	pABMR	0	0	**43**	22	**5**	0	*70*
	pTCMR	0	0	0	0	69	0	*69*
	TCMR	0	0	0	0	0	71	*71*
	Column totals	*120*	*29*	*307*	*72*	*74*	*88*	*690*

Bolding and dark gray shading indicates discordant cases.

Light gray shading indicates agreement.

Italics denote rows or columns with sum totals.

ABMR, antibody-mediated rejection. TCMR, T cell-mediated rejection. NR, no rejection. pABMR, possible ABMR. pTCMR, possible TCMR. MFI, mean fluorescence intensity.

[a]Insufficient lesions/features for a Banff rejection assessment, ie, missing some/all of the i-, t-, v-, ptc-, cg-, g-lesion scores, DSA status (unknown or ambiguous), or C4d status were labeled "inadequate" and excluded from this table (N = 85).

interpretation. In addition, 25 of the 68 cases had already been assigned SOC ABMR diagnoses by the local pathologists. Calling all DSA-positive had minimal effect on the interpretation of biopsies called NR by AutoBanff.

The cases called ABMR by local SOC histology but not by AutoBanff (even after assuming all DSA was positive) often had high dd-cfDNA, molecular ABMR, and even DSA in some cases, indicating that the AutoBanff model misses some true ABMR (data not shown). This is expected: as noted in the "Introduction" section, pathologists interpret the biopsy based on the total information available to them in addition to the Banff guidelines. Noting that the local SOC pathologists will sometimes differ from strict application of the guidelines when diagnosing ABMR, we found that the strict adherence to Banff 2022 guidelines in AutoBanff (using conventional DSA interpretation) compared with the SOC local histology diagnosis did not improve the agreement with dd-cfDNA (NRI, $P = .46$) or with MMDx ABMRM (NRI, $P = .63$) (see footnote in Table 4). Thus, assigning diagnoses using strict adherence to Banff rules did not improve the relationship of the histology diagnoses to either dd-cfDNA or MMDx.

In summary, when all DSA is considered positive, the AutoBanff model and the Banff 2022 histology guidelines detect 46% more ABMR and mixed rejection than when using conventional DSA thresholds. These newly found DSA-negative ABMR cases were as likely to be molecular ABMRM and dd-cfDNA positive as those diagnosed using the conventional DSA interpretation. Considering all DSA as positive also improved the agreement

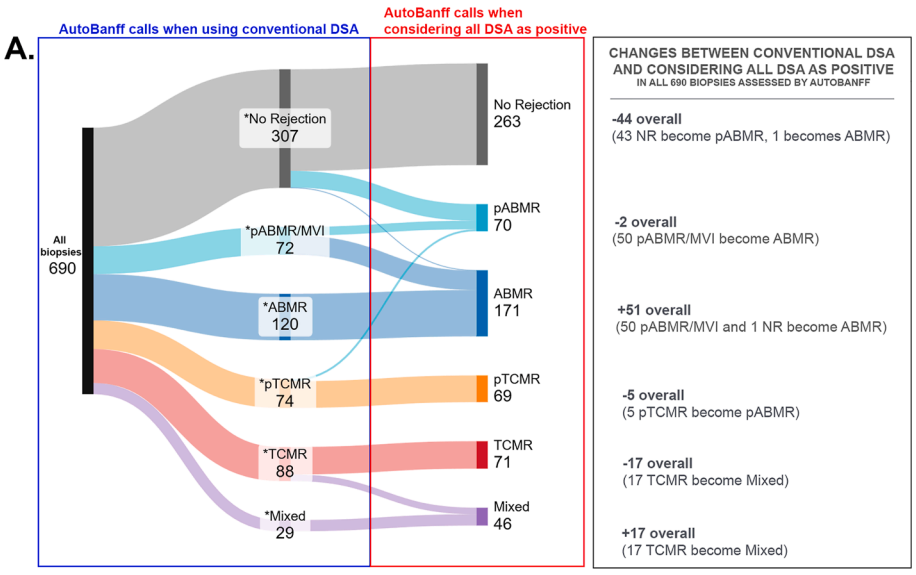

Figure 3. Sankey plot showing the redistribution of AutoBanff 6 class calls when considering all DSA as positive instead of conventional DSA (set at MFI500 for positivity). The total 690 biopsies with available AutoBanff calls are shown as the black node on the far left. The middle nodes show the distribution of those 690 biopsies into 6 classes (pABMR/MVI, ABMR, pTCMR, TCMR, Mixed rejection "Mixed". and No rejection "NR") when using conventional DSA-positivity inputs, and the far-right nodes show redistribution by AutoBanff when all DSA was considered positive. ABMR, antibody-mediated rejection; ABMR-Prob, ABMR probability classifier score; DSA, donor-specific antibody; pABMR, possible ABMR; pTCMR, possible TCMR; TCMR, T cell-mediated rejection.

between histologic diagnoses and both dd-cfDNA and molecular ABMRM.

3.4. Validation testing

We examined the AutoBanff ABMR diagnoses in an independent cohort collected in the INTERCOMEX study (ClinicalTrials.gov NCT#01299168). In 1709 biopsies with available data, AutoBanff diagnosed 166 more ABMR cases (a 45% increase) when all DSA was called positive than when conventional DSA was used (Supplementary Table S3). (INTERCOMEX analyses used DSA assessed locally by each center because central DSA was not tested in this study). By NRI, these newly found DSA-negative ABMR cases agreed more with the molecular ABMR diagnoses than those made using conventional DSA assessment.

