

Biomarkers for Rejection and Allograft Function in Kidney Transplantation: Results from the Clinical Trials in Organ Transplantation (CTOT*)

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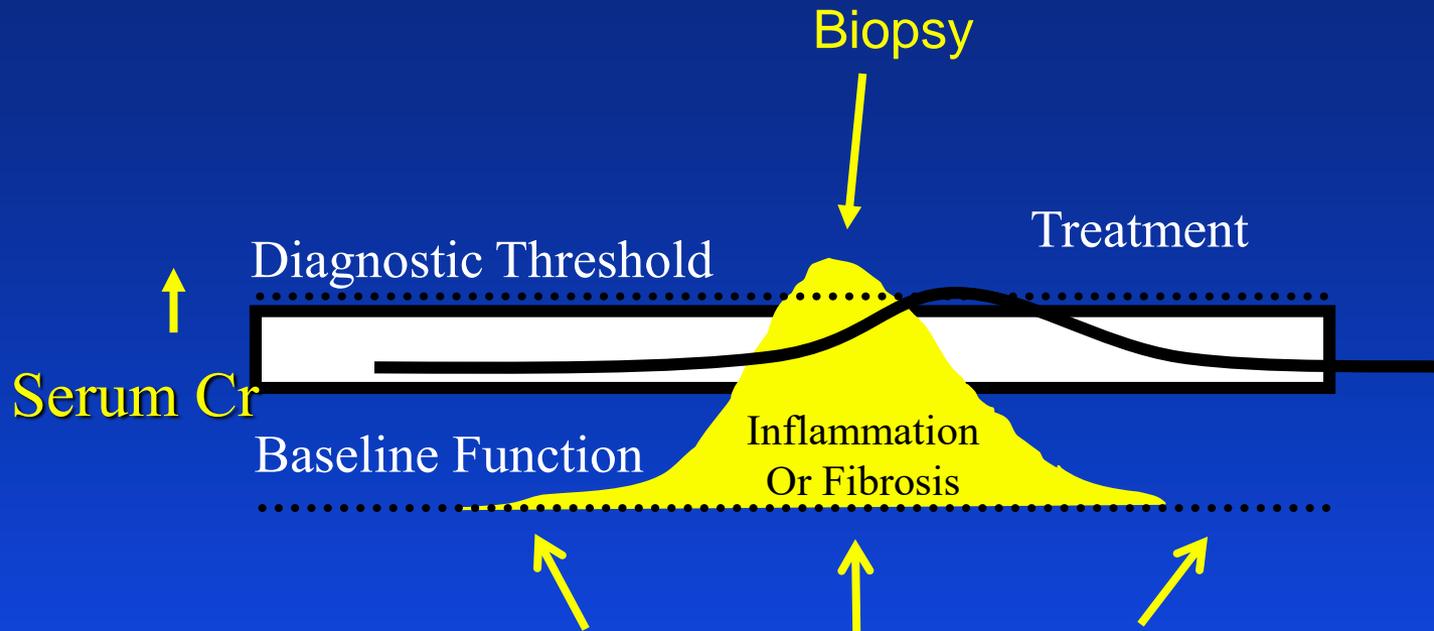
Cleveland, Ohio



*A cooperative consortium of transplant centers carrying out clinical and mechanistic studies in organ transplantation, sponsored by the National Institute of Allergy and Infectious Diseases (NIAID).



Biomarkers in Kidney Transplantation



Can we use biomarkers 1) as a surrogate for acute rejection, 2) to detect early signs of immune activation before overt rejection occurs, 3) to predict a decline in renal function? or 4) to guide immunosuppression

CTOT-04 – questions 1,2; CTOT-01 (CTOT-17)– questions 1,2, and 3; CTOT-09 – questions 1,2,3 and 4

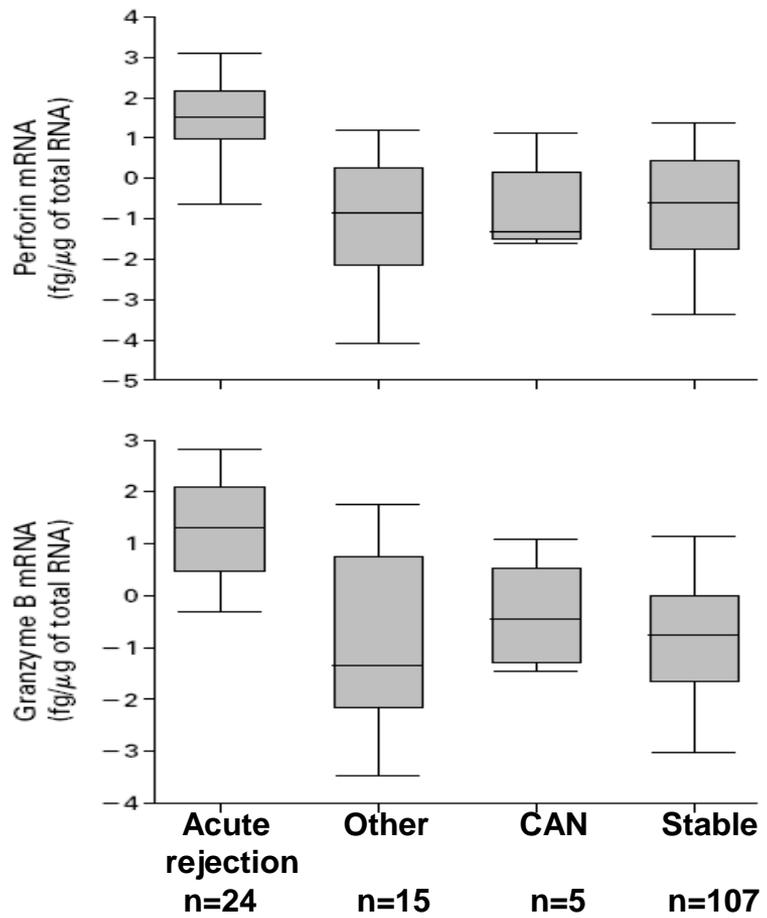
Comparison of CTOT- 4 and CTOT-01

CTOT-04 (485 adults)

- 5 centers
- Adults
- Assays
 - 8 Urine mRNAs: perforin, granzyme, CD3, proteinase inhibitor 9, CD103, IP-10 (CXCL10), CXCR3, TGF β
- Core Lab – Cornell (Suthanthiran)

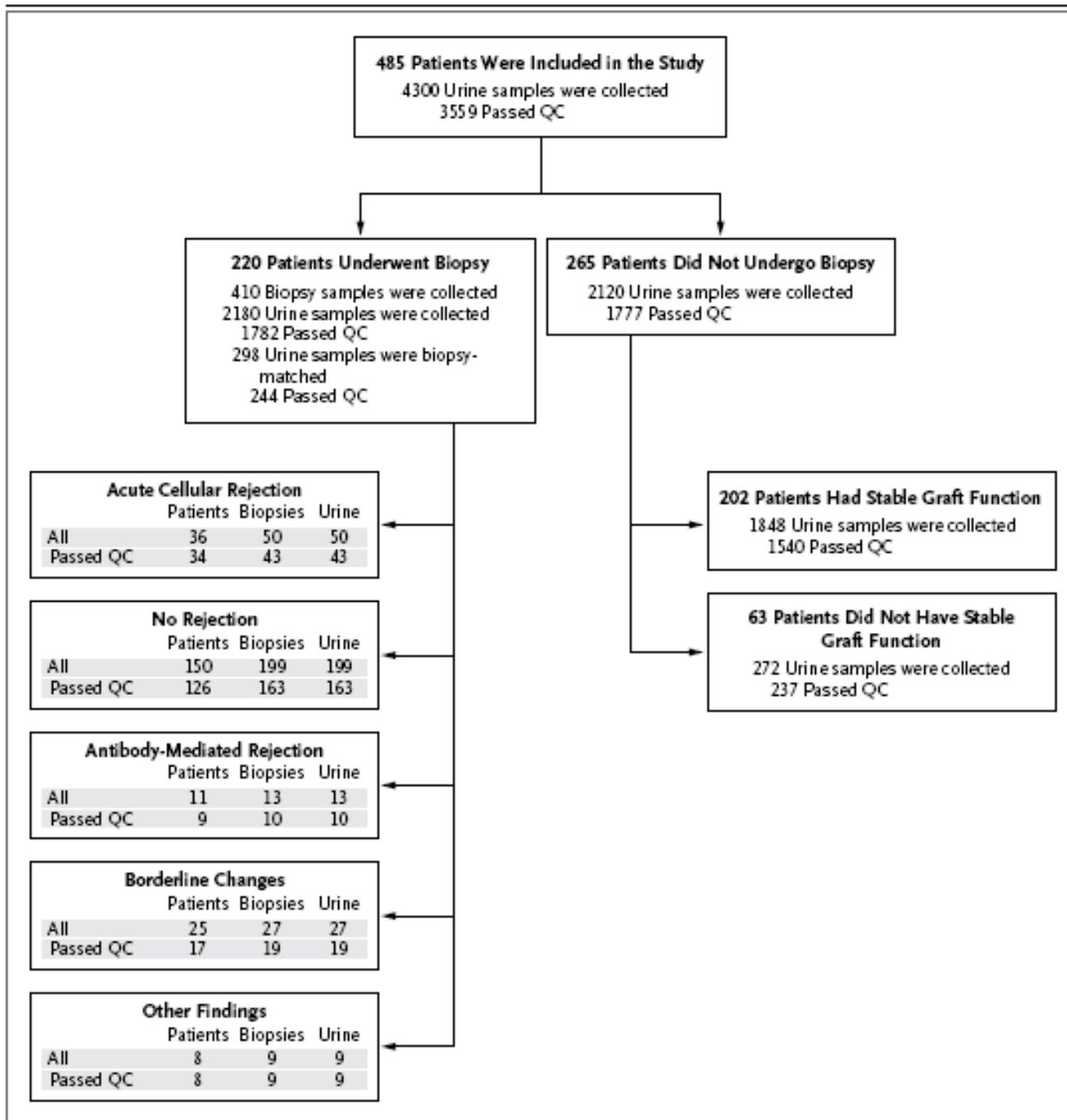
CTOT-01 (282 adults and children)

