Supplemental Material

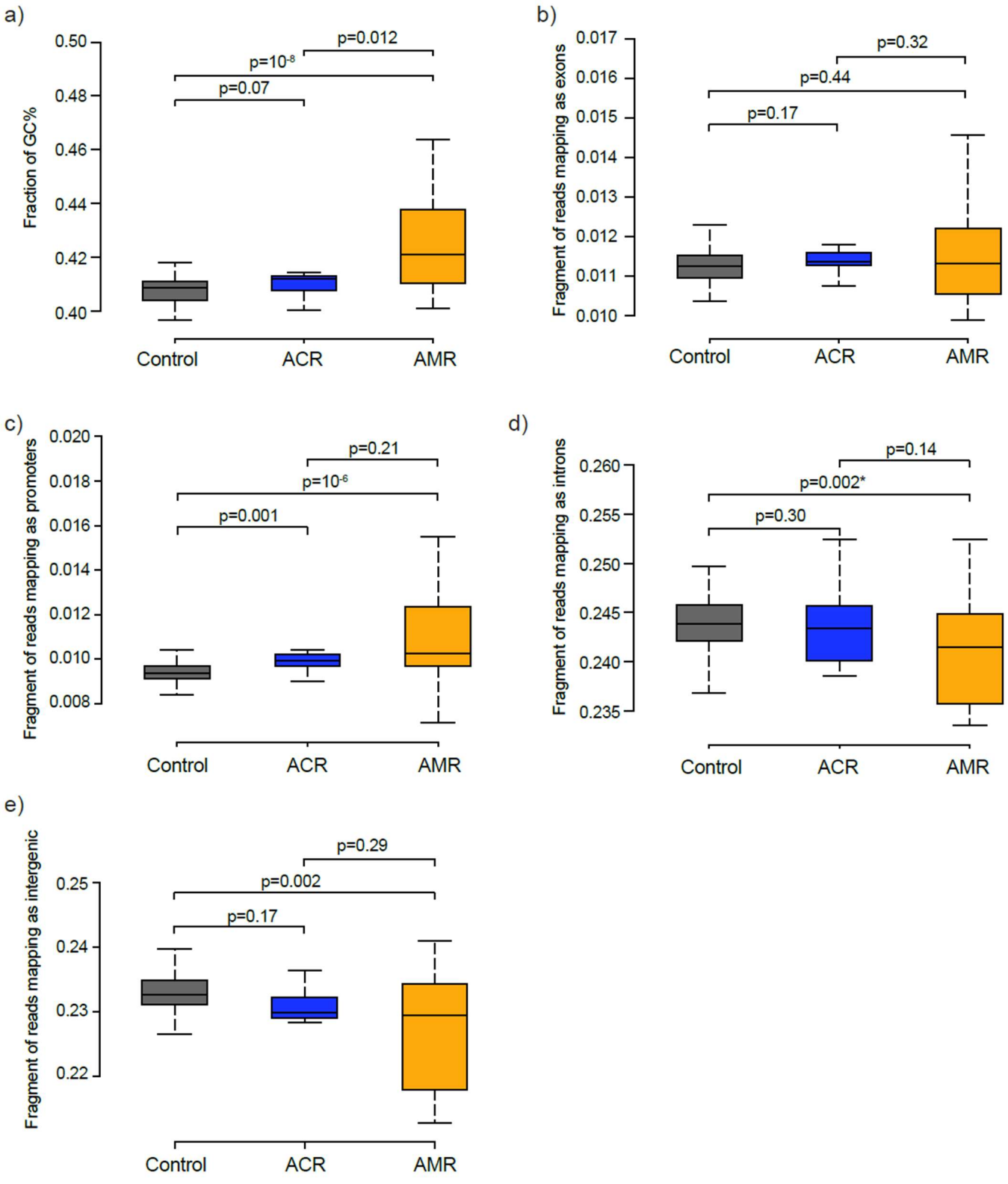
Cell-free DNA to Detect Heart Allograft Acute Rejection

Supplemental Methods

Patient monitoring schedule and sample collection schedules

Subjects at all five centers with surveillance endomyocardial biopsy (EMBx), donor specific antibodies and echocardiography. Routine clinical labs such as basic metabolic panel and complete count, as well as labs to monitor tacrolimus and other immunosuppression drugs troughs, cytomegalovirus PCR were also performed. EMBx schedule at each of the 5 centers is shown in Supplemental Tables I a-e. The study collected blood samples coincident to surveillance EMBx. Additional samples were collected in the early transplant period on Days 1 and 7, and also when subjects presented with clinical or echocardiographic signs of allograft dysfunction.

Supplemental Figure I: Genomic Element Characterization in ACR and AMR



Genomic composition of ddcfDNA measures was compared in ACR, AMR and controls. AMR showed higher fraction of guanosine-cytosine base content (a), but similar fraction of reads mapping as exons (b) compared to ACR or controls. AMR also showed higher fraction of reads mapping as promoters (c) and lower fraction of reads mapping as introns (d) or intergenic regions (e) compared to ACR and no rejection controls, repeat elements are not included in intronic and intergenic regions.