Although Trifecta-Kidney was too recent and had too few post-biopsy failures to assess outcomes, the larger INTERCOMEX study had longer available follow-up and more failures. We compared graft outcomes in the ABMRM cases diagnosed by AutoBanff using conventional DSA to new DSA-negative ABMR cases found by AutoBanff when all DSA was considered to be positive. Both the newly diagnosed DSA-negative ABMR group and the ABMR diagnosed using conventional DSA interpretation had significantly increased graft failure compared with NR (Fig. 4, black line, log-rank test comparing conventional DSA ABMRM to NR, $P = .0014$, comparing all DSA as positive ABMRM with NR, $P = .0038$). There was no difference in risk for graft loss between the ABMR groups (Fig. 4, green and blue lines, post hoc analysis using log-rank test, $P = .5$).

In the INTERCOMEX data set, compared with local histology, AutoBanff considering all DSA-positive agreed better with dd-cfDNA (NRI, $P = 4.2E{-}3$) and MMDx (NRI, $P = 3.1E{-}5$). AutoBanff using conventional DSA interpretation did not improve agreement (NRI, $P > .05$ in both comparisons).

3.5. Effect of lowering MFI thresholds on central DSA-positive calls

We examined the impact of using lower MFI thresholds on the detection of ABMR. Lowering the MFI500 threshold to MFI300 or MFI100 resulted in relatively few additional cases being called central DSA-positive. In 690 cases, the MFI300 threshold produced 12 more DSA-positive sera and MFI100 found 30 more DSA-positive sera compared with conventional MFI500 (Supplementary Table S4). MFI300 called 4 additional DSA-positive ABMRM cases (+3%), and MFI100 called 11 additional DSA-positive ABMRM cases (+9%). The lower MFI thresholds did not improve agreement with dd-cfDNA or molecular ABMR because the DSA-positive cases with no molecular ABMRM ("false positives") also rose.

Lowering the MFI500 threshold to MFI300 or MFI100 increased sensitivity but decreased specificity, with no effect on balanced accuracy for the prediction of either molecular ABMR or dd-cfDNA by DSA (Supplementary Table S5).

Thus, reducing the MFI threshold for DSA positivity from 500 to 300 or 100 found somewhat more DSA-positive cases but did not improve the overall ability of DSA positivity to predict either dd-cfDNA or molecular ABMR.

Although Trifecta-Kidney is not intended to address the relative value of DSA screening versus dd-cfDNA screening, the DSA and dd-cfDNA findings at the time of biopsy in this study indicate that dd-cfDNA predicts ABMRM in the biopsy better than DSA, and that adding DSA to dd-cfDNA does not improve the prediction of ABMRM by dd-cfDNA alone (Supplementary Table S6).

3.6. PRA-positivity is not an alternative to DSA-positivity

Because considering all DSA as positive improved the agreement between the AutoBanff histology calls and local

Table 4
Comparing the simulated histology diagnoses by the AutoBanff algorithm to local histology diagnoses, MMDx diagnoses (compressed classes: ABMRM versus No ABMRM), and %dd-cfDNA (in samples with known AutoBanff classes assigned and known local histology, N = 690).

Simulated histology diagnoses using the AutoBanff algorithm[d,e,f,g]		Local histology diagnoses[a] (summarized from 6 classes)		MMDx[b] (summarized from 6 classes)		%dd-cfDNA[c]		Row totals (# added compared with using conventional DSA)
		ABMRM[h]	No ABMRM[i]	ABMRM[h]	No ABMRM[i]	≥1.0	<1.0	
Using conventional DSA	ABMRM[h]	85	64	98	51	98	51	*149*
	No ABMRM[i]	51	490	123	418	181	360	*541*
Considering all DSA as positive	ABMRM[h]	110 (+25)	107 (+43)	141 (+43)	76 (+25)	146 (+48)	71 (+20)	*217 (+68)*
	No ABMRM[i]	26 (−25)	447 (43)	80 (−43)	393 (−25)	133 (−48)	340 (−20)	*473*
Column totals		*136*	*554*	*221*	*469*	*279*	*411*	*690*

Grey shading indicates agreement.

Italics denote rows or columns with sum totals.

[a] Net Reclassification Improvement: AutoBanff ABMRM calls per conventional DSA vs considering all DSA as positive - agreement with local histology ABMRM P=0.003 (directionality – considering all DSA as positive is better).

[b] Net Reclassification Improvement: AutoBanff ABMRM calls per conventional DSA vs all DSA as positive - agreement with MMDx P = 7.69E-7 (directionality – considering all DSA as positive is better).

[c] Net Reclassification Improvement: AutoBanff ABMR/Mixed calls per conventional DSA vs all DSA as positive agreement with dd-cfDNA ≥ 1.0 P = 7.72E-7 (directionality – considering all DSA as positive is better).

[d] Net Reclassification Improvement comparing AutoBanff using conventional DSA vs local histology agreement with MMDx: P = 0.63.

[e] Net Reclassification Improvement comparing AutoBanff considering all DSA as positive vs local histology - agreement with MMDx: P = 3.1E-5.

[f] Net Reclassification Improvement comparing AutoBanff using conventional DSA vs local histology - agreement with dd-cfDNA: P = 0.46.

[g] Net Reclassification Improvement comparing AutoBanff considering all DSA as positive vs local histology - agreement with dd-cfDNA: P = 4.2E-3.

[h] ABMRM includes clear cases of ABMR or Mixed rejection only.

