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Supplementary Materials for

Circulating Cell-Free DNA Enables Noninvasive Diagnosis of Heart Transplant Rejection

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The PDF file includes:

METHODS

Post-transplant therapeutic protocol

Adult heart transplant recipients. The post-transplant therapeutic protocol was previously described in (*20*). Post-transplant immunosuppression consisted of methylprednisolone (500 mg) administered immediately post-operatively followed by 125 mg every 8 hours for three doses. Anti-thymocyte globulin (rATG; 1 mg/kg) was administered on post-operative days 1, 2, and 3. Maintenance immunosuppression consisted of prednisone (20 mg) twice daily starting on post-operative day 1 and tapered to <0.1 mg/kg/day by the 6^{th} post-operative month and tapered further if endomyocardial biopsies showed no evidence of cellular rejection. Tacrolimus was started on post-operative day 1 and dosing was further adjusted to maintain a level of 10-15 ng/ml during months 0 to 6, 7-10 ng/ml during months 6 to 12, and 5-10 ng/ml thereafter. Mycophenolatemofetil was started at 1000 mg twice daily on post-operative day 1 and dose adjustments were made, if required, in response to leukopenia. All patients received standard CMV (antiviral) prophylaxis consisting of ganciclovir (5 mg/kg i.v.), adjusted for renal function, every 12 hours starting on post-operative day 1 unless both donor and recipient were CMV-negative. When able to tolerate oral medications, recipients were started on valganciclovir(900 mg) twice daily for 2 weeks, then 900 mg daily until 6 months post-transplant, followed by 450 mg daily until 12 months post-transplant, at which point anti-viral prophylaxis was discontinued. Valganciclovir dose reductions were made in the setting of leukopenia. CMV- recipients of a CMV+ allograft also received CMV hyperimmune globulin (150 mg/kg i.v.) within 72 hours of transplant; 100 mg/kg at post-transplant weeks 2, 4, 6, and 8; and 50 mg/kg at weeks 12 and 16 post-transplant. CMV- recipients of CMV- allografts were not treated with antiviral prophylaxis until May 2012; subsequently, these recipients were treated with acyclovir (400 mg) twice daily for 1 year. Antifungal prophylaxis consisted of itraconazole (300 mg) daily for the first 3 months post-transplant, and prophylaxis against pneumocystis jiroveci infection consisted of trimethoprim/sulfamethoxazole, 80 mg TMP component daily. Prophylaxis against pneumnocystis infection was continued indefinitely, and

patients intolerant of TMP-SMX were treated with atovaquone, dapsone, or inhaled pentamidine.

Pediatric heart transplant recipients. Induction immunosuppression initially consisted of daclizumab (1 mg/kg i.v.) every 2 weeks for a total of 5 doses, and was switched to basiliximab (10-20 mg i.v.) on post-operative days 0 and 4 beginning in August 2011. Recipients were also treated immediately with pulse methylprednisolone (10 mg/kg i.v.) every 8 hours for 3 doses, followed by prednisone (0.5 mg/kg) twice daily for the first 14 days post-transplant; corticosteroids were subsequently tapered off during the first post-transplant year, in the absence of acute rejection. Calcineurin inhibition consisted primarily of cyclosporine, with goal levels of 300-350 ng/ml for months 0 to 3, 275-325 ng/ml for months 4 to 6, 250-300 ng/ml for months 7 to 12, and 200-250 after month 12 post-transplant. Patients intolerant of cyclosporine were treated with tacrolimus. Protocols for prophylaxis against opportunistic infections and surveillance endomyocardial biopsies were similar to adult heart transplant recipients.

SUPPLEMENTARY FIGURES

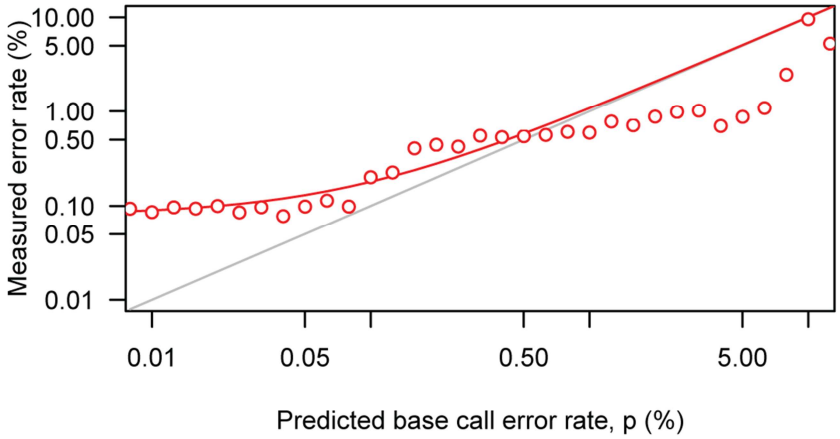

Fig. S1. Measured versus predicted error rate. Error rate measured in the assay as a function of the base call error rate as predicted by the sequencer (red circles). The predicted base call error rate, p, was calculated from the Illumina Phred score (Q_s) as indicated in the fastq sequencing data format [$p = 10^{(-Qs-33)/10}$]. The actual error rate was measured by examining SNP positions for which both the donor and recipient are homozygous and carry the same allele. The matched error rate is the frequency at which a base other than the donor and recipient allele is measured at one of these positions. The gray line indicates the expected error rate, with errors from sequencing only. The red line indicates the error rate as function of the predicted error rate for the case of an additional source of error (genotyping or PCR).

