

## Background

- Anti-HLA DQ donor specific antibodies (DQ-DSA) are associated with chronic graft damage
- Presence and strength of DQ-DSA does not predict the immediate development of antibody mediated rejection (AMR)
- We hypothesize that levels of HLA-DQ expression on endothelium could influence the pathogenicity of HLA antibodies

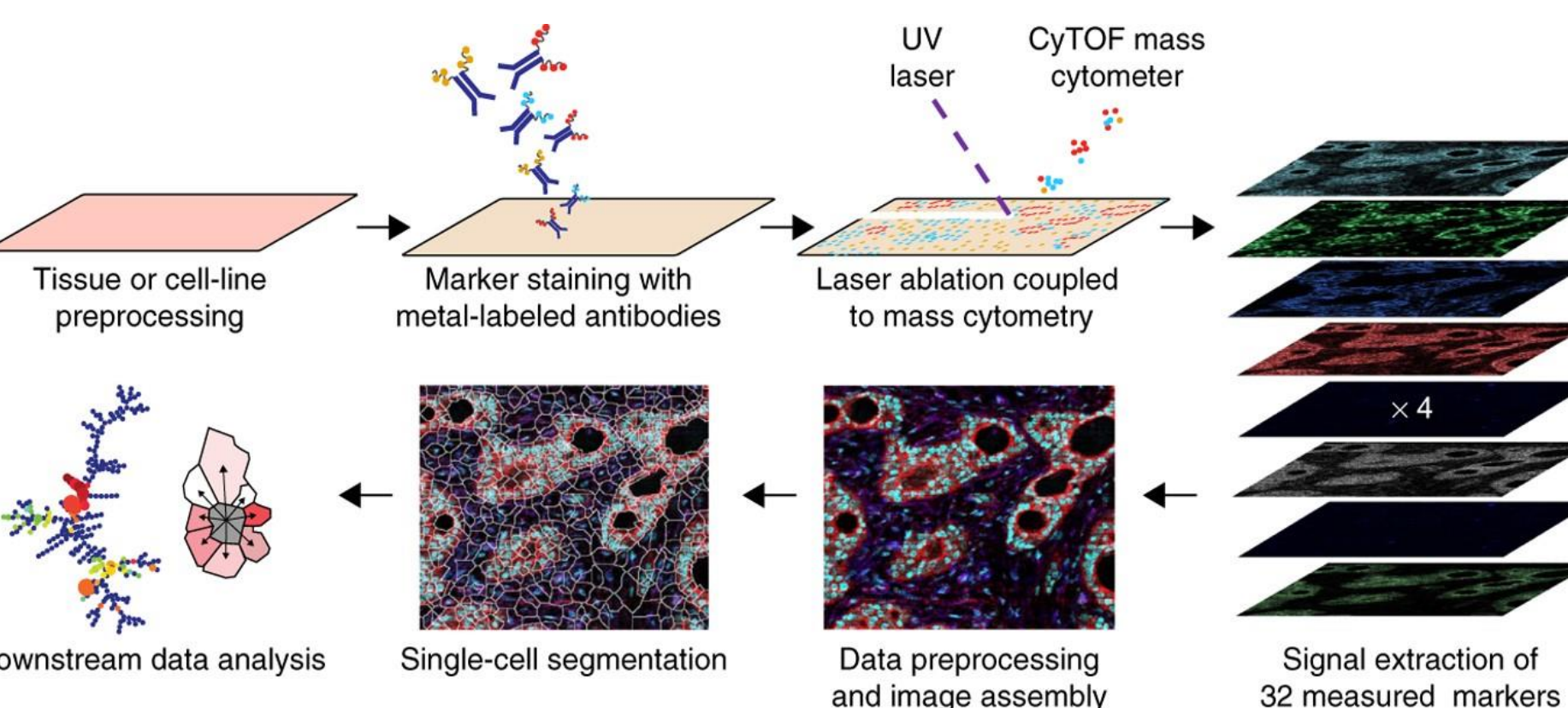
## Objective

- Characterize HLA-DQ expression in endothelial cells in cardiac allografts
- Determine if HLA-DQ expression is increased during episodes of AMR
- Assess feasibility of using IMC to visualize relative HLA-DQ expression