[i] Samples with insufficient lesions/features for a Banff rejection assessment i.e. missing some/all of the i-, t-, v-, ptc-, cg-, g-lesion scores, DSA status (unknown or ambiguous), or C4d status were labeled 'Inadequate' and excluded from this table (N = 85). 'No ABMRM' included TCMR, pTCMR, pABMR and NR calls. Abbreviations: ABMRM, antibody-mediated rejection plus Mixed rejection; dd-cfDNA, donor-derived cell-free DNA; DSA, donor-specific antibody; MMDx, the Molecular Microscope Diagnostic System.

histology ABMR diagnoses, MMDx ABMR diagnoses, and dd-cfDNA positivity, we also considered whether substituting PRA-positivity for conventional DSA would improve the agreement. However, when comparing conventional DSA-positivity with PRA-positivity, conventional DSA agreed better with MMDx ABMRM (NRI, $P = 2.17E{-}4$), indicating that PRA-positivity was not an appropriate substitute for DSA measurements within the Banff 2022 guidelines.

4. Discussion

The diagnosis of ABMR and Mixed rejection has been a challenge[39] since the first recognition that DSA can be associated with microcirculation inflammation 35 years ago,[4,40] and the potential availability of new effective ABMR treatments makes histologic assessment of ABMR even more important. A recent editorial by Cornell et al.[41] states that "even before the most recent iteration of the Banff schema defining milder forms of AMR for investigation, physicians found difficulties with Banff AMR categories, rendering cases vulnerable to misclassification." The present analysis of 690 biopsies in Trifecta-Kidney examines potential improvements in the histologic assessment of ABMR in cases with no measurable DSA. Calling all DSA as positive allowed the existing Banff 2022 guidelines as modeled in AutoBanff to identify 68 additional histologic ABMRM cases (a 46% increase over the same algorithm using conventionally assessed DSA), and the new cases were as often positive for dd-cfDNA and molecular ABMR as those already diagnosed using conventional thresholds for DSA-positivity. A validation analysis in the INTERCOMEX cohort confirmed these conclusions and showed that the newly found ABMR cases (all with no measurable conventional DSA) had increased risk of graft loss similar to that in ABMR cases diagnosed using the conventional DSA interpretation. The AutoBanff model indicates that the existing Banff rules can diagnose more ABMRM by simply considering all DSA to be positive—and these new cases had typical histologic ABMR lesions, elevated dd-cfDNA, molecular ABMR activity, and comparable increased risk of graft loss compared to No rejection. We propose that the next Banff

Table 5
Features of AutoBanff diagnostic groups using conventional DSA versus those when all DSA is considered positive (N = 690).

AutoBanff diagnosis	AutoBanff version	Fraction called ABMRM by local histology	Fraction called ABMRM by MMDx	Fraction called conventional DSA-positive (central conventional DSA)	Fraction called central %dd-cfDNA-positive (≥1)	Continuous %dd-cfDNA (mean, standard deviation)
No rejection	Using conventional DSA (N = 307)	9/307 (3%)	29/307 (9%)	51/307 (17%)	61/307 (20%)	0.33 (0.09-1.34)
	Considering all DSA as positive (N = 263)	7/263 (3%)	21/263 (8%)	51/263 (19%)	53/263 (20%)	0.34 (0.09-1.39)
pABMR/MVI	Using conventional DSA (N = 72)	21/72 (29%)	44/72 (61%)[a]	22/72 (31%)[c]	44/72 (61%)[e]	1.06 (0.29-3.87)[g]
	Considering all DSA as positive (N = 70)	5/70 (7%)	24/70 (34%)[a]	22/70 (31%)[c]	18/70 (26%)[e]	0.45 (0.12-1.64)[g]
ABMRM	Using conventional DSA (N = 149)	85/149 (57%)	98/149 (66%)[b]	98/149 (66%)[d]	98/149 (66%)[f]	1.39 (0.48-3.98)[h]
	Considering all DSA as positive (N = 217)	110/217 (51%)	141/217 (65%)	98/217 (45%)	146/217 (67%)	1.32 (0.42-4.17)
	Newly identified cases only (N = +68)	*25/68 (35%)*	*43/68 (63%)[b]*	*0/68 (0%)[d]*	*48/68 (71%)[f]*	*1.19 (0.31-4.52)[h]*
pTCMR	Using conventional DSA (N = 74)	5/74 (7%)	12/74 (16%)	15/74 (20%)	27/74 (36%)	0.58 (0.15-2.24)
	Considering all DSA as positive (N = 69)	5/69 (7%)	9/69 (13%)	15/69 (22%)	26/69 (38%)	0.58 (0.15-2.26)
TCMR	Using conventional DSA (N = 88)	15/88 (17%)	38/88 (43%)	22/88 (25%)	49/88 (56%)	0.89 (0.25-3.21)
	Considering all DSA as positive (N = 71)	9/71 (13%)	26/71 (37%)	22/71 (31%)	36/71 (51%)	0.81 (0.22-2.94)

Italics denote new ABMRM cases only.

Samples identified as TG (including TG in combination with other histologic findings) by local histology were typically identified as 'pABMR' by the AutoBanff algorithm.

ABMRM, antibody-mediated rejection plus mixed rejection; dd-cfDNA, donor-derived cell-free DNA; DSA, donor-specific antibody; pTCMR, possible T cell-mediated rejection; MMDx, the Molecular Microscope Diagnostic System, TCMR, T cell-mediated rejection.

[a]Chi-squared, $P = .002$.

[b]Chi-squared, $P = .84$.

[c]Chi-squared, $P = 1.0$.

[d]Chi-squared, $P = .0099$.

[e]Chi-squared, $P = 4.45E{-}5$.

[f]Chi-squared, $P = .59$.

[g]Welch's t-test, $P = .4$.

