Integrated biomarkers reveal coordinated biologic states of humoral injury and response to plasma cell-targeted therapy after heart transplantation

Matthew Cusick[1], Amanda MCCormick[2], Heang Lim[2], Kurt Schumacher[2], Hala El Mikati[2], Anusha Konduri[2], Mary Zamberlan[2], Ashley Huebschman[2], Bethany Giacobbe[2], Natalie Sinicropi[2], Kyle Jennings[1], Danielle Ross[1], Steven Hrycaj[1], David Peng[2], Matthew Najor[1]

1. Pathology, University of Michigan, Ann Arbor, MI, United States; 2. Pediatric Cardiology, University of Michigan Medicine, Ann Arbor, MI, United States.

INTRODUCTION & RATIONALE

Circulating donor-specific antibodies (DSA) against HLA-DQ isotypes are key drivers of antibody-mediated rejection (AMR) and long-term graft loss in solid organ transplant recipients.

Pediatric heart transplant recipients frequently exhibit persistent, high-level circulating class II DSA (specifically HLA-DQ), but their **clinical courses range widely** from acute allograft rejection and vascular remodeling to long-term chronic stability without tissue injury (accommodation).

Standard pathological monitoring (protocol biopsy) relies heavily on C4d immunohistochemistry. However, **C4d is an insensitive marker**, and current classifications fail to resolve subclinical microvascular injury, potentially delaying life-saving interventions.

STUDY OBJECTIVES

1. To determine whether an integrated assessment of class II DSA, donor-derived cell-free DNA (dd-cfDNA), and tissue-level endothelial HLA-DQA1 expression defines distinct biological phenotypes of humoral injury.

2. To distinguish active microvascular injury from stable chronic accommodation.

3. To evaluate the utility of paired HLA-DQA1/dd-cfDNA monitoring to guide clinical management and track response to plasma cell-targeted therapy (Daratumumab).

STUDY METHODS & TECHNIQUES

Study Cohort: Longitudinal follow-up of 9 pediatric heart transplant recipients monitored using protocol and clinical endomyocardial biopsies (EMB).

Circulating Biomarker Assays:

HLA DSA: Single Antigen Beads (SAB) monitored longitudinally, reporting Mean Fluorescence Intensity (MFI).

- **dd-cfDNA:** Quantified via AlloSure Heart (CareDx) at routine and clinical timepoints.

Tissue Biomarker Assays:

- **Histopathology:** Traditional ISHLT cellular (ACR 0R-3R) and antibody-mediated (pAMR 0-3) grading, including C4d and CD68 immunohistochemistry.
- **HLA-DQA1 Staining:** Retrospective and serial paired immunophenotyping performed on formalin-fixed, paraffin-embedded (FFPE) EMB biopsy tissues to evaluate endothelial HLA-DQ upregulation.

CONCORDANCE RISK STRATIFICATION

Integrated analysis of circulating antibodies and tissue-level injury markers resolves the clinical heterogeneity of HLA-DQ donor-specific antibodies (DSA) into three major phenotypes:

1. Concordant Positive (+) Phenotype (Active Injury):

Characterized by high circulating DQ DSA (typically >15k MFI), elevated dd-cfDNA (>1.0%), and diffuse, intense microvascular HLA-DQA1 endothelial expression. Correlates with active, biopsy-proven AMR and clinical instability, signaling the need for immediate, targeted intervention.

2. Discordant Phenotype (Stable Accommodation):

Characterized by high circulating class II DSA but persistently low or non-endothelial HLA-DQA1 staining and low/stable dd-cfDNA. This represents graft-level accommodation where circulating antibodies are tolerated without active tissue-level injury.

3. Concordant Negative (-) Phenotype (Healthy Control):

Characterized by low or absent circulating DSA (<1k MFI), minimal or absent endothelial HLA-DQA1 staining, and baseline dd-cfDNA (≤0.21%), demonstrating allograft stability.

COHORT CLINICAL & PATHOLOGICAL PROFILES

Table 1: Clinical, immunogenetic, and pathological characteristics of the 9-patient pediatric heart transplant cohort, stratified by biomarker concordance.

Patient ID	Dominant DSA	Peak MFI	Peak dd-cfDNA	HLA-DQA1 IHC	Clinical Outcome / Status	Phenotype
Patient 1	DQ5	32,148	3.60%	Diffuse Strong	Dara (10 doses); patchy staining but persistent high MFI	Concordant (+)
Patient 2	DQ2/DR53	25,603	4.40%	Endothelial Up	Dara (4 doses); gradual histological improvement	Concordant (+)
Patient 3	DQ5	~20,000	3.10%	Diffuse Intense	Dara (15 doses); complete biologic response; resolved to pAMR0	Concordant (+)
Patient 4	DQ8	~24,000	9.00%	Upregulated	Under-recognized C4d(-) injury; progressed to CAV	Concordant (+)
Patient 5	DQ2/DQ5	>30,000	4.70%	Focal / Low	Chronically stable pAMR0 (>20-year pediatric survivor)	Discordant
Patient 6	DQ6	8,784	0.95%	Focal / Low	Early stable (PTLD workup; EBV-positive hyperplasia)	Discordant
Patient 7	DQ2	<1,000	≤0.15%	Minimal / Absent	Consistent 0R/pAMR0; biomaker-negative baseline control	Concordant (-)
Patient 8	None	<1,000	0.21%	Minimal / Absent	Early cellular (2R/3A) resolved; no AMR control	Concordant (-)
Patient 9	DQ2	28,341	0.82%	Diffuse Intense	Dara (4 doses); late-onset (10-yr) complete responder	Concordant (+)