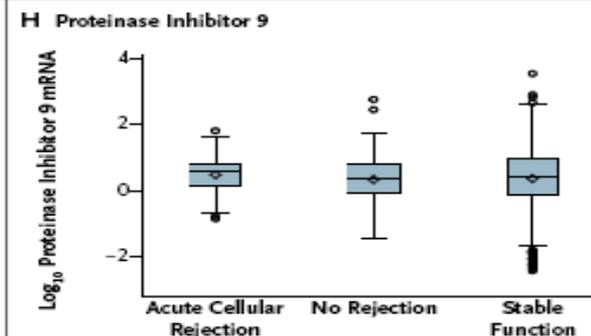
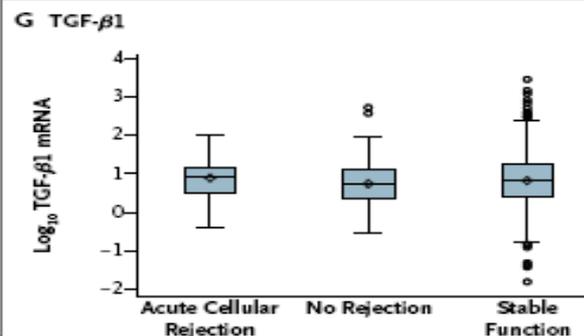
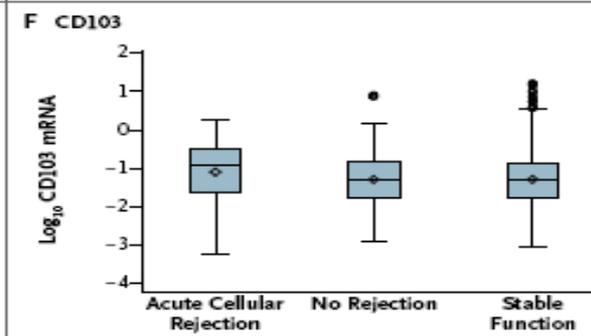
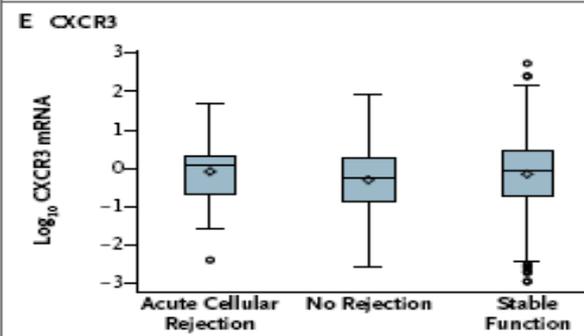
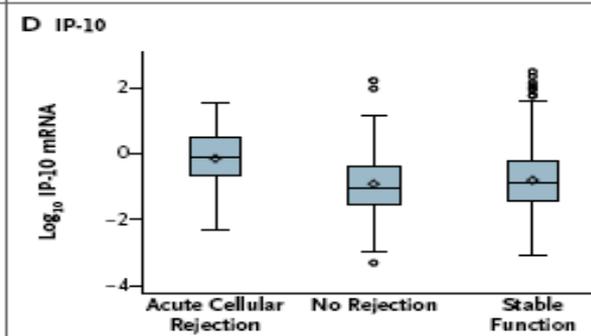
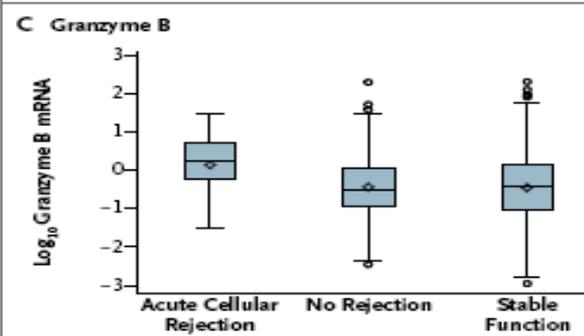
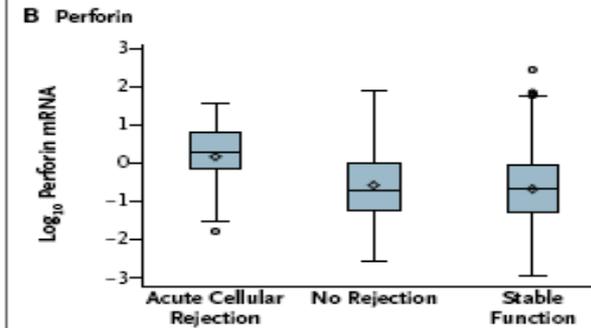
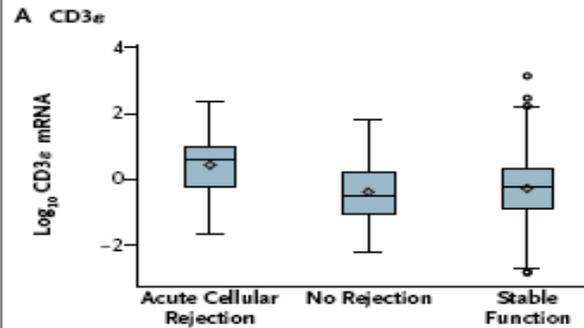
- 8 centers
- Adults and children
- Assays
 - 9 Urine mRNAs: perforin, granzyme, CCR5, CCL5, IL-8, CXCR3, CCR1, MIG (CXCL9), IP-10 (CXCL10)
 - 2 urine proteins: CXCL9, CXCL10 by ELISA
 - Others (ELISPOTs, Immunknow[®], blood mRNAs)
- Core Labs - mRNAs – CCF (Fairchild); ELISAs – Mt.Sinai (Heeger)



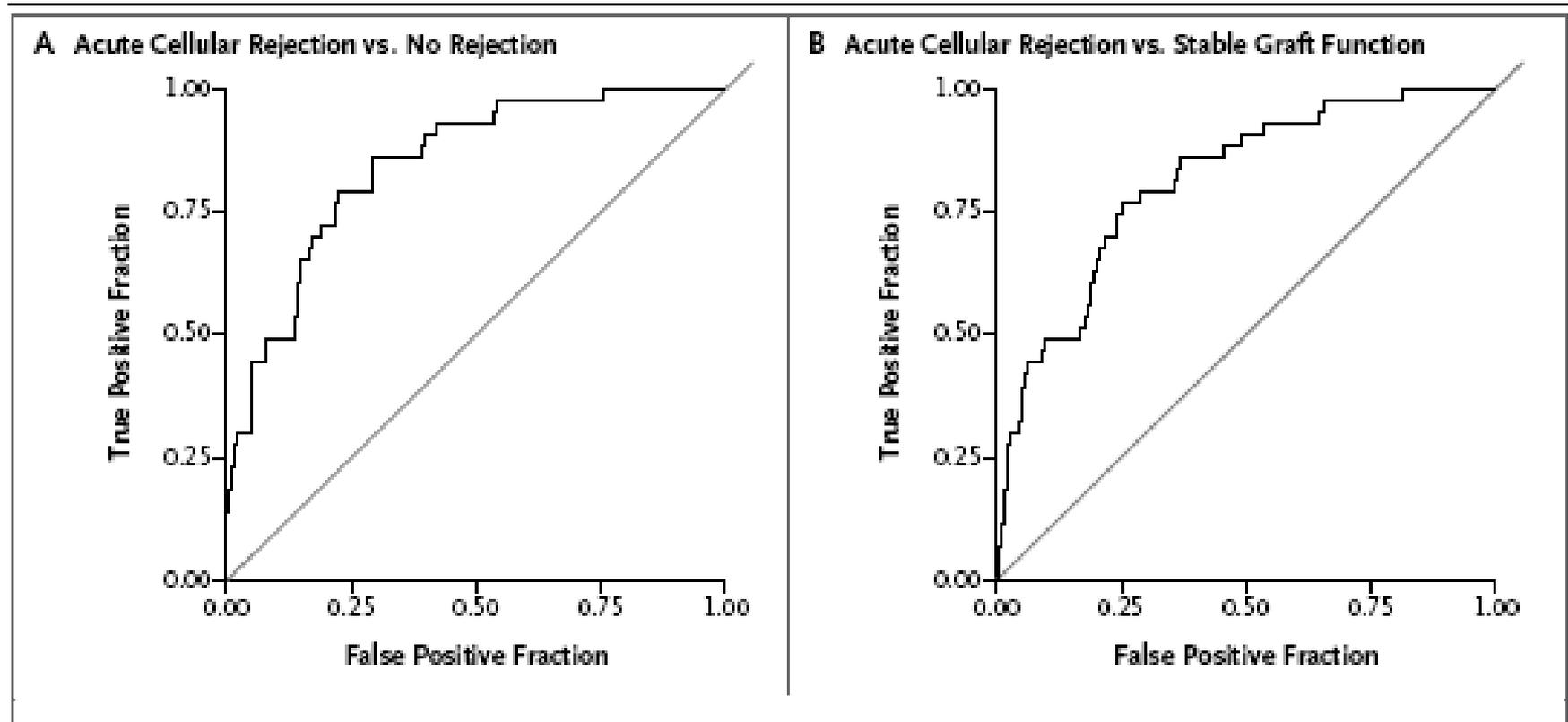
Li et al. N Engl J Med 2001

CTOT-04



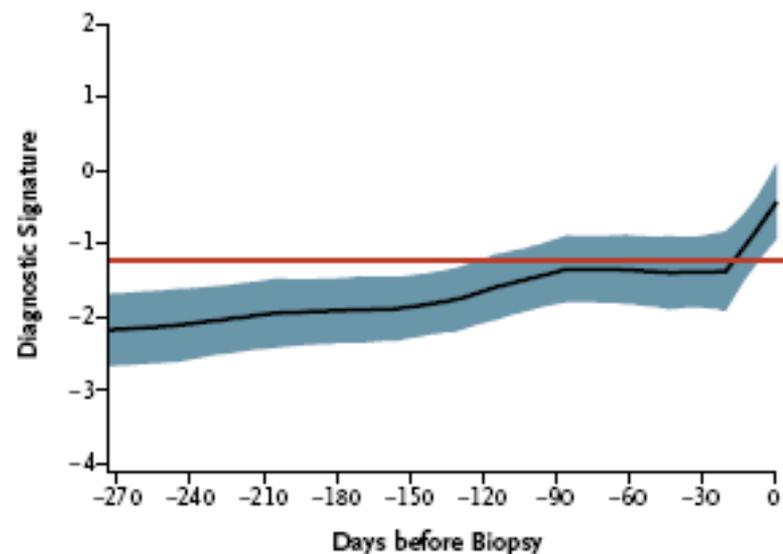


Three Gene Signature (CD3, IP-10 (CXCL10) and 18s ribosomal RNA) as a Diagnostic Test for Acute Rejection

