Supplementary Materials for:

Elevation of donor-derived cell-free DNA prior to biopsy proven rejection in kidney transplant

Authors: Jonathan S. Bromberg, MD, PhD[1]; Suphamai Bunnapradist, MD, MS[2]; Milagros Samaniego-Picota, MD[3]; Sanjiv Anand, MD[4]; Erik Stites, MD[5]; Philippe Gauthier, MD, MBA[6]; Zachary Demko, PhD[6]; Adam Prewett, MBA[6]; Madeleine Armer-Cabral, MS[6]; Kyle Marshall, MS[6]; Navchetan Kaur, PhD[6]; Michelle S. Bloom, PhD[6]; Hossein Tabriziani, MD[6]; Sangeeta Bhorade, MD[6]; Matthew Cooper, MD[7] on behalf of the ProActive investigators[*]

Affiliations:

1. University of Maryland, School of Medicine, Department of Surgery, Baltimore, MD, USA.

2. UCLA School of Medicine, Los Angeles, CA, USA

3. Henry Ford Transplant Institute, Detroit, MI, USA

4. Intermountain Healthcare, Salt Lake City, UT, USA

5. University of Colorado Anschutz Medical Campus, School of Medicine, Denver, CO, USA

6. Natera, Inc. Austin, TX, USA

7. Medical College of Wisconsin, Milwaukee, WI, USA

* A full list of the ProActive investigators is included under Acknowledgments, and in Table S1.