Supplemental Tables

Supplemental Table I: Post-Transplant Allograft Surveillance Schedule

Suppl Table Ia-Center 1 post-heart transplant care-biopsy schedule and proposed blood draws for cell-free DNA assay																					
	Weeks post-transplant										Months/years' post-transplant										
	1	2	4	6	8	10	12	16	20	24	7	8	9	10	11	12	15	18	21	24	2-5 yrs.
EMB	X	X	X	X	X	X	X	X		X		X		X		X		X		X	Xc
Clinic visit	X	X	X	X	X	X	X	X_1	X	X	X	X	X	X	X	X	X	X	X	X	Xb
Blood draw	X	X	X	X	X	X	X	X_1		X		X		X		X		X		X	Xb

Suppl Table Ib-Center 2 post-heart transplant care-biopsy schedule and proposed blood draws for cell-free DNA assay																					
	Weeks post-transplant										Months/years' post-transplant										
	1	2	3	4	6	8	12	16	20	24	7	8	9	10	11	12	15	18	21	24	2-5 yrs.
EMB	X	X	X	X	X	X	X	X	X	X				X		X				X	Xa
AlloMap										X		X				X	X		X		Xc
Clinic visit	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	Xb
Blood draw	X	X	X	X	X	X	X	X	X	X		X		X		X	X		X	X	Xb

Suppl Table Ic-Center 3 post-heart transplant care-biopsy schedule and proposed blood draws for cell-free DNA assay																					
	Weeks post-transplant										Months/years' post-transplant										
	1	2	4	6	8	10	12	16	20	24	7	8	9	10	11	12	15	18	21	24	2-5 yrs.
EMBx	X	X	X	X	X	X	X	X		X		X		X		X		X		X	Xa
AlloMap										X		X				X	X		X		
Clinic visit	X	X	X	X	X	X	X	X_1	X	X	X	X	X	X	X	X	X	X	X	X	Xb
Blood draw	X	X	X	X	X	X	X	X_1		X		X		X		X		X		X	Xb

Suppl Table Id-Center 4 post-heart transplant care-biopsy schedule and proposed blood draws for cell-free DNA assay																					
	Weeks post-transplant										Months/years' post-transplant										
	1	2	3	4	6	8	12	16	20	24	7	8	9	10	11	12	15	18	21	24	2-5 yrs.
EMBx	X	X	X	X	X	X	X	X	X	X						X				X	Xa
AlloMap										X	X	X	X	X	X	X		X		X	
Clinic visit	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	Xb
Blood draw	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X		X	Xb

Suppl Table Ie-Center 5 post-heart transplant care-biopsy schedule and proposed blood draws for cell-free DNA assay		
	Weeks post-transplant	Months/years' post-transplant

	1	2	3	4	6	10	14	16	20	24	7	8	9	10	11	12	15	18	21	24	2-5 yrs.
EMBx	X	X	X	X	X	X	X	X		X		X		X		X		X		X	Xa
AlloMap										X		X				X	X		X		
Clinic visit	X	X	X	X	X	X	X	X_1	X	X	X	X	X	X	X	X	X	X	X	X	Xb
Blood draw	X	X	X	X	X	X	X	X_1		X		X		X		X		X		X	Xb

EMBx = endomyocardial biopsy (scheduled), Xa = every 6 months, Xb = every 3 months. In addition to the pre-specified time-points, samples were also collected at clinically-indicated EMBx, which were performed when subjects present with clinical or echocardiographic signs of allograft dysfunction.

Supplemental Table II: Induction and Maintenance Immunosuppression Regimen and Treatment for Acute Rejection

Supplemental Table IIa: Induction and maintenance immunosuppression regimen

Centers	Induction		Maintenance
	Initial steroid	Other regimens	
Center 1	High dose methylprednisolone	Basiliximab/ATG* for all patients	FK/MMF/Pred, Prednisone is tapered by 1 year of transplantation unless there is concern for rejection
Center 2	High dose methylprednisolone	Basiliximab for Selected patients with PRA>25%, GFR<40	FK/MMF/Pred, Prednisone is tapered by 6 months of transplantation unless there is concern for rejection
Center 3	High dose methylprednisolone	Basiliximab for selected patients with PRA (>25%), renal insufficiency/CKD (GFR < 40), age (<50), AA race, or inpatients	FK/MMF/Pred, Prednisone is tapered by 6 months of transplantation unless there is concern for rejection
Center 4	High dose methylprednisolone	Basiliximab or ATG for selected patients with any of the following PRA (>25%), renal insufficiency/CKD (GFR < 40), age (<50), combined kidney/heart transplants	FK/MMF/Pred, Prednisone is tapered by 6 months of transplantation unless there is concern for rejection
Center 5	High dose methylprednisolone	ATG for selected patients with positive crossmatch, renal insufficiency/CKD (GFR < 40), combined kidney/heart transplants	FK/MMF/Pred, Prednisone is tapered by 6 months of transplantation unless there is concern for rejection or underlying disease is sarcoidosis