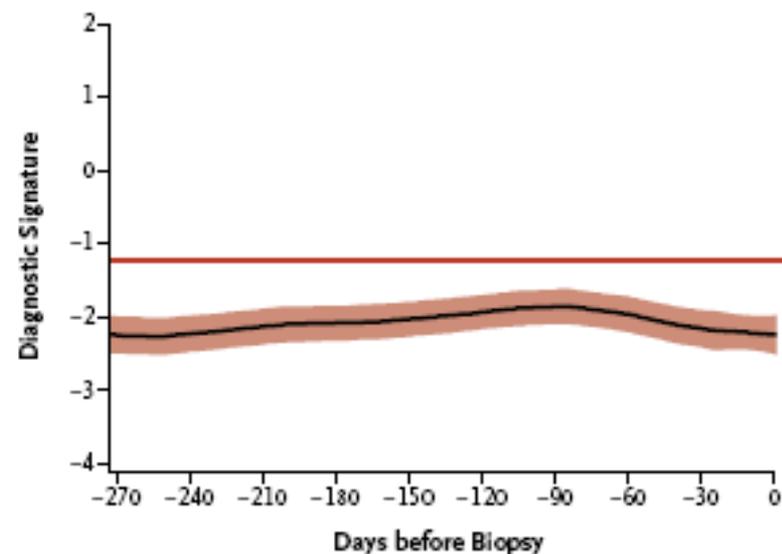


**AUC 0.85; Sensitivity 79%;
Specificity 78%**

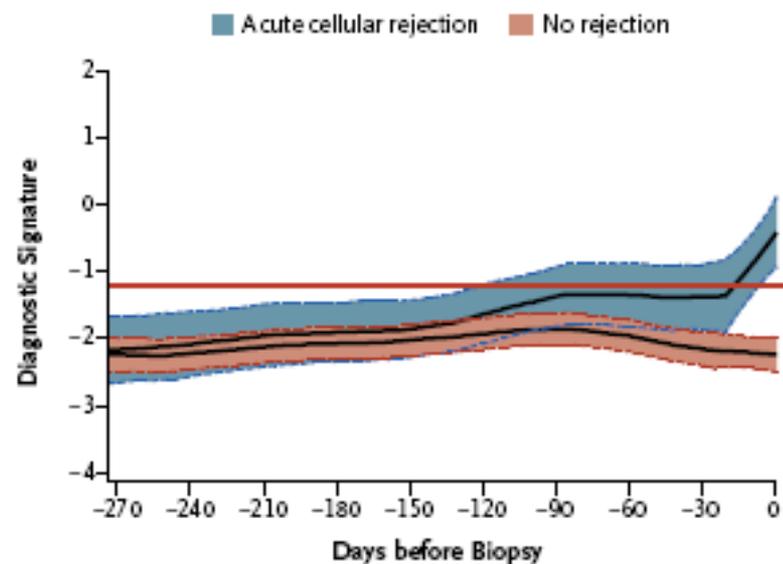
A First Biopsy Sample Showing Acute Cellular Rejection