[h]Welch's t-test, $P = .0001$.

consensus meeting consider adding this nuance to the Banff guidelines, to avoid missing ABMR cases when DSA is negative but ABMR lesions are present. In contrast, lowering MFI cutoffs did not improve the ability of DSA to predict ABMR because while sensitivity increased, specificity fell.

We acknowledge that AutoBanff is a model that cannot fully simulate how pathologists actually assess ABMR or DSA in SOC. The fact that local pathologists are already diagnosing some of the new DSA-negative ABMR cases with high dd-cfDNA and MMDx ABMR reminds us that pathologists use judgment rather than simply following guidelines. We found that strictly using guidelines was not better at predicting dd-cfDNA or molecular ABMR diagnoses compared with the SOC histologic diagnosis by the pathologist.[37] The AutoBanff approach makes no assumptions[42] but simply assigns the diagnoses according to Banff 2022 guidelines, which were never intended to replace the professional expertise of pathologists. Trifecta-Kidney does not attempt to evaluate the merits of the Banff guidelines themselves.

Although the mechanisms of DSA-negative ABMR remain elusive despite extensive studies,[15,17,18,21] the ultimate mechanism is likely to have a crucial role for NK cells and their products such as IFNG. NK cell localization in MVI lesions and ABMR NK cell activation is well-recognized,[42-44] presumably controlled by

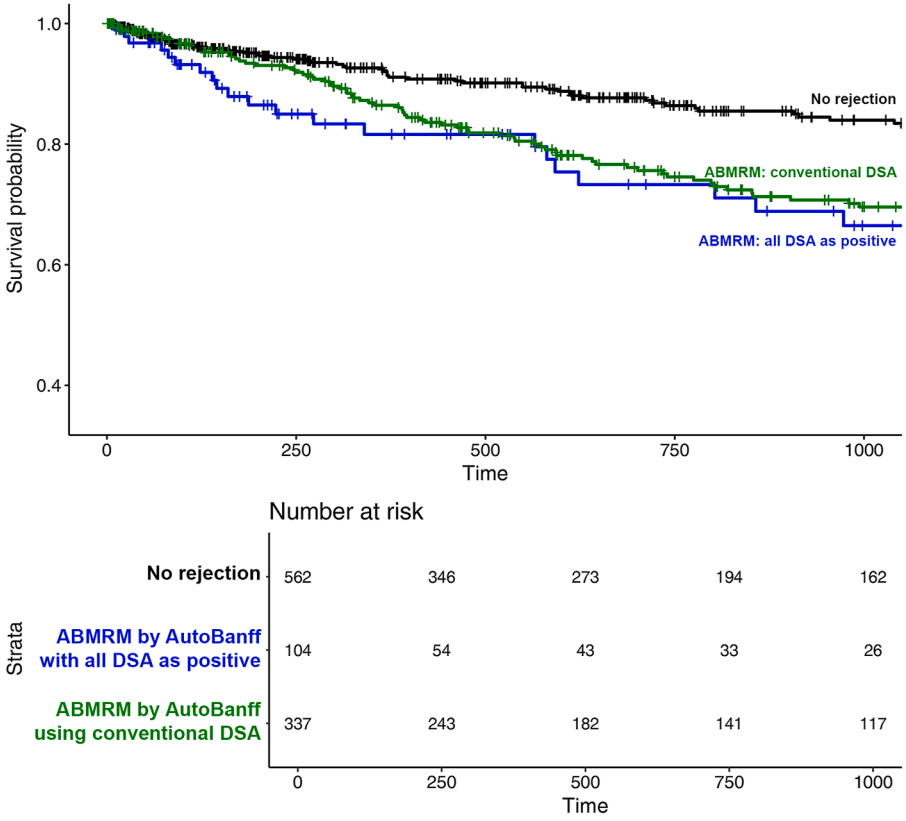

Figure 4. Outcomes analysis of groups in the independent INTERCOMEX population by Kaplan-Meier curves, using one random biopsy per transplant, and excluding any cases where 3 years post-biopsy outcome data were unavailable. Two ABMRM groups are compared: (1) all ABMRM called by the AutoBanff algorithm incorporating conventional DSA (green line), (2) new ABMRM cases identified by the AutoBanff algorithm when all DSA was considered to be positive (blue line, no overlap with the group in "1"), and (3) No rejection cases agreed upon by AutoBanff using conventional DSA positivity and AutoBanff considering all DSA as positive (black line). Post hoc analyses using a log rank test found that the ABMRM groups were not significantly different from each other in terms of likelihood of graft failure ($P = .5$), but both were significantly more likely to fail than the No rejection group (conventional DSA ABMRM, $P = .0014$, considering all DSA as positive ABMRM, $P = .0038$). Abbreviations: ABMRM, antibody-mediated rejection plus Mixed rejection.

the balance between activating and inhibitory signals due to donor-recipient HLA class I differences and others. NK cells are localized in microcirculation lesions in ABMR,[45] and NK cell-expressed genes are increased in biopsies with ABMR or Mixed rejection, presumably reflecting NK cell localization and activation through their primary Fc receptor CD16a.[43] The recent felzartamab study offers more evidence for the role of NK cells in DSA-positive ABMR[1,2]: felzartamab therapy was associated with regression of ABMR NK and IFNG-inducible gene activity, with minimal change in DSA, and recovery of blood NK cells was associated with relapse.