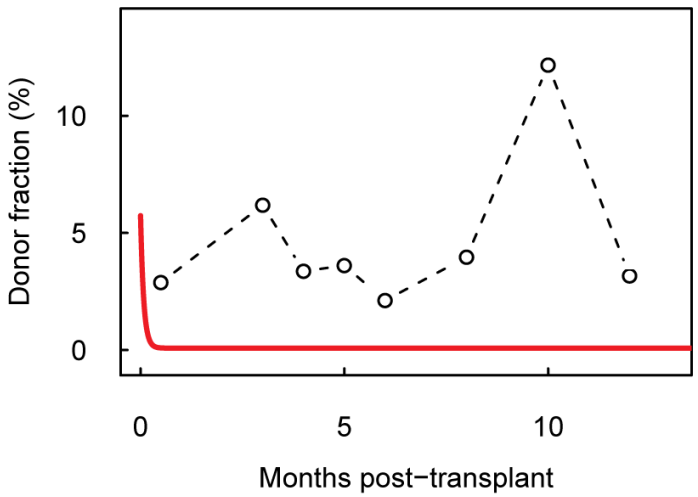

Fig. S2. cfdDNA time course for patient A36. Elevated levels of cfdDNA were observed in the absence of biopsy-proven acute rejection post-transplant throughout the transplant course of an adult transplant patient transplanted due to giant cell myocarditis (solid line is fit to data for non-rejecting patients, Fig. 4A). The data for this subject were not included in the analysis of the detection quality of cfdDNA (Fig. 5).

Table S1: Heart transplant recipient demographics.

Table 1. **Heart Transplant Recipient Demographic Characteristics**				
Adult Recipients	**(N=44)**		**Pediatric Recipients**	**(N=21)**
Characteristic			**Characteristic**	
Age -- yr			Age -- yr	
Mean ± StdDev	50±15		Mean ± StdDev	8± 6.6
Range	20-69		Range	0-19
Male sex – no. (%)	30 (70)		Male sex – no. (%)	9 (43)
Race or ethnic groups - no. (%)			Race or ethnic groups - no. (%)	
White	24 (56)		White	14 (66)
Hispanic	4 (9)		Hispanic	5 (24)
Black	5 (12)		Black	1 (5)
Asian or Pacific Islander	10 (23)		Asian or Pacific Islander	1 (5)
Other	0		Other	0
Indication for cardiac transplantation – no. (%)			Indication for cardiac transplantation – no. (%)	
Ischemic cardiomyopathy	7 (16)		Ischemic cardiomyopathy	0
Dilated cardiomyopathy	17 (40)		Dilated cardiomyopathy	13 (62)
Valvular heart disease	0		Valvular heart disease	0
Congenital heart disease	5 (12)		Congenital heart disease	4 (19)
Graft vasculopathy or retransplantation	1 (2)		Graft vasculopathy or retransplantation	0
Arrhythmias	0		Arrhythmias	0
Hypertrophic cardiomyopathy	4 (9)		Hypertrophic cardiomyopathy	4 (19)
Other	9 (21)		Other	0
Cytomegalovirus status – no. (%)			Cytomegalovirus status – no. (%)	
Donor and recipient positive	21 (49)		Donor and recipient positive	6 (28)
Donor and recipient negative	6 (15)		Donor and recipient negative	1 (5)
Donor positive and recipient negative	8 (18)		Donor positive and recipient negative	5 (24)
Donor negative and recipient positive	8 (18)		Donor negative and recipient positive	9 (43)
Unknown	0		Unknown	0
Hospitalization status- no. (%)			Hospitalization status- no. (%)	
Inpatient, intensive care	11 (26)		Inpatient, intensive care	13 (62)
Inpatient, not intensive care	12 (28)		Inpatient, not intensive care	4 (19)
Outpatient	20 (46)		Outpatient	4 (19)
Cardiopulmonary support- no. (%)			Cardiopulmonary support- no. (%)	
Ventricular assist device	14 (33)		Ventricular assist device	7 (33)
Intra aortic balloon pump	1 (2)		Intra aortic balloon pump	1 (5)
Extracorporeal membrane oxygenation	0		Extracorporeal membrane oxygenation	0
Mechanical ventilation	0		Mechanical ventilation	1 (5)
Hemodialysis - no. (%)	2 (5)		Hemodialysis - no. (%)	0
Panel reactive antibodies >20% - no. (%)	20 (47)		Panel reactive antibodies >20% - no. (%)	17 (85)
Diabetes at time of transplant - no. (%)	8 (19)		Diabetes at time of transplant - no. (%)	0
History of hypertension- no. (%)	31 (72)		History of hypertension- no. (%)	4 (19)
History of hyperlipidemia - no. (%)	11 (26)		History of hyperlipidemia - no. (%)	0
UNOS Status- no. (%)			UNOS Status- no. (%)	
1A	15 (35)		1A	17 (81)
1B	21 (49)		1B	3 (14)
2	7 (16)		2	1 (5)
Induction therapy- no. (%)			Induction therapy- no. (%)	
rATG	42 (98)		rATG	0
Daclizumab	0		Daclizumab	15 (71)
Basiliximab	0		Basiliximab	6 (29)
None	0		None	0
Unknown	1 (2)		Unknown	0
Maintenance immunosuppression*			Maintenance immunosuppression*	
Cyclosporine - no. (%)	9 (21)		Cyclosporine - no. (%)	19 (95)
Tacrolimus - no. (%)	43 (100)		Tacrolimus - no. (%)	8 (40)
Mycophenolate mofetil - no. (%)	42 (98)		Mycophenolate mofetil - no. (%)	20 (100)
Sirolimus - no. (%)	15 (35)		Sirolimus - no. (%)	5 (25)
Everolimus - no. (%)	3 (7)		Everolimus - no. (%)	0
* includes all agents used during the study period			* includes all agents used during the study period	