KEY CLINICAL PATHWAYS

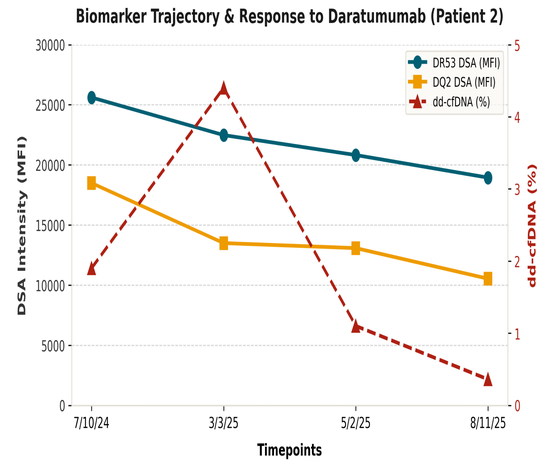
The complete biologic responders (Patients 3 & 9): These cases illustrate the ultimate potential of the biomarker-guided approach. Both patients had 'explosive' de novo DSA rises with acute cellular and humoral rejection (Patient 3 at early post-txp, Patient 9 at 10-years post-txp). Targeted therapy with **Daratumumab (anti-CD38)** successfully cleared circulating antibodies, normalized dd-cfDNA, and down-regulated tissue DQA1 staining back to baseline pAMR0 status.

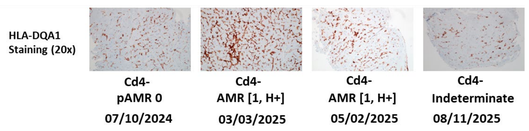
The partial / refractory responders (Patients 1 & 2): Patients who exhibit persistently high circulating DSA despite anti-plasma cell therapy represent a refractory cohort. In Patient 1, 10 doses of Daratumumab reduced tissue-level injury (HLA-DQA1 staining transitioned from ***diffuse strong*** to ***patchy*** and dd-cfDNA normalized), yet circulating MFI remained high (>27k). This reveals a **discordant treatment response** where clinical stability was achieved without antibody clearance.

Clinical takeaway: Active tissue-level injury is a dynamic coordinate process requiring intensive therapy, but clinical resolution does not always require complete clearance of circulating antibodies if tissue-level accommodation can be induced.

BIOMARKER TRAJECTORY ANALYSIS

In Patient 2, active humoral injury was identified on 3/3/2025 based on histological EMB findings (pAMR2) and a significant dd-cfDNA spike (4.40%) despite negative C4d. Retrospective HLA-DQA1 IHC confirmed diffuse microvascular staining. Initiation of Daratumumab in May 2025 resulted in a rapid biologic response, with dd-cfDNA resolving to 0.36% and HLA-DQA1 staining showing marked down-regulation, demonstrating successful therapeutic monitoring using integrated tissue and molecular biomarkers.





AVOIDING DIAGNOSTIC PITFALLS

Pitfall 1: Relying Solely on C4d Immunostaining

In standard biopsy protocols, a negative C4d result often leads to a diagnosis of 'no rejection'. However, **Patient 4** demonstrated persistent high DQ8 DSA (~24k MFI) and severe, subclinical graft injury (peaking at **9.0% dd-cfDNA**) which was consistently C4d-negative. This unrecognized microvascular injury went untreated, leading to permanent concentric intimal proliferation and fibrosis—resulting in a biopsy diagnosis of **Chronic Allograft Vasculopathy (CAV)** at year 6. Utilizing dd-cfDNA and HLA-DQA1 staining reveals this under-recognized C4d-negative injury window.

Pitfall 2: Over-treating Stable Accommodation

High-MFI DSA is frequently assumed to signify active danger, leading to aggressive immunotherapy. However, **Patient 5** represents a pediatric success story, living **over 20 years post-transplant with 'VERY HIGH' DQ2 and DQ5 DSA (>30k MFI)**. Despite high antibodies, protocol biopsies show pAMR0/0R and HLA-DQA1 staining remains strictly focal and non-endothelial. This is a classic case of **chronic accommodation**. Utilizing tissue-level DQA1 upregulation as a 'tie-breaker' prevents the hazard of unnecessarily over-treating these stable grafts.

Therapeutic Monitoring: Paired serial HLA-DQA1 staining allows clinicians to visualize whether plasma cell-targeted therapies like Daratumumab are successfully down-regulating injury at the tissue level, providing a reliable measure of biologic efficacy.

CONCLUSIONS & CLINICAL IMPACT

1. **Multimodal Integration:** Combining circulating antibodies (DSA), molecular markers of cell injury (dd-cfDNA), and tissue-level expression (HLA-DQA1 IHC) resolves the clinical heterogeneity of humoral alloreactivity.

2. **Tissue Tie-Breaker:** HLA-DQA1 endothelial upregulation acts as a critical tissue-level indicator of active injury, separating active, graft-threatening AMR from benign chronic accommodation.

3. **Personalized Management:** This framework informs clinical decision-making ('To Treat or Not to Treat'), preventing unnecessary immunotherapy in accommodated patients while enabling the timely initiation of life-saving plasma cell-targeted therapy (Daratumumab) to halt progression to CAV.

Disclosures

The authors have no financial conflicts to disclose