Supplementary Figures

Figure S1: Histogram showing propensity scores of the AKP1631 cohort and the subcohort used in this study. Propensity scores (probabilities) were estimated by fitting a series of three logistic regression models to the study population data and averaging the resultant scores. The data consisted of 55 covariates that included medical procedures or hospitalizations, laboratory measurements, medications, demographics and histology assessments of each patient.
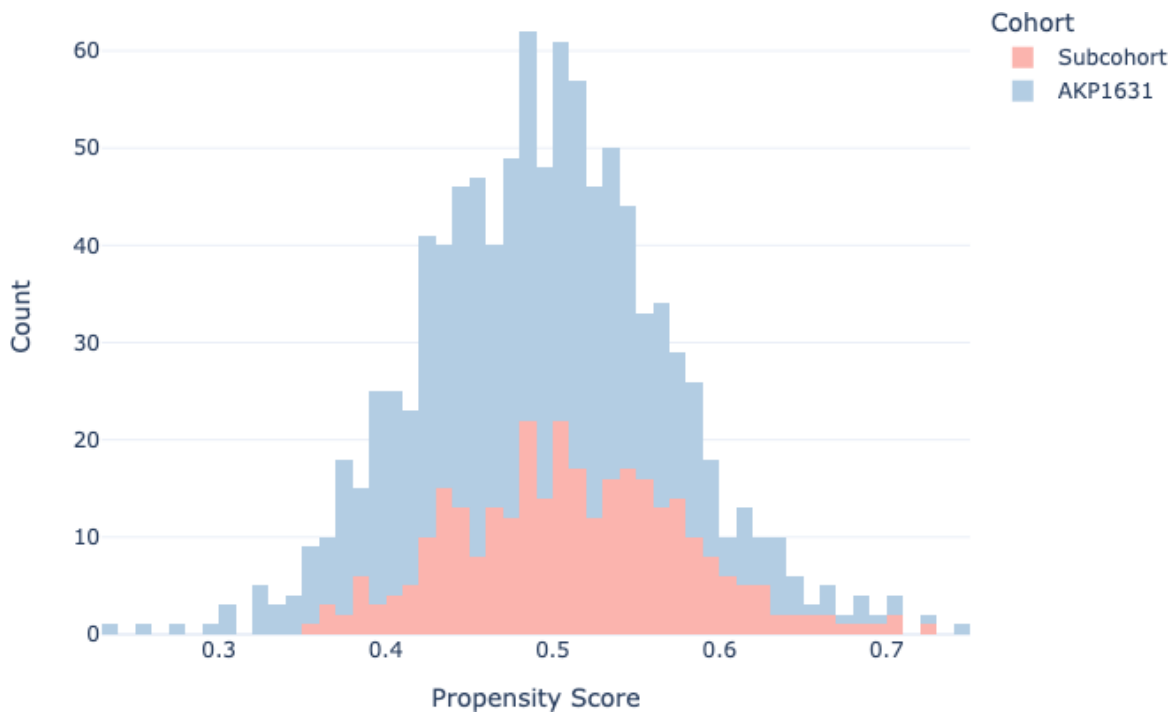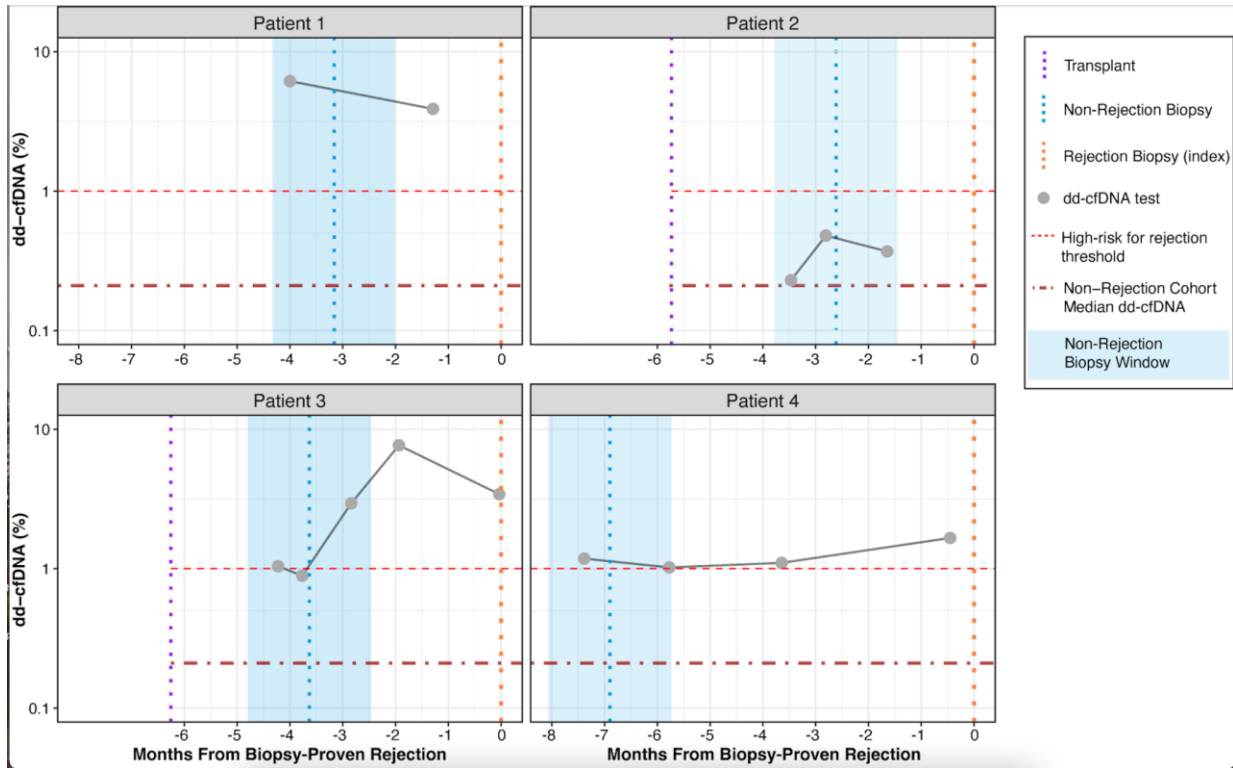
Figure S2: dd-cfDNA% trends in patients with a non-rejection biopsy prior to an ABMR biopsy.

dd-cfDNA test results are plotted for 4 unique patients in the study cohort with a non-rejection biopsy (dotted blue line) within the eight months prior to the index biopsy diagnosing ABMR (dotted orange line). Shaded blue box indicates the 2-month window before and after the non-rejection biopsy.