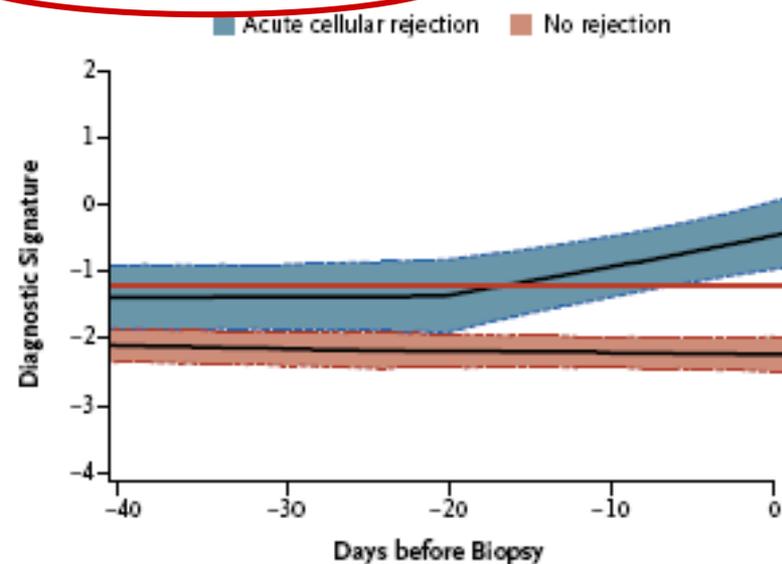
B Biopsy Sample Showing No Rejection



C Both Groups, 270 Days before Biopsy

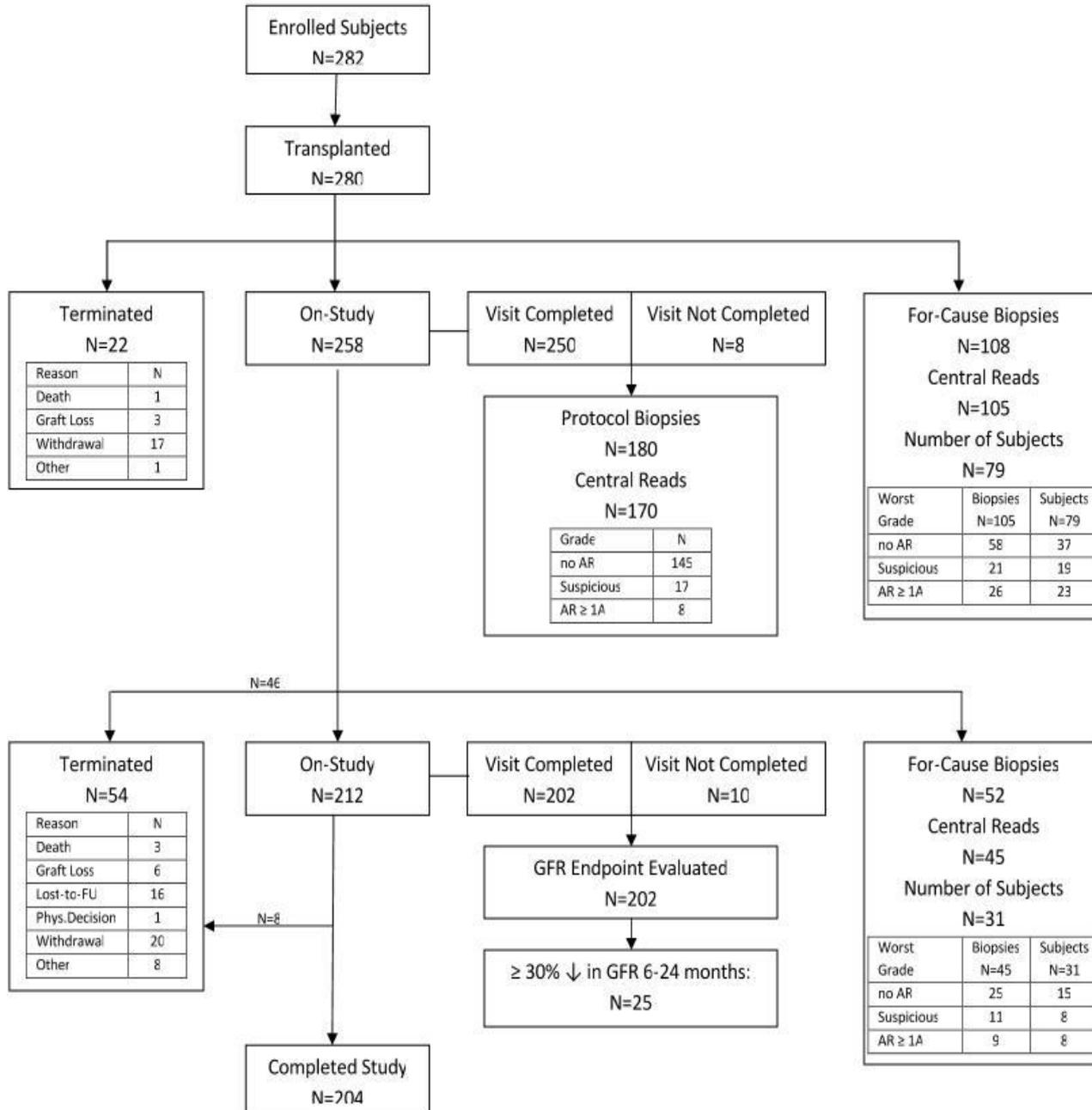


D Both Groups, 40 Days before Biopsy

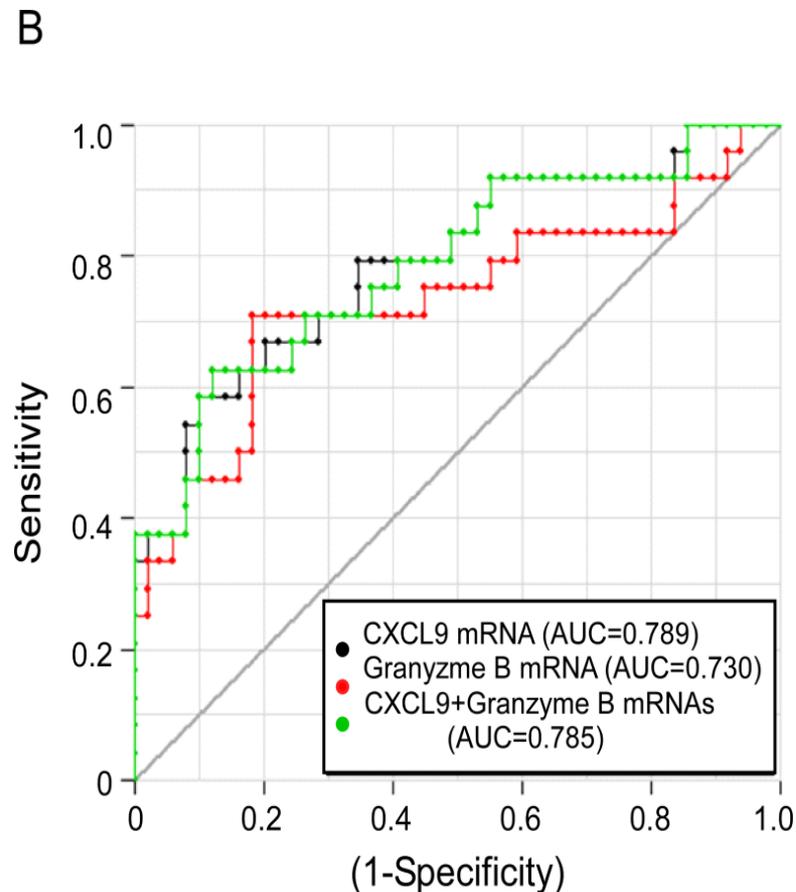
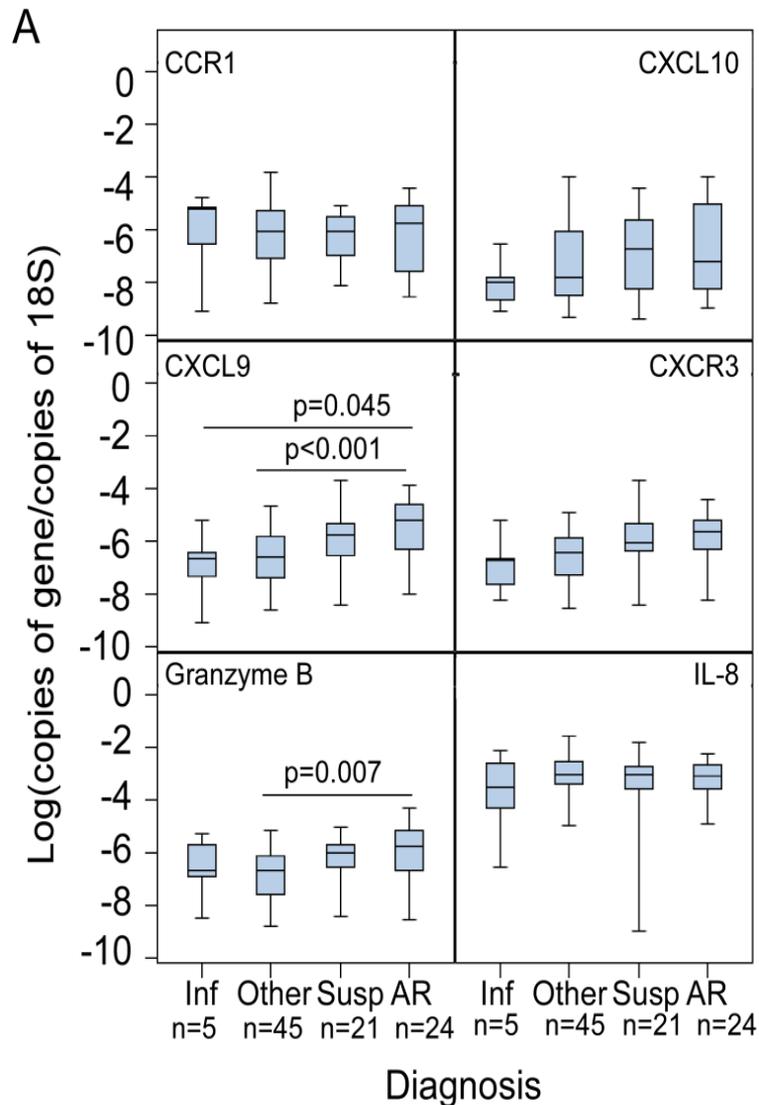


CTOT-01

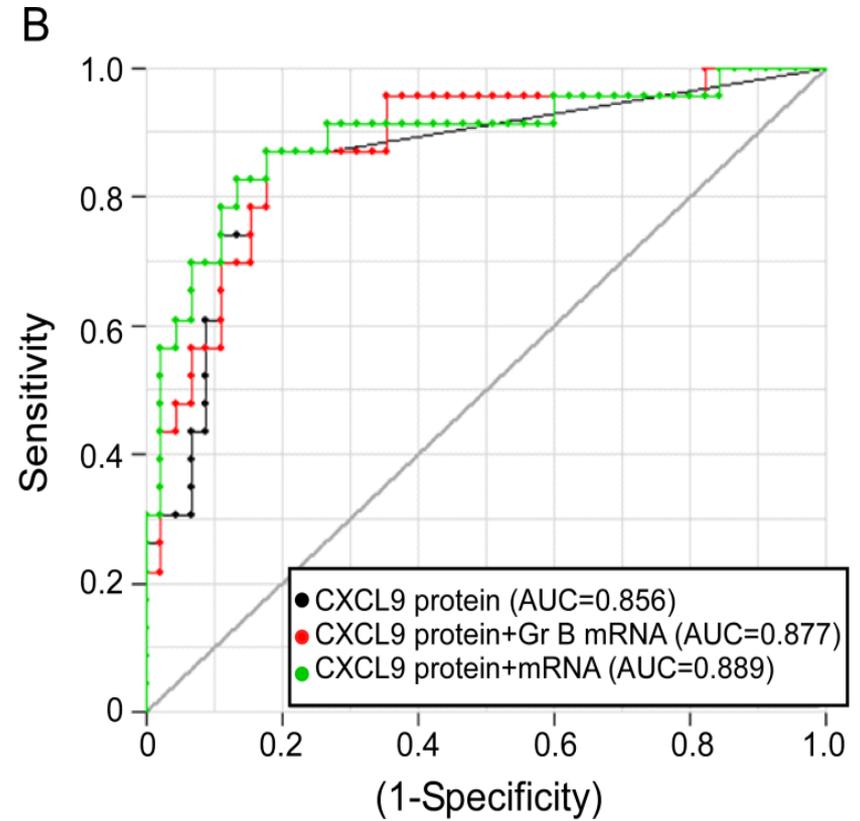
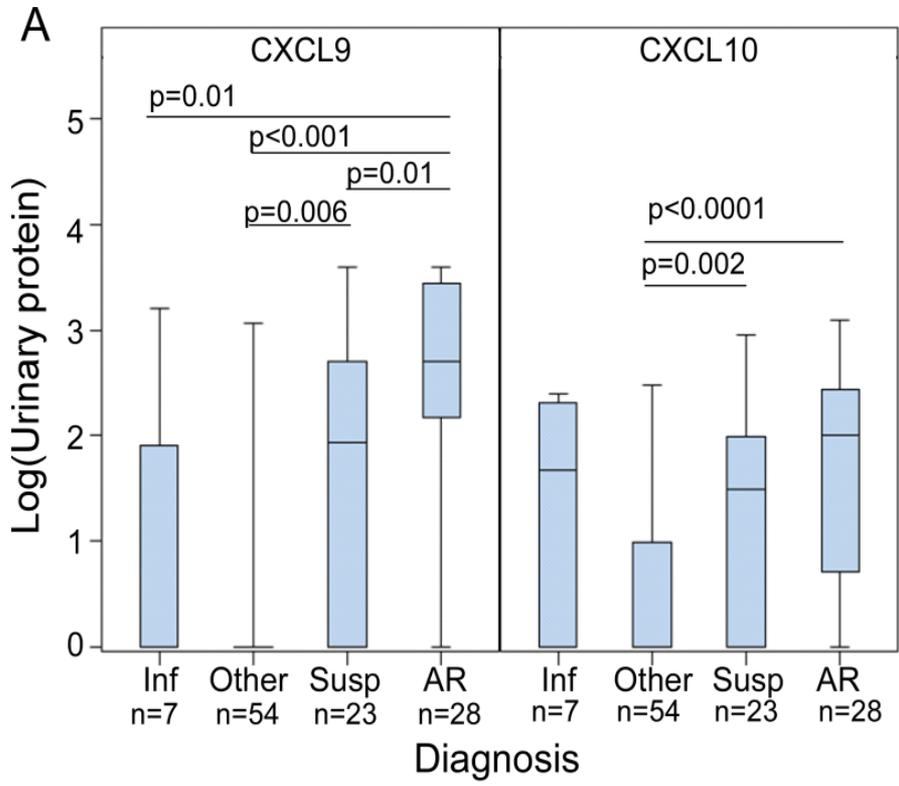
Enrollment



Among **mRNAs**, those for **CXCL9** and **Granzyme B** provide best correlations with **AR**



Urine chemokine **proteins** CXCL9 and CXCL10 measured by ELISA correlate with diagnosis of AR



Urine CXCL9 Protein at 6 months Predicts Subsequent Decline in Allograft Function