With detection and management of ABMR now a central issue for clinicians in kidney and heart transplantation, the fact that DSA-negative and DSA-positive ABMR have identical molecular phenotypes and long-term outcomes[15] leads naturally to the possibility that certain novel therapies effective in DSA-positive ABMR will be tested in DSA-negative ABMR. For example, anti-CD38 suppresses ABMR through its NK-depleting properties, and DSA-negative ABMR should be included in the trials of anti-CD38. Some ABMR cases that are called "DSA--negative" may be mediated by DSA undetected by current platforms, and thus may benefit from depletion of plasma cells, reducing IgG half-life using efgartigimod to inhibit the neonatal Fc receptors or using imlifidase to temporarily inactivate all IgG.[46] It is probable that both antibody-dependent and antibody-independent mechanisms of NK cell activation are involved in all ABMR. Moreover, therapies that reduce DSA levels may improve outcomes in DSA-negative ABMR by depleting antibodies not detected as DSA, such as alloantibodies against non-HLA polymorphisms. Moreover, the utility of other treatments often used for ABMR such as plasmapheresis, rituximab, anti-IL6, or anti-IL-6R, and proteasome inhibitors need to be re-examined.

We do not advocate for omitting DSA from patient follow-up or from the Banff histology classification. DSA is an intrinsic component of the cognate immune response to alloantigens and is strongly associated with molecular ABMR in kidney and heart transplants. The detection of DSA, like the detection of dd-cfDNA, increases the probability that ABMR is operating. We cannot rule out either subthreshold emerging DSA or waning DSA levels as a cause of DSA-negative ABMR due to the cross-sectional nature of the study. Early-stage and late-stage molecular ABMR are both more likely to be DSA-negative than fully developed ABMR.[15] More granularity in DSA assessment and details such as eplets finds concealed DSA associated with ABMR.[47] Our studies also found more DSA with lower MFI thresholds (although this did not improve the balanced accuracy of predictions). In addition, other details of DSA improve its utility, eg, de novo anti-DQ versus a weak anti-Class I.

In the context of recent literature, our findings are compatible with those in Sablik et al.,[48] which found no significant differences in clinical features, allograft function, or graft survival between DSA-positive and DSA-negative cases called suspicious for chronic active ABMR versus DSA-positive cases called fully developed chronic active ABMR. Our analyses also found minimal molecular, histologic, or dd-cfDNA-associated differences between DSA-negative cases with otherwise consistent ABMR

features, versus full ABMR with DSA-positivity. In contrast with our results, a recent study[47] found a mild "borderline ABMR" phenotype with MVI lesions and negative DSA of which the characteristics fell between full ABMR and NR. Our findings cannot be directly compared with that study because we focused exclusively on DSA-negative ABMR and did not include any eplet-directed or misclassified anti-HLA DSA. In the most recent iteration of the Banff guidelines, most of our cases called pABMR/MVI that transitioned to full ABMR when all DSA was considered positive meet the criteria for "MVI,"[12] ie, they have all the necessary criteria for full ABMR only excluding DSA-positivity. Further discussion of these criteria could consider the results presented in this manuscript—primarily that many of these MVI cases have molecular ABMR and elevated dd-cfDNA.

In conclusion, the histologic identification of DSA-negative ABMR can be addressed within the existing Banff rules by adding a step in which all DSA is considered positive. Both in the Trifecta-Kidney cohort and in the INTERCOMEX validation analysis, this step found 46% and 45% more ABMR cases with typical lesions, molecular ABMR, dd-cfDNA, and risk of graft loss despite lacking measurable conventional DSA. However, simply adjusting MFI thresholds is not useful. Although the AutoBanff algorithm was not designed as a clinical tool, it could be made available to pathologists for use as a model, eg, in cases with MVI $\geq$ 2 but no detectable DSA.[47] We believe that our proposal to detect DSA-negative ABMR using Banff guidelines but calling all DSA-positive—and indeed, all changes proposed to the Banff guidelines—should be subjected to prospective testing in controlled trials to determine the actual impact on real-time SOC histology diagnoses in the hands of expert pathologists.

Acknowledgments

The authors thank our valued clinicians in the Trifecta study group who partnered with us for this study by contributing biopsies and feedback. The authors also thank Anna Hutton for biopsy processing for the microarray biopsy assessment component of this study, and Natera, Inc, for blood sample processing and the Prospera™ test results. The authors would like to thank Dr Ryszard Grenda (Warsaw, Poland) and Dr Millie Samaniego-Picota for the biopsies they contributed.

Funding

This study was supported in part by a grant from Natera, Inc. The Trifecta-Kidney study is an investigator-initiated study supported in part by a grant from Natera, Inc, to Transcriptome Sciences Inc/Alberta Transplant Applied Genomics Centre. The microarray biopsy assessment project is supported in part by a licensing agreement with One Lambda/Thermo Fisher Scientific.

Declaration of competing interest

P. Halloran has shares in Transcriptome Sciences Inc, a University of Alberta research company with an interest in molecular diagnostics, and is a consultant to Natera, Inc, and Argenx BV. All Natera, Inc, authors are employees and own equity at Natera, Inc. The authors of this manuscript have conflicts of interest to disclose as described by *American Journal of Transplantation*.

Data availability statement

CEL files will be available on Gene Expression Omnibus upon publication (GSE303670).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajt.2025.08.029.