Figure S3: Boxplots for dd-cfDNA measurements drawn within 14 days of biopsy, stratified by biopsy result. P values from Wilcoxon rank-sum test with FDR correction for multiple testing.
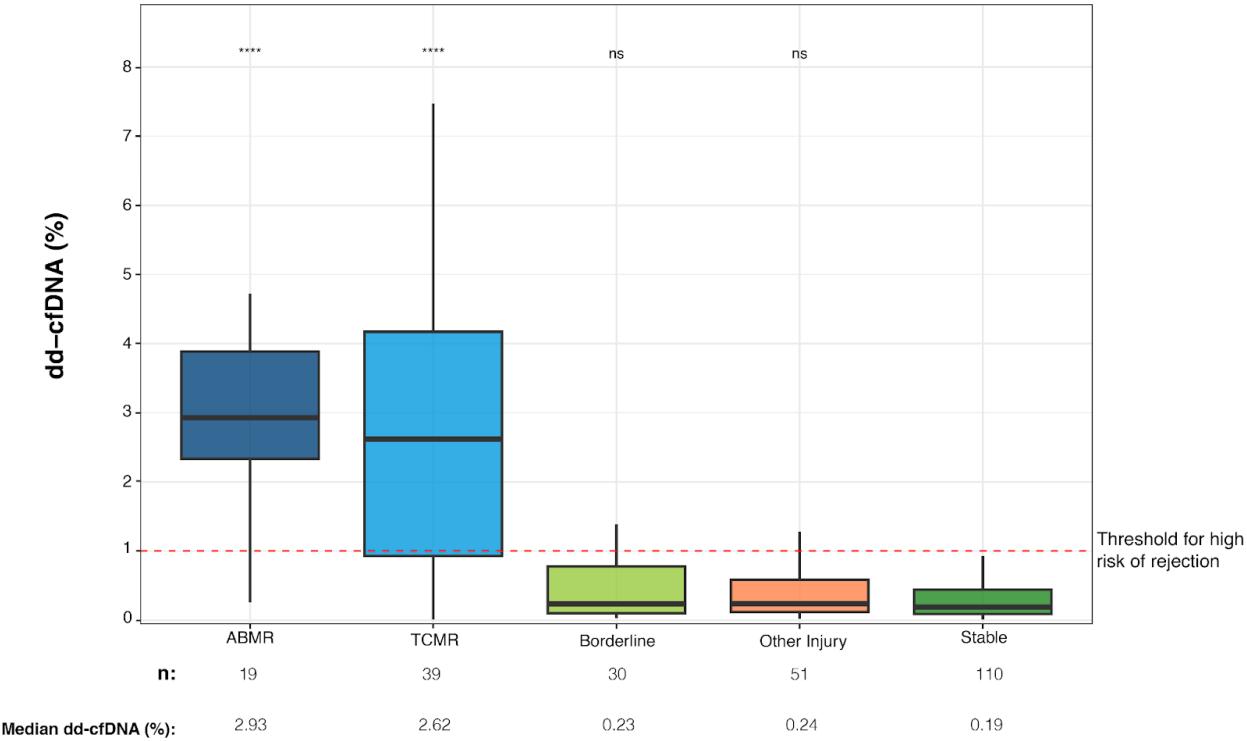
Figure S4: AUC for detection of rejection by dd-cfDNA for samples within 14 daysof biopsy. (N = 249 samples; 58 Rejection, 191 non-rejection)
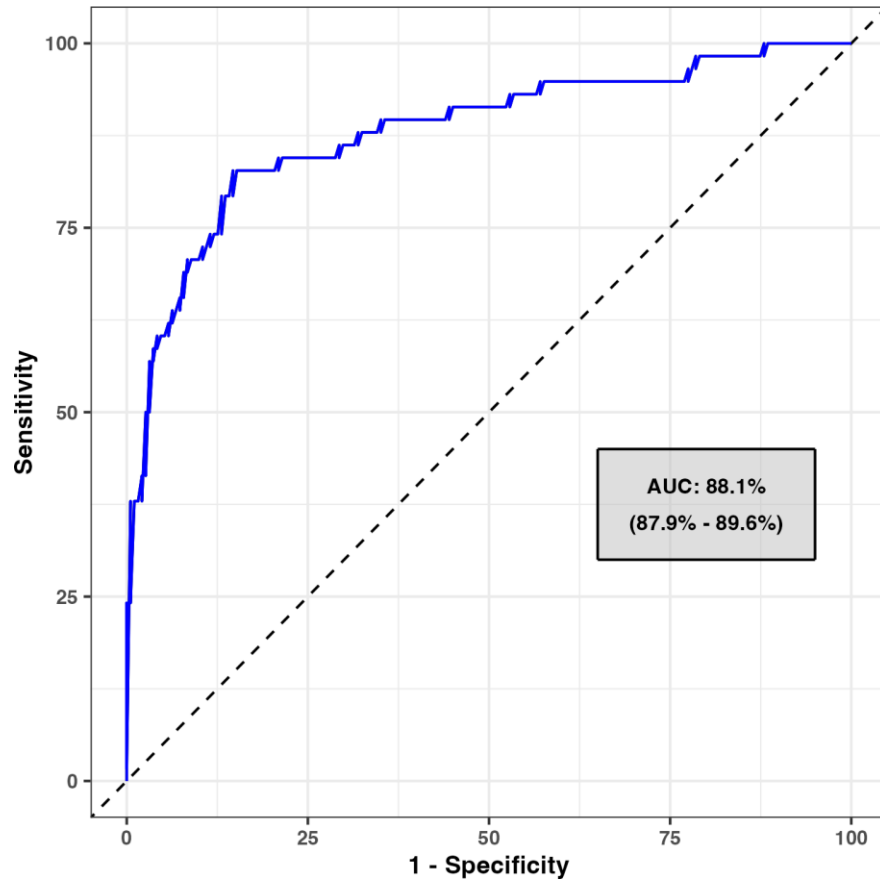
Table S1: A list of the ProActive study investigators with their respective centers and the IRB approval protocols numbers. This list represents 40 centers from which the ProActive AKPI 1631 cohort was derived.