2015 – 2018 = Basiliximab, 2018 – present ATG, ATG = anti-thymocyte globulin, GFR = glomerular filtration rate, FK = FK-506, also called tacrolimus, MMF = mycophenolate mofetil, Pred = prednisone, PRA = AA = African American. Center 1 = Johns Hopkins Hospital, Center 2

= Medstar Washington Hospital Center, Center 3 = Inova Heart and Vascular Institute, Center 4 = Virginia Commonwealth University Hospital, Center 5 = University of Maryland Medical Center

Supplemental Table IIb: Treatment for acute rejection by center

Centers	Acute rejection	
	ACR	AMR
Center 1	IV steroids if hemodynamically unstable or if rejection occurs within 3months. Oral steroids for others	IV Steroids/Plex/Rituximab/IVIG
Center 2	IV steroids. ATG with hemodynamic instability	IV steroids/Plex/IVIG/ Bortezomib
Center 3	Oral steroid or IV steroid + ATG with low EF or hemodynamic instability	IV Steroid, Plex/IVIG/Thymo+Ritux or Bortezomib
Center 4	Oral steroid or IV steroid + ATG with low EF or hemodynamic instability	IV Steroid, Plex/IVIG/Ritux and/or Bortezomib
Center 5	Oral steroid or IV steroid + ATG with low EF or hemodynamic instability	IV Steroid, Plex/IVIG.

IV = intravenous, Plex = plasmapheresis, IVIG = Intravenous immunoglobulin, Thymo = thymoglobulin, Ritux = rituximab

Supplemental Table III: %ddcfDNA post-transplant kinetics

Supplemental Table IIIa: %ddcfDNA sequencing characteristics

Parameters	Average	Standard deviation
Total reads/sample (million)	30.26	20.65
Reads after removing duplicates or low-quality reads (million)	20.00	8.09
Reads with donor or recipient SNPs (thousand)	14.96	9.29
Error rate (%)	0.04	0.02

Supplemental Table IIIb: %ddcfDNA decay parameters

Decay parameter	Measure	95% CI
Y0	3.44	3.06 - 3.85
Plateau	0.07	0.048 - 0.10
K	0.74	0.64 - 0.84
Half Life	0.94	0.82 - 1.08
Tau	1.36	1.19- 1.56

Supplemental Table IIIc: %ddcfDNA trend after transplantation

Time after transplantation (Days)	Median %ddcfDNA	%ddcfDNA IQR (%)
1	2.83	1.68 – 4.02
7	0.19	0.07 – 0.30
14	0.21	0.11 – 0.40
28	0.13	0.03 - 0.21
60	0.02	0.01 - 0.13
90	0.01	01 - 0.08
180	0.01	0.01 - 0.04
270	0.03	0.01 – 0.10
360	0.01	0.01 – 0.08
450	0.01	0.01 – 0.03
540	0.05	0.01 – 0.14
630	0.05	0.01 – 0.12
720	0.04	0.01 – 0.14

Supplemental Table IV: Changes in %ddcfDNA performance overtime post-transplantation

Supplemental Table IVa: %ddcfDNA test characteristics to detect biopsy-positive acute rejection: Eliminated biopsies before Day 7 after transplantation

%ddcfDNA threshold		Sensitivity (%)			Specificity (%)			AUROC (95% CI)
		0.1	0.25	0.5	0.1	0.25	0.5	
AR diagnosis								
	AR	97	88	48	61	80	91	0.88 (0.85 – 0.91)
	AMR	100	90	67	61	81	91	0.92 (0.88 – 0.95)
	ACR	93	82	29	60	80	91	0.84 (0.79 – 0.89)

Supplemental Table IVb: %ddcfDNA test characteristics to detect biopsy-positive acute rejection - Eliminated biopsies before Day 14 after transplantation

%ddcfDNA threshold		Sensitivity (%)			Specificity (%)			AUROC (95% CI)
		0.1	0.25	0.5	0.1	0.25	0.5	
AR diagnosis								
	AR	96	88	45	63	82	91	0.88 (0.85 – 0.92)
	AMR	100	88	64	64	82	91	0.92 (0.88 – 0.95)
	ACR	92	83	25	64	82	91	0.85 (0.80 – 0.90)

Supplemental Table IVc: %ddcfDNA test characteristics to detect biopsy-positive acute rejection - Eliminated biopsies before Day 45 after transplantation

%ddcfDNA threshold		Sensitivity (%)			Specificity (%)			AUROC (95% CI)
		0.1	0.25	0.5	0.1	0.25	0.5	
AR diagnosis								
	AR	93	81	44	72	87	93	0.90 (0.85 – 0.94)
	AMR	100	91	73	72	87	93	0.94 (0.90 – 0.99)
	ACR	88	75	25	72	87	93	0.87 (0.81 – 0.93)