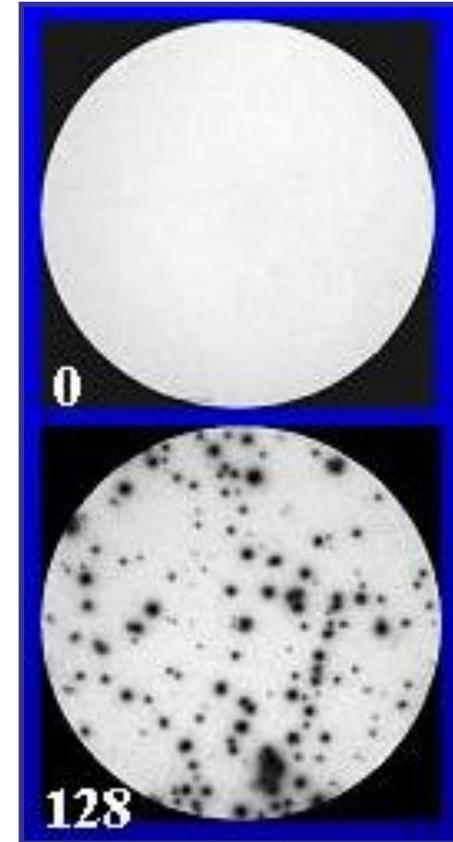
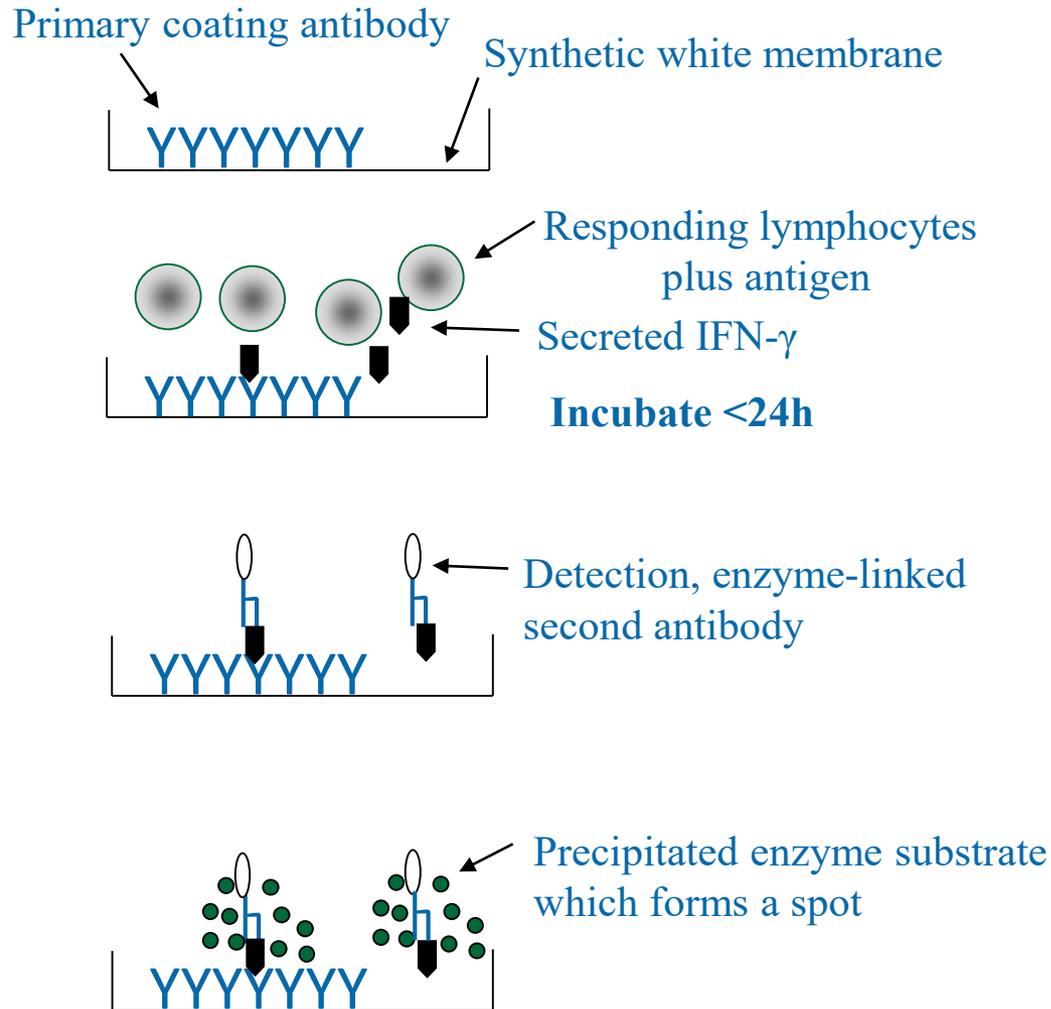
Dichotomized Decrease in GFR >30% from 6 to 24 Months Imputed Endpoint Modeled by CXCL9 Protein

Visit	#Meeting Endpoint/# Not Meeting Endpoint	Parameter Estimates and tests		ROC-based Discrimination Measures			Positive/Negative Predictive Value	
		CXCL9 Protein OR	P-value	AUC	Sensitivity	Specificity	PPV	NPV
3 Months	20/157	1.273 (0.817, 1.985)	0.2859	0.545	N/A	N/A	N/A	N/A
4 Months	19/154	0.894 (0.552, 1.447)	0.6474	0.516	N/A	N/A	N/A	N/A
5 Months	17/145	1.637 (1.034, 2.592)	0.0355	0.624	35.3	81.4	18.2	91.5
6 Months	23/163	1.929 (1.300, 2.861)	0.0011	0.681	43.5	82.2	25.6	91.2

Interim Summary and Conclusions

- Results of CTOT-04 and CTOT-01 are complementary in finding biomarkers for acute rejection
- CTOT-04 focused on the **positive predictive value** of a 3 gene signature requiring RT PCR techniques – i.e. a *possible* replacement for biopsy as the gold standard for AR
- CTOT-01 focused on the **negative predictive value** of single urinary protein (CXCL9) measurable by a simple ELISA – low or normal values in noninfected patients make AR unlikely and predict stable graft function.

ELISPOT Assay



Pre-transplant *donor-reactive* T cells and post-transplant outcome – Univariate analysis

Pre-transplant donor reactive ELISPOT (n=37)			
Variable	Negative (<25/300K)	Positive (> 25/300K)	p value
Acute cellular rejection	17%	50%	.036
GFR (MDRD) 12 months	55 ± 20 ml/min/1.73m ²	37 ± 16 ml/min/1.73m ²	.006
DGF	23%	31%	NS

Pre-transplant positive anti-donor ELISPOT is independently associated with ACR

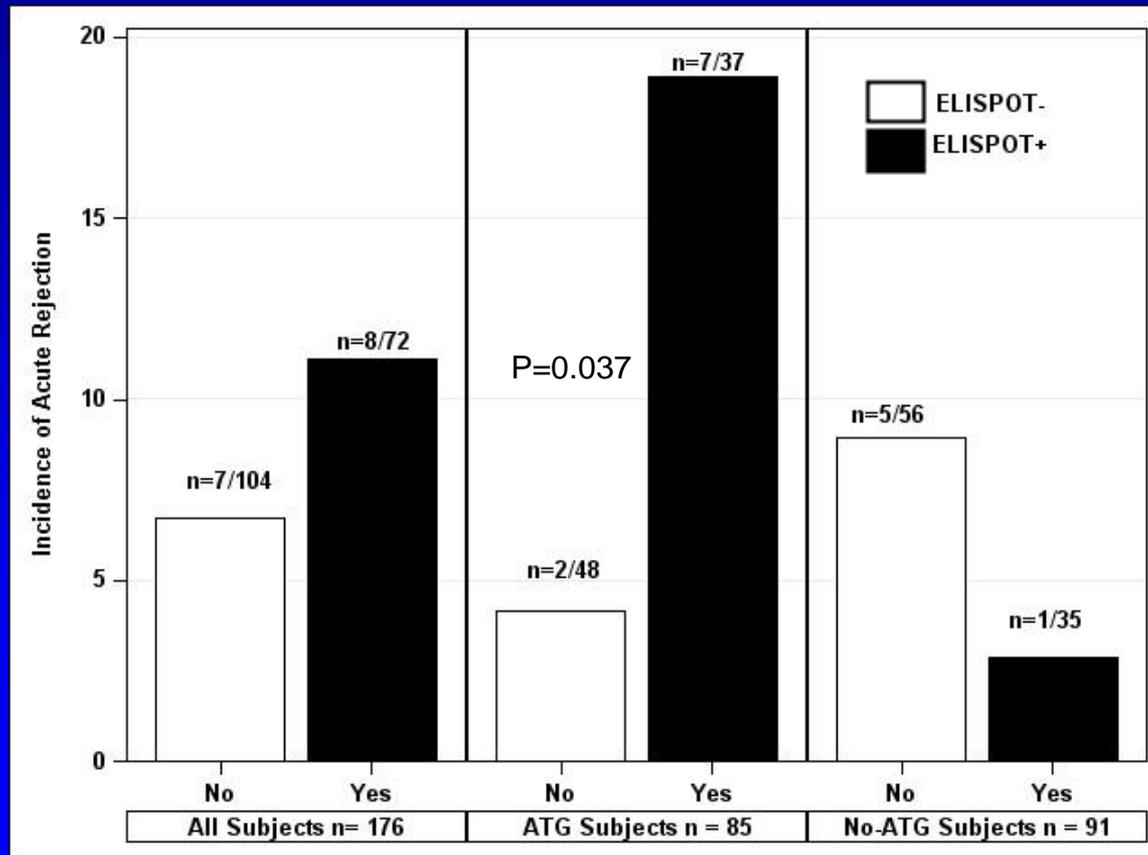
Table 2: Logistic regression analysis for acute rejection

Variable	Odds ratio	95% CI	p-value
HLA mismatch	0.5	0.2–1.4	ns
Sensitized	1.1	0.1–13.1	ns
Induction Rx	0.2	0.1–4.8	ns
DGF	15.1	1.6–145.0	0.019
ELISPOT (+)	6.3	1.0–41.1	0.055