ORCiD

Katelynn S. Madill-Thomsen https://orcid.org/0000-0003-2781-6934
Tarek Alhamad https://orcid.org/0000-0003-4289-0817
Sanjiv Anand https://orcid.org/0000-0003-0147-0138
Miha Arnol https://orcid.org/0000-0002-2379-7820
Mirosław Banasik https://orcid.org/0000-0002-0588-1551
Christopher D. Blosser https://orcid.org/0000-0001-8368-6682
Daniel Brennan https://orcid.org/0000-0002-4818-7455
Klemens Budde https://orcid.org/0000-0002-7929-5942
Andrzej Chamienia https://orcid.org/0000-0002-9213-4801
Michał Ciszek https://orcid.org/0000-0003-0641-5920
Dominika Dęborska-Materkowska https://orcid.org/0000-0002-7282-0177
Alicja Debska-Ślizień https://orcid.org/0000-0001-8210-8063
Leszek Domański https://orcid.org/0000-0002-4712-7821
Richard Fatica https://orcid.org/0000-0003-2455-641X
Justyna Fryc https://orcid.org/0000-0002-7895-1012
John Gill https://orcid.org/0000-0002-8837-6875
Jagbir Gill https://orcid.org/0000-0001-8072-265X
Maciej Glyda https://orcid.org/0000-0002-0566-870X
Sita Gourishankar https://orcid.org/0000-0002-6442-895X
Gaurav Gupta https://orcid.org/0000-0003-1919-1970
Petra Hruba https://orcid.org/0000-0002-8663-413X
Peter Hughes https://orcid.org/0009-0004-7659-0585
Arskarapurk Jittirat https://orcid.org/0009-0003-9215-5674
Zeljka Jurekovic https://orcid.org/0000-0003-0690-2577
Layla Kamal https://orcid.org/0000-0002-3797-5544
Sam Kant https://orcid.org/0000-0001-5863-3562
Nika Kojc https://orcid.org/0000-0003-1893-4349
Dhiren Kumar https://orcid.org/0000-0002-6816-9297
Joanna Mazurkiewicz https://orcid.org/0000-0003-3560-3402
Marius Miglinas https://orcid.org/0000-0002-0017-468X
Irfan Moinuddin https://orcid.org/0000-0002-9314-4649
Marek Myślak https://orcid.org/0000-0002-9569-031X
Beata Naumnik https://orcid.org/0000-0001-7090-8856
Leszek Pączek https://orcid.org/0000-0003-0160-3009
Anita Patel https://orcid.org/0000-0003-0359-1929
Agnieszka Perkowska-Ptasińska https://orcid.org/0000-0001-5524-3534
Grzegorz Piecha https://orcid.org/0000-0001-8371-0195
Emilio Poggio https://orcid.org/0000-0003-1492-5103
Silvie Rajnochová Bloudíčkova https://orcid.org/0000-0003-0270-571X
Thomas Schachtner https://orcid.org/0000-0001-5549-4798
Soroush Shojai https://orcid.org/0000-0002-2969-9121
Majid L.N. Sikosana https://orcid.org/0000-0001-9477-432X
Janka Slatinská https://orcid.org/0000-0001-5095-8338
Katarzyna Smykal-Jankowiak https://orcid.org/0009-0001-7091-7215

Željka Veceric Haler https://orcid.org/0000-0003-3318-9850
Ondrej Viklicky https://orcid.org/0000-0003-1049-2195
Ksenija Vucur https://orcid.org/0000-0002-8255-4933
Matthew R. Weir https://orcid.org/0000-0001-8820-5702
Andrzej Wiecek https://orcid.org/0000-0002-8625-4188
Ziad Zaky https://orcid.org/0000-0001-9752-0050
Philip F. Halloran https://orcid.org/0000-0003-1371-1947