## Methods

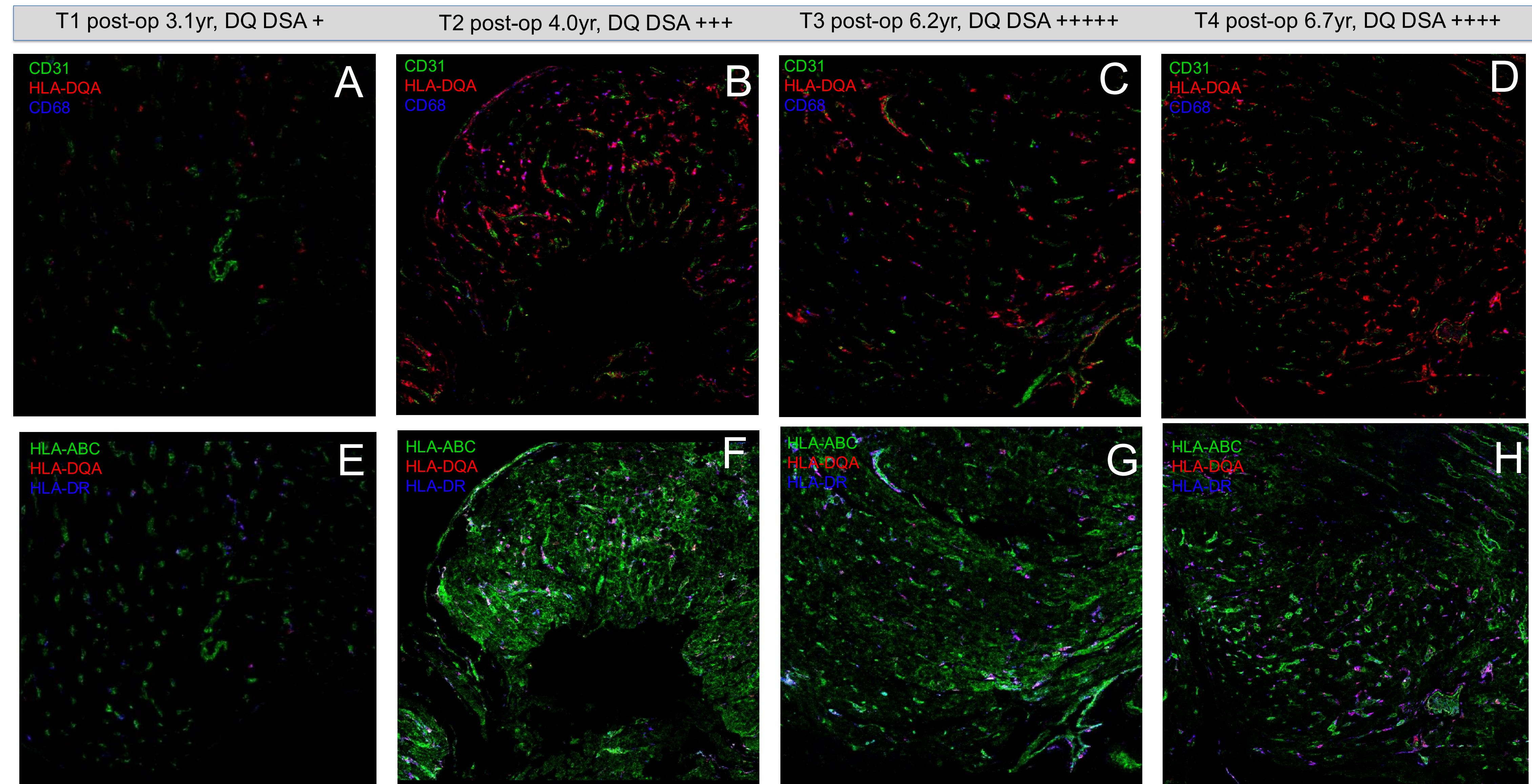
Four heart biopsies at different time points were selected from one pediatric heart recipient to exemplify different clinical scenarios:

- 1) states of both clinical antibody-mediated rejection (AMR) with strong presence of HLA-DQ donor-specific antibodies (DQ-DSA),
- 2) no AMR with presence of DQ-DSA.
- We used a 33-marker IMC panel which included an Anti-HLA-DQA1 Antibody [EPR7300] and markers to detect immune cells, HLA Class I, HLA-DR, and nonimmune cell markers for cell type segmentation.
- DSA assignments were based on donor and recipient HLA types determined by DNA-based HLA typing methods, primarily next-gen sequencing
- All types were determined using next generation sequencing with the exception of DQA for the recipient. This is a prediction based upon allele and haplotype frequencies



Giesen C, Wang HA, Schapiro D, Zivanovic N, Jacobs A, Hattendorf B, Schüffler PJ, Grolimund D, Buhmann JM, Brandt S, Varga Z, Wild PJ, Günther D, Bodenmiller B. Highly multiplexed imaging of tumor tissues with subcellular resolution by mass cytometry. Nat Methods. 2014 Apr;11(4):417-22. doi: 10.1038/nmeth.2869. Epub 2014 Mar 2. PMID: 24584193.

## Results



**Figure 1.** (above) Relative changes in HLA expression through post-transplant course. A-D, HLA-DQA co-localized on CD31 cells with varying CD68 infiltration. E-H, HLA Class I and II co-localization

HLA Typing			Patient 1			
	Recipient	Donor	Biopsy 1	Biopsy 2	Biopsy 3	Biopsy 4
A	02:05, 30:02	03:01, 32:01	2/5/18	1/4/19	3/10/21	9/22/21
B	15:220 (B70), 41:01	15:01 (B62), 44:02	1139	1472	2280	2463
C	12:03:17:01	03:04,05:03	pAMR0	pAMR1	pAMR0	pAMR2
DRB1	08:04, ---	04:01, 12:01	Negative	Positive	Negative	Positive
DRB3	---	02:02	<10%	>10%	Negative	>10%
DRB4	---	01:03	-	-	-	+
DQB1/DQA1	04:02/04:01	03:01/03:01(DQ7) 03:01/05:05(DQ7) 03:03/03:01(DQ8) 03:02/05:05(DQ8)*	Absent	Absent	Present	Present
DQA1	04:01, ---	03:01, 05:05	DSA Titers/MFIs			
			A*03:01	---	400	---
			A*32:01	---	900	---
			B*44:02	---	700	---
			C*03:04	---	3500	---
			DRB1*04:01	---	2,100	1200
			DRB1*12:01	---	800	2700
			DRB4*01:03 (DR53)	---	2,200	6300
			DQ7	2,500-3,000	1:64	1:4,096-1:8192
			DQ8	---	---	1:2,048
					1:4,096	1:2,048

**Table 1.** HLA typing comparison between the recipient and donor, showing alleles across loci. DSA strength expressed as MFI values (black text) or titers (red text). \*not included in reagents

## Limitations/Future Aims

- Future aims include continued development of segmentation techniques in pursuit of quantitative approaches in measuring HLA antigen expression specifically in endothelial cells
- Challenges with quantitative single-cell segmentation on endothelial cells include cell morphology (linear/contiguous shapes) and 2D segmentation which often miss portions of the cytoplasm and mistakenly include cytoplasm from adjacent cells, due to planar limitations.
- Imaging intensity and thresholds were standardized for this study, but other factors may influence staining patterns.

## Conclusions

- IMC and other spatial imaging technology may provide new insights into the relationship between HLA expression and AMR in allografts
- Preliminary/qualitative analysis suggests that DQ expression was extremely low in a stable graft and highest during episodes of rejection
- IMC and other spatial imaging techniques may provide new insights into how the pathogenicity of DSA can be influenced by HLA expression in allografts
- IMC can simultaneously detect 30-40 markers without signal overlap while preserving high resolution tissue architecture enabling characterization of infiltrating immune cells