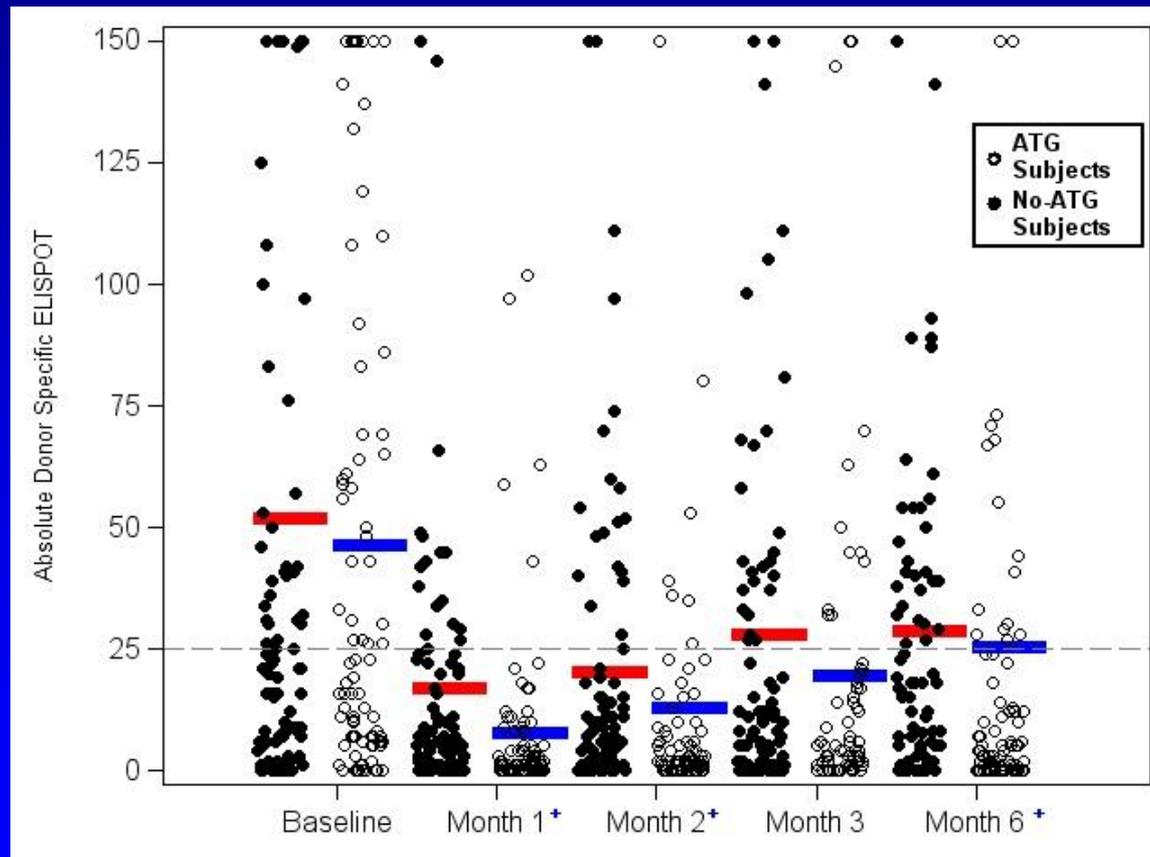
Clinical Characteristics of Patients Enrolled in CTOT-01

Characteristics	All CTOT-01 Enrollees (N=280)	Subjects w/ Pre-Trans ELISPOT (N=176)	Subject w/Pre-Trans ELISPOT		P value
			ATG Subjects (N=85)	No-ATG Subjects (N=91)	
Donor Age (years)					
Mean (SD)	39.8 (13.02)	40.0 (12.93)	41.5 (12.97)	38.6 (12.81)	
Donor Gender					
Male	134 (47.9)	85 (48.3)	42 (49.4)	43 (47.3)	
Female	146 (52.1)	91 (51.7)	43 (50.6)	48 (52.7)	
Donor Type					
Deceased	88 (31.4)	56 (31.8)	43 (50.6)	13 (14.3)	<0.01
Living	192 (68.6)	120 (68.2)	42 (49.4)	78 (85.7)	
Recipient Age (years)					
Mean (SD)	43.5 (17.57)	46.6 (15.59)	50.1 (12.60)	43.4 (17.37)	<0.01
Recipient Gender					
Male	168 (60.0)	105 (59.7)	59 (69.4)	46 (50.5)	
Female	112 (40.0)	71 (40.3)	26 (30.6)	45 (49.5)	
Recipient Race					
Black or African American	80 (28.6)	55 (31.3)	38 (44.7)	17 (18.7)	<0.01
Other Race	199 (71.1)	120 (68.2)	47 (55.3)	73 (80.2)	
Unknown or Not Reported	1 (0.4)	1 (0.6)	0	1 (1.1)	
Recipient Age Group					
Adult	240 (85.7)	164 (93.2)	83 (97.6)	81 (89.0)	<0.01
Pediatric	40 (14.3)	12 (6.8)	2 (2.4)	10 (11.0)	
Peak PRA					
Mean (SD)	12.9 (24.58)	10.2 (20.27)	11.4 (23.75)	9.0 (16.43)	
Number of HLA Mismatches					
Mean (SD)	3.5 (1.81)	3.5 (1.81)	3.9 (1.83)	3.1 (1.71)	<0.05
Induction Therapy					
Yes	233 (83.2)	151 (85.8)	85 (100)	66 (72.5)	<0.01
No	47 (16.8)	25 (14.2)	0	25 (27.5)	
DeNovo DSA					
Yes	11 (3.9)	4 (2.3)	1 (1.2)	3 (3.3)	
No	269 (96.1)	172 (97.7)	84 (98.8)	88 (96.7)	

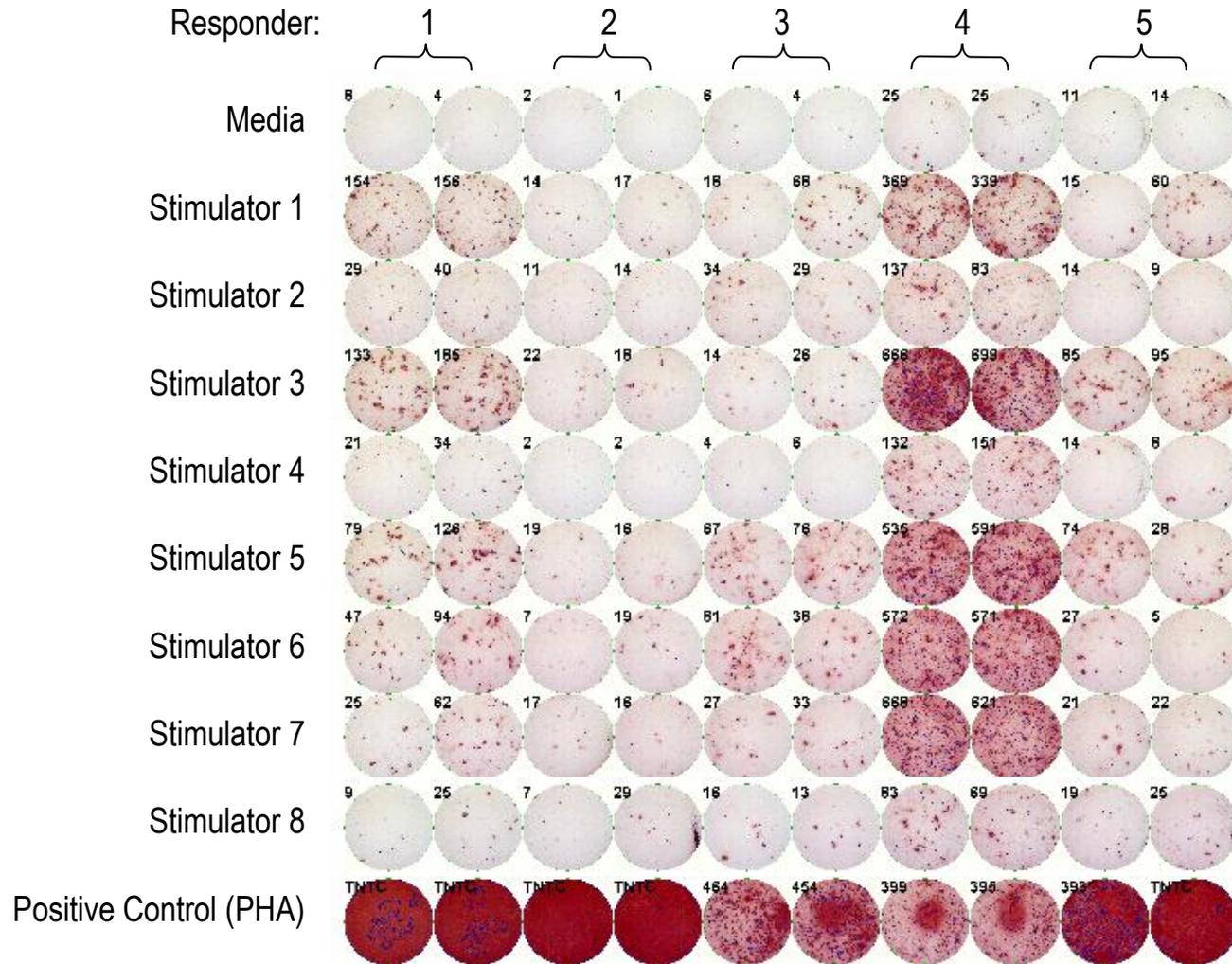
Incidence of Acute Rejection Based on Pretransplant ELISPOT Status and ATG vs No ATG