References

1. Mayer KA, Schrezenmeier E, Diebold M, et al. A randomized phase 2 trial of felzartamab in antibody-mediated rejection. *N Engl J Med*. 2024;391(2):122–132. https://doi.org/10.1056/NEJMoa2400763.
2. Diebold M, Gauthier P, Mayer KA, et al. Effect of felzartamab anti-CD38 treatment on the molecular phenotype of antibody-mediated rejection in kidney transplant biopsies. *Swiss Med Wkly*. 2024;154(11):4290. https://doi.org/10.57187/s.4290.
3. Haas M, Loupy A, Lefaucheur C, et al. The Banff 2017 Kidney Meeting Report: revised diagnostic criteria for chronic active T cell-mediated rejection, antibody-mediated rejection, and prospects for integrative endpoints for next-generation clinical trials. *Am J Transplant*. 2018;18(2):293–307. https://doi.org/10.1111/ajt.14625.
4. Halloran PF, Wadgymar A, Ritchie S, Falk J, Solez K, Srinivasa NS. The significance of the anti-class I antibody response. I. Clinical and pathologic features of anti-class I-mediated rejection. *Transplantation*. 1990;49(1):85–91. https://doi.org/10.1097/00007890-199001000-00019.
5. Bohmig GA, Exner M, Habicht A, et al. Capillary C4d deposition in kidney allografts: a specific marker of alloantibody-dependent graft injury. *J Am Soc Nephrol*. 2002;13(4):1091–1099. https://doi.org/10.1681/ASN.V1341091.
6. Regele H, Bohmig GA, Habicht A, et al. Capillary deposition of complement split product C4d in renal allografts is associated with basement membrane injury in peritubular and glomerular capillaries: a contribution of humoral immunity to chronic allograft rejection. *J Am Soc Nephrol*. 2002;13(9):2371–2380. https://doi.org/10.1097/01.asn.0000025780.03790.0f.
7. Collins AB, Schneeberger EE, Pascual MA, et al. Complement activation in acute humoral renal allograft rejection: diagnostic significance of C4d deposits in peritubular capillaries. *J Am Soc Nephrol*. 1999;10(10):2208–2214. https://doi.org/10.1681/ASN.V10102208.
8. Mauiyyedi S, Pelle PD, Saidman S, et al. Chronic humoral rejection: identification of antibody-mediated chronic renal allograft rejection by C4d deposits in peritubular capillaries. *J Am Soc Nephrol*. 2001;12(3):574–582. https://doi.org/10.1681/ASN.V123574.
9. Einecke G, Sis B, Reeve J, et al. Antibody-mediated microcirculation injury is the major cause of late kidney transplant failure. *Am J Transplant*. 2009;9(11):2520–2531. https://doi.org/10.1111/j.1600-6143.2009.02799.x.
10. Halloran PF, Chang J, Famulski K, et al. Disappearance of T cell-mediated rejection despite continued antibody-mediated rejection in late kidney transplant recipients. *J Am Soc Nephrol*. 2015;26(7):1711–1720. https://doi.org/10.1681/ASN.2014060588.
11. Sis B, Allanach K, Bunnag S, Mueller TFF, Halloran PF. Microarrays detect deteriorating C4d(−) human renal allografts with ongoing antibody-mediated injury. *Am J Transplant*. 2008;8, 275-275.
12. Naesens M, Roufosse C, Haas M, et al. The Banff 2022 Kidney Meeting Report: reappraisal of microvascular inflammation and the role of biopsy-based transcript diagnostics. *Am J Transplant*. 2024;24(3):338–349. https://doi.org/10.1016/j.ajt.2023.10.016.
13. Gauthier PT, Madill-Thomsen KS, Demko Z, et al. Distinct molecular processes mediate donor-derived cell-free DNA release from kidney transplants in different disease states. *Transplantation*. 2024;108(4):898–910. https://doi.org/10.1097/TP.0000000000004877.
14. Halloran PF, Reeve J, Madill-Thomsen KS, et al. Antibody-mediated rejection without detectable donor-specific antibody releases donor-derived cell-free DNA: results from the Trifecta study. *Transplantation*. 2023;107(3):709–719. https://doi.org/10.1097/TP.0000000000004324.
15. Halloran PF, Madill-Thomsen KS, Pon S, et al. Molecular diagnosis of ABMR with or without donor-specific antibody in kidney transplant biopsies: differences in timing and intensity but similar mechanisms and outcomes. *Am J Transplant*. 2022;22(8):1976–1991. https://doi.org/10.1111/ajt.17092.
16. Halloran PF, Madill-Thomsen KS, Reeve J. The molecular phenotype of kidney transplants: insights from the MMDx project. *Transplantation*. 2024;108(1):45–71. https://doi.org/10.1097/TP.0000000000004624.
17. Senev A, Coemans M, Lerut E, et al. Histological picture of antibody-mediated rejection without donor-specific anti-HLA antibodies: clinical presentation and implications for outcome. *Am J Transplant*. 2019;19(3):763–780. https://doi.org/10.1111/ajt.15074.
18. Senev A, Callemeyn J, Lerut E, Emonds MP, Naesens M. Histological picture of ABMR without HLA-DSA: temporal dynamics of effector mechanisms are relevant in disease reclassification. *Am J Transplant*. 2019;19(3):954–955. https://doi.org/10.1111/ajt.15234.
19. Callemeyn J, Lerut E, de Loor H, et al. Transcriptional changes in kidney allografts with histology of antibody-mediated rejection without anti-HLA donor-specific antibodies. *J Am Soc Nephrol*. 2020;31(9):2168–2183. https://doi.org/10.1681/ASN.2020030306.
20. Halloran PF, Famulski KS, Chang J. A probabilistic approach to histologic diagnosis of antibody-mediated rejection in kidney transplant biopsies. *Am J Transplant*. 2017;17(1):129–139. https://doi.org/10.1111/ajt.13934.
21. Reeve J, Bohmig GA, Eskandary F, et al. Assessing rejection-related disease in kidney transplant biopsies based on archetypal analysis of molecular phenotypes. *JCI Insight*. 2017;2(12):e94197. https://doi.org/10.1172/jci.insight.94197.
22. Koenig A, Mezaache S, Callemeyn J, et al. Missing self-induced activation of NK cells combines with non-complement-fixing donor-specific antibodies to accelerate kidney transplant loss in chronic antibody-mediated rejection. *J Am Soc Nephrol*. 2021;32(2):479–494. https://doi.org/10.1681/ASN.2020040433.
23. Koenig A, Chen CC, Marcais A, et al. Missing self triggers NK cell-mediated chronic vascular rejection of solid organ transplants. *Nat Commun*. 2019;10(1):5350. https://doi.org/10.1038/s41467-019-13113-5.
24. Callemeyn J, Senev A, Coemans M, et al. Missing self-induced microvascular rejection of kidney allografts: a population-based study. *J Am Soc Nephrol*. 2021;32(8):2070–2082. https://doi.org/10.1681/ASN.2020111558.
25. Bachelet T, Couzi L, Lepreux S, et al. Kidney intragraft donor-specific antibodies as determinant of antibody-mediated lesions and poor graft outcome. *Am J Transplant*. 2013;13(11):2855–2864. https://doi.org/10.1111/ajt.12438.
26. Dragun D, Catar R, Philippe A. Non-HLA antibodies in solid organ transplantation: recent concepts and clinical relevance. *Curr Opin Organ Transplant*. 2014;18(4):430–435.
27. Halloran PF, Reeve J, Madill-Thomsen KS, et al. The Trifecta study: comparing plasma levels of donor-derived cell-free DNA with the molecular phenotype of kidney transplant biopsies. *J Am Soc Nephrol*. 2022;33(2):387–400. https://doi.org/10.1681/ASN.2021091191.
28. Halloran PF, Madill-Thomsen KS, Bohmig G, et al. Subthreshold rejection activity in many kidney transplants currently classified as having no rejection. *Am J Transplant*. 2025;25(1):72–87. https://doi.org/10.1016/j.ajt.2024.07.034.
29. Venner JM, Hidalgo LG, Famulski KS, Chang J, Halloran PF. The molecular landscape of antibody-mediated kidney transplant rejection: evidence for NK involvement through CD16a Fc receptors. *Am J Transplant*. 2015;15(5):1336–1348. https://doi.org/10.1111/ajt.13115.
30. Halloran PF, Venner JM, Madill-Thomsen KS, et al. Review: the transcripts associated with organ allograft rejection. *Am J Transplant*. 2018;18(4):785–795. https://doi.org/10.1111/ajt.14600.
31. Gupta G, Moinuddin I, Kamal L, et al. Correlation of donor-derived cell-free DNA with histology and molecular diagnoses of kidney transplant