Influence of ATG on Posttransplant ELISPOTS

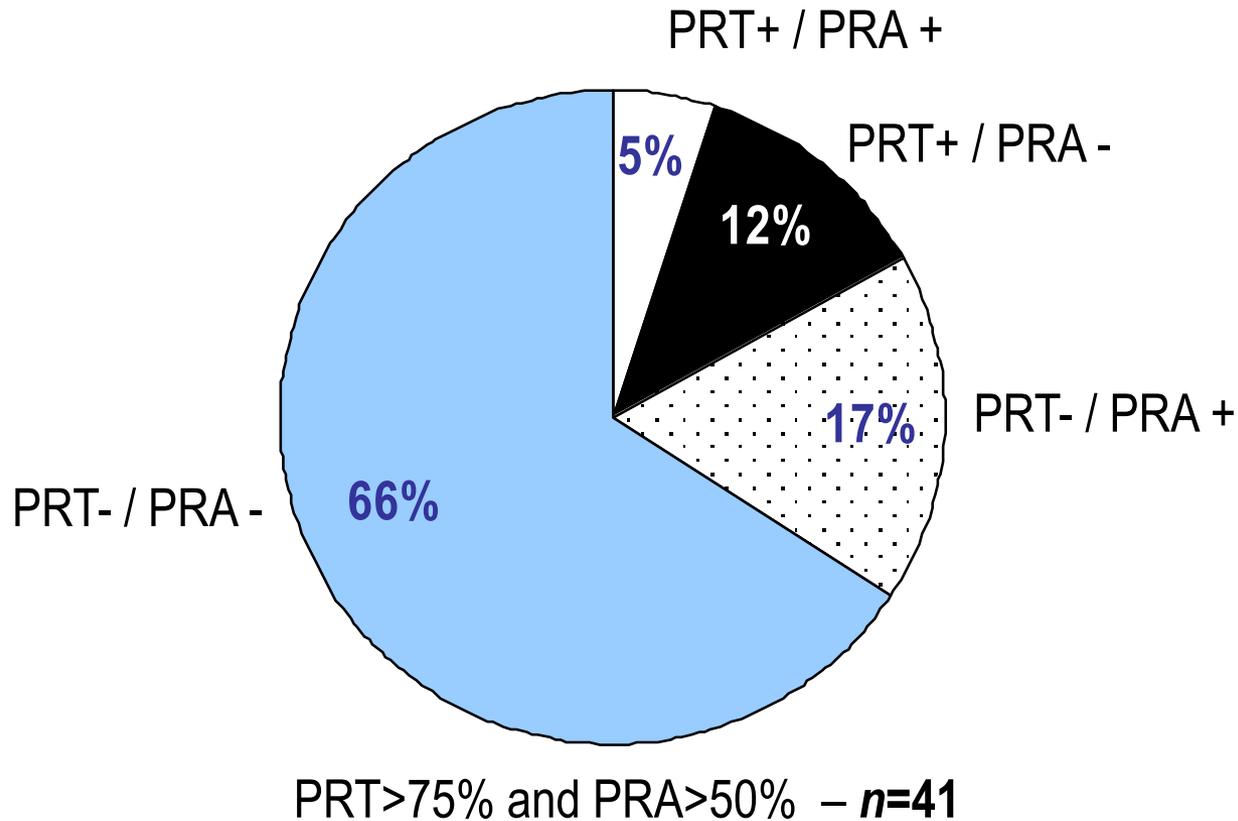


Representative PRT ELISPOT Assay

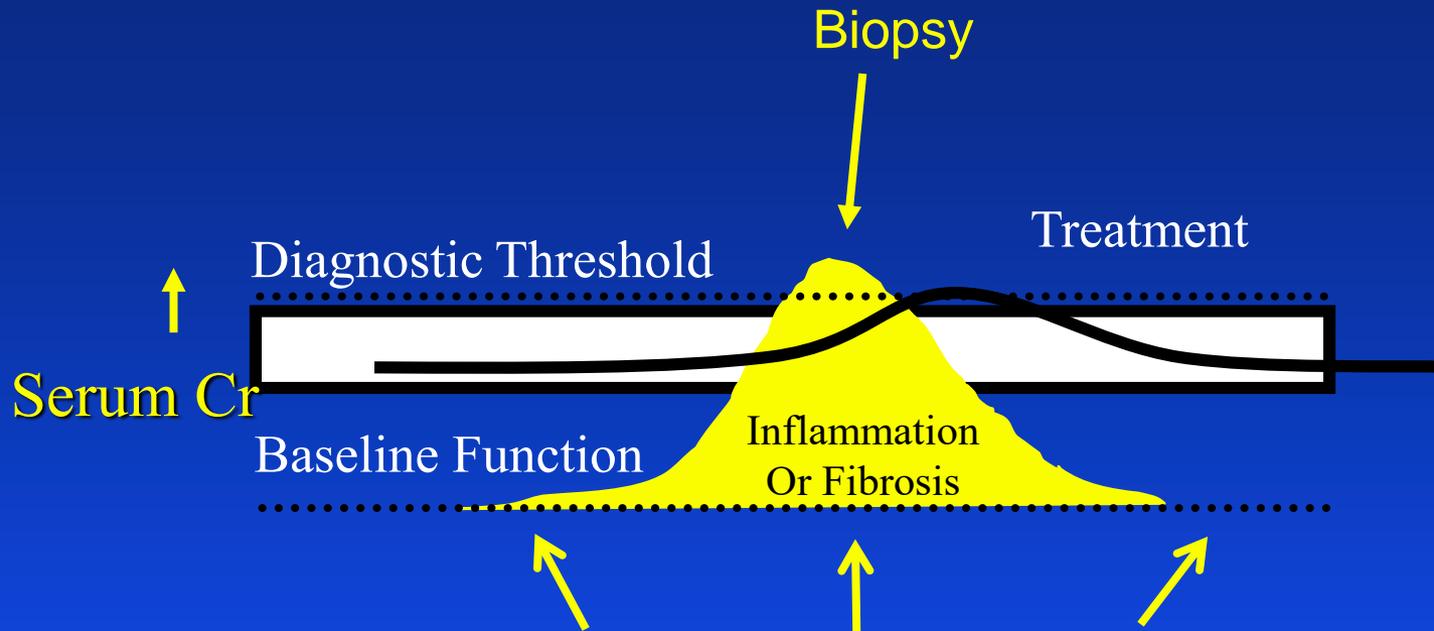


Relationship between PRA and PRT

T cell sensitization does not imply B cell sensitization and vice versa



Biomarkers in Kidney Transplantation



Can we use biomarkers 1) as a surrogate for acute rejection, 2) to detect early signs of immune activation before overt rejection occurs, 3) to predict a decline in renal function? or

4) to guide immunosuppression

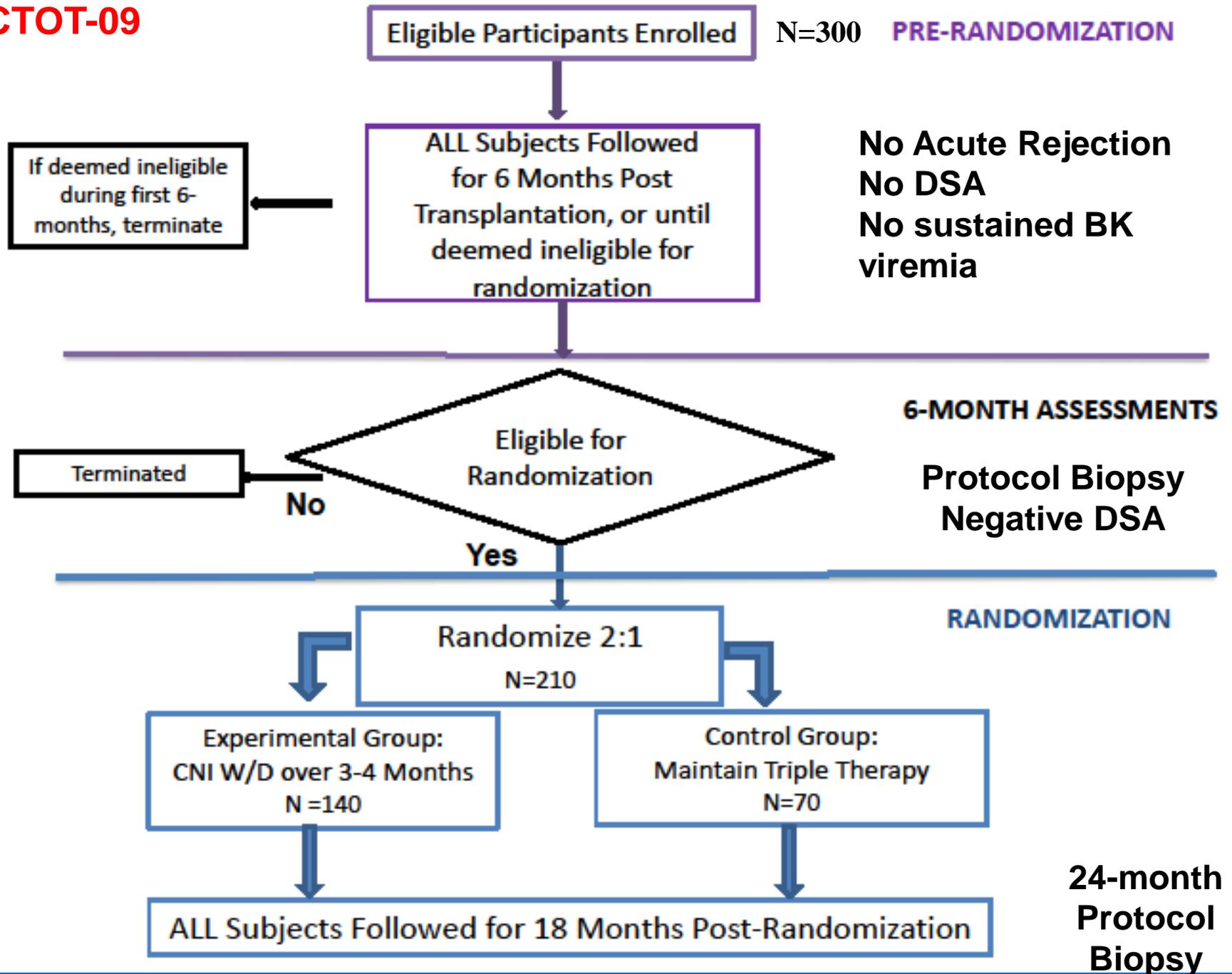
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CTOT-09 – questions 1,2,3 and 4

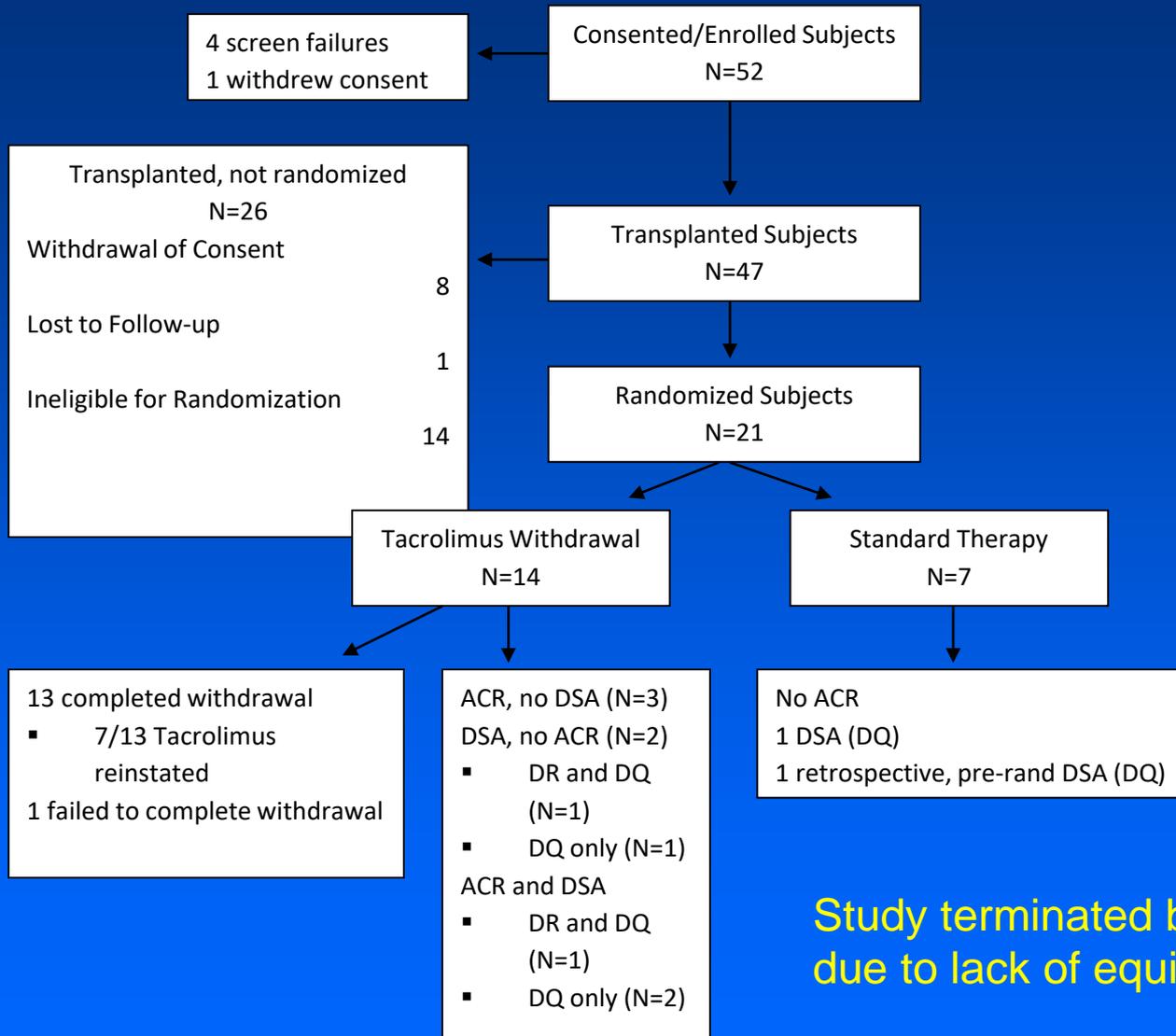
CTOT09

- Tacrolimus withdrawal in stable kidney transplant recipients
- Living donors, DSA negative, peak PRA < 20%
- Thymo, MMF, Tac, low dose prednisone for 6 mo
- Eligible for randomization (2:1 withdrawal) at 6 mo if
 - Absence of AR within first 6 mo
 - Absence of anti HLA antibody
 - 6 mo biopsy no rejection
- Withdraw tacrolimus over 3 months, serial urine for chemokines
- Biopsies driven by elevated urinary MIG (CXCL9) or IP10 (CXCL10) on 2 consecutive repeat studies

CTOT-09

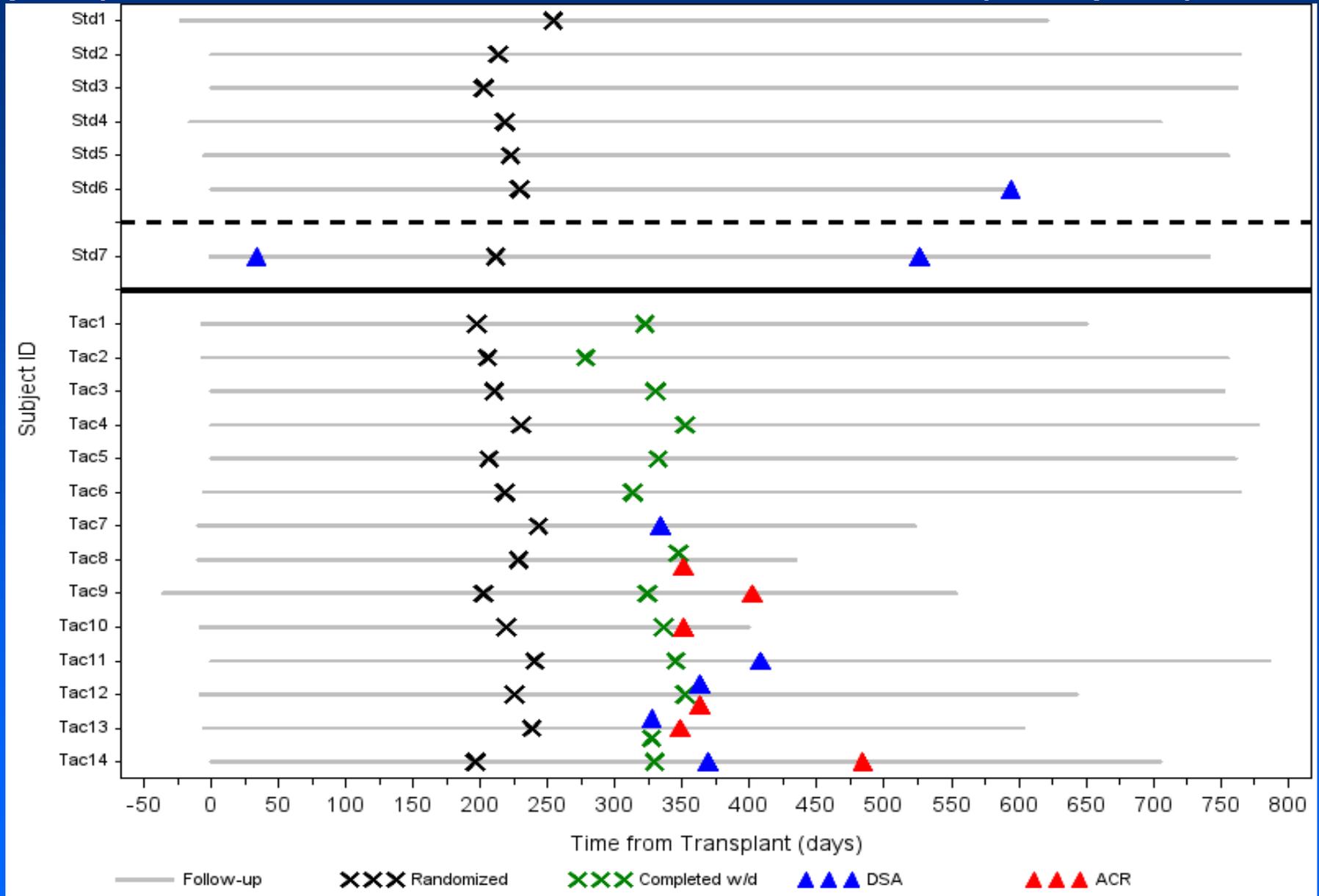


CTOT-09 CONSORT DIAGRAM

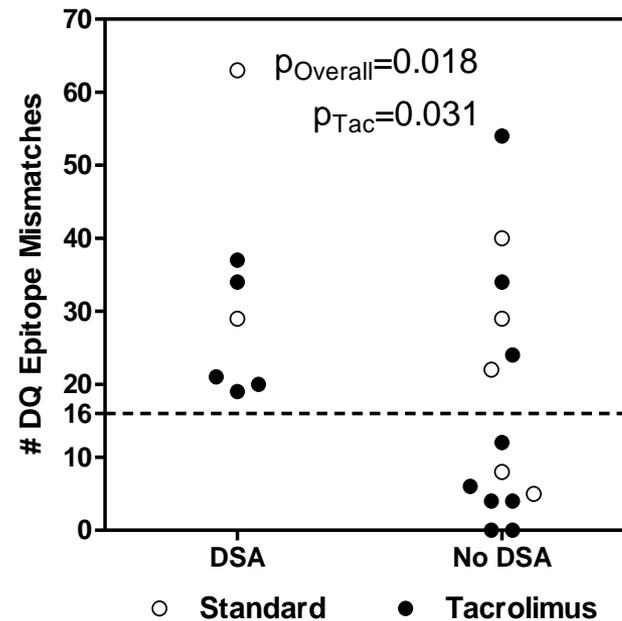
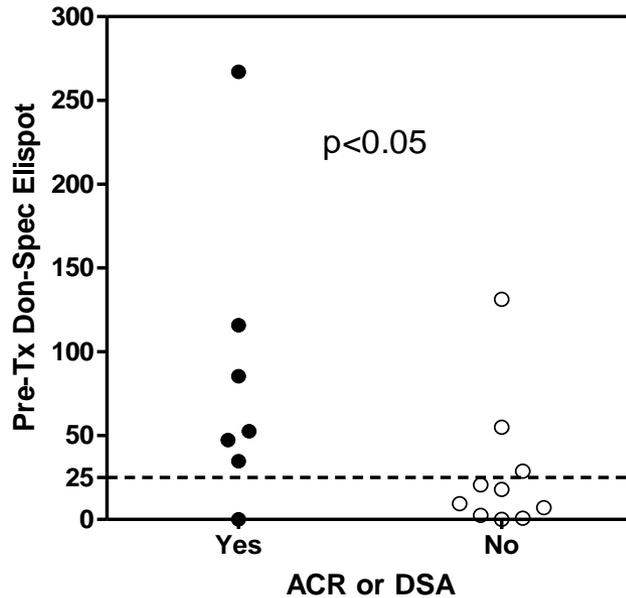


**Study terminated by DSMB
due to lack of equipoise**

Timing of Adverse Outcomes (DSA and/or ACR) in Control Patients (upper panel) vs Patients Randomized to Tacrolimus Withdrawal (lower panel)



Post-hoc analyses: ELISPOTs and HLA Epitope Mismatching



CTOT09

- Biomarker analyses show
 - Urinary CXCL9 detectable prior to rejection in all cases of rejection. False positives were 2 cases of infection, one BK the other “viral illness”
 - The numbers of epitope mismatches at DQ strongly correlated with the risk of developing new DQ DSA
 - Pretransplant ELISPOT positive (anti donor >25 correlated with increased risk of developing either AR or de novo DSA

Summary and Conclusions

- Several urine biomarkers emerging as reliable surrogates for ongoing or imminent AR.
- ELISA for CXCL9 easier to perform and less labor intensive than mRNA assays
- ELISPOT assay for IFN gamma promising as a pretransplant immune response assessment tool
- Whether these biomarkers can be used as a guide to immunosuppression remains to be proven