biopsies. *Transplantation*. 2022;106(5):1061–1070. https://doi.org/10.1097/TP.0000000000003838.
32. Halloran PF, Reeve J, Akalin E, et al. Real time central assessment of kidney transplant indication biopsies by microarrays: the INTERCOMEX study. *Am J Transplant*. 2017;17(11):2851–2862. https://doi.org/10.1111/ajt.14329.
33. Madill-Thomsen K, Perkowska-Ptasinska A, Bohmig GA, et al. Discrepancy analysis comparing molecular and histology diagnoses in kidney transplant biopsies. *Am J Transplant*. 2020;20(5):1341–1350. https://doi.org/10.1111/ajt.15752.
34. Morales-Buenrostro LE, Terasaki PI, Marino-Vazquez LA, Lee JH, El-Awar N, Alberu J. "Natural" human leukocyte antigen antibodies found in nonalloimmunized healthy males. *Transplantation*. 2008;86(8):1111–1115. https://doi.org/10.1097/TP.0b013e318186d87b.
35. P Core Team. *RCT. R: a language and environment for statistical computing*. R Foundation for Statistical Computing; 2019. Available from http://www.r-project.org/.
36. Harrell Jr FE, Dupont C. *Hmisc: Harrell miscellaneous*. R package version 4.3-0. Published; 2020. Available from: https://CRAN.R-project.org/package=Hmisc. Accessed August 24, 2025.
37. Madill-Thomsen KS, Halloran PF. Precision diagnostics in transplanted organs using microarray-assessed gene expression: concepts and technical methods of the Molecular Microscope® Diagnostic System (MMDx). *Clin Sci (Lond).* 2024;138(11):663–685. https://doi.org/10.1042/CS20220530.
38. Madill-Thomsen KS, Reeve J, Bohmig G, et al. Automated histology lesion interpretation in kidney transplant biopsies shows that pathologists often deviate from Banff guidelines. *Am J Transplant*. 2019;19(S3):666–667.
39. Schinstock CA, Sapir-Pichhadze R, Naesens M, et al. Banff survey on antibody-mediated rejection clinical practices in kidney transplantation: diagnostic misinterpretation has potential therapeutic implications. *Am J Transplant*. 2019;19(1):123–131. https://doi.org/10.1111/ajt.14979.
40. Lefaucheur C, Suberbielle-Boissel C, Hill GS, et al. Clinical relevance of preformed HLA donor-specific antibodies in kidney transplantation. *Am J Transplant*. 2008;8(2):324–331.
41. Cornell LD, Helantera I. Exploring microvascular inflammation and the spectrum of antibody-mediated rejection. *Am J Transplant.* 2025;25(1):9–12. https://doi.org/10.1016/j.ajt.2024.08.028.
42. Varol H, Wagenmakers A, Hoeft K, et al. Expanding the scope of microvascular inflammation: unveiling its presence beyond antibody-mediated rejection into T-cell mediated contexts. *Transpl Int.* 2024;37. https://doi.org/10.3389/ti.2024.13464.
43. Parkes MD, Halloran PF, Hidalgo LG. Evidence for CD16a-mediated NK cell stimulation in antibody-mediated kidney transplant rejection. *Transplantation*. 2017;101(4):e102–e111. https://doi.org/10.1097/TP.0000000000001586.
44. Bohmig GA, Loupy A, Sablik M, Naesens M. Microvascular inflammation in kidney allografts: new directions for patient management. *Am J Transplant*. 2025;25(7):1410–1416. https://doi.org/10.1016/j.ajt.2025.03.031.
45. Hidalgo LG, Sis B, Sellares J, et al. NK cell transcripts and NK cells in kidney biopsies from patients with donor-specific antibodies: evidence for NK cell involvement in antibody-mediated rejection. *Am J Transplant*. 2010;10(8):1812–1822. https://doi.org/10.1111/j.1600-6143.2010.03201.x.
46. Huang E, Maldonado AQ, Kjellman C, Jordan SC. Imlifidase for the treatment of anti-HLA antibody-mediated processes in kidney transplantation. *Am J Transplant*. 2022;22(3):691–697. https://doi.org/10.1111/ajt.16828.
47. Nankivell BJ, Taverniti A, Viswanathan S, Ronquillo J, Carroll R, Sharma A. The relationship of microvascular inflammation with antibody-mediated rejection in kidney transplantation. *Am J Transplant*. 2025;25(1):115–126. https://doi.org/10.1016/j.ajt.2024.07.023.
48. Sablik M, Sannier A, Raynaud M, et al. Microvascular inflammation of kidney allografts and clinical outcomes. *N Engl J Med*. 2025;392(8):763–776. https://doi.org/10.1056/NEJMoa2408835.