#	Investigator(s) (Credentials)	Center	Location (City, State)	Central IRB#	Local IRB#
1	Jonathan Bromberg (MD, PhD)	University of Maryland	Baltimore, MD	Central IRB Advarra Protocol ID Pro00037470	Human Research Protection Program Study # HP-00087496
2	Tarek Alhamad (MD, MS, FACP, FASN)	Washington University School of Medicine	St. Louis, MO	Central IRB Advarra Protocol ID Pro00037470	Washington University in St. Louis, Human Research Protection Office, IRB # 201909033
3	Anup Patel (MD)	RWJ Barnabas Medical Center	Livingston, NJ	Central IRB Advarra Protocol ID Pro00037470	-
4	Liise Kayler (MD, MS, FACS)	Erie County Medical Center	Buffalo, NY	Central IRB Advarra Protocol ID Pro00037470	University of Buffalo IRB Study # 00003845
5	Nadiesda Costa (MD, MPH) Mathew Cooper (MD)	MedStar Georgetown University Hospital	Washington, DC	Central IRB Advarra Protocol ID Pro00037470	-
6	David Wojciechowski (DO)	University of Texas Southwestern Medical Center	Dallas, TX	Central IRB Advarra Protocol ID Pro00037470	UT Southwestern Medical Center IRB, Protocol # STU-2019-1440
7	Milagros Samaniego-Picota (MD)	Henry Ford Hospital	Detroit, MI	Central IRB Advarra Protocol ID Pro00037470	-
8	Yasir Qazi (MD) Neeraj Sharma (MD)	Keck Hospital of USC	Los Angeles, CA	Central IRB Advarra Protocol ID Pro00037470	University of Southern California IRB Proposal # HS-19-00746
9	Christine Du (MD) Michael Moritz (MD)	Lehigh Valley Health Network	Allentown, PA	-	Lehigh Valley Health Network IRB ID STUDY00000353

10	Ali Zarrinpar (MD, PhD)	University of Florida	Gainesville, FL	Central IRB Advarra Protocol ID Pro00037470	-
11	Thuy Le (MD) / William Bennett (MD, FASN)	Legacy Good Samaritan Hospital and Medical Center	Portland, OR	-	Legacy Health IRB # 1769-2019
12	Erik Stites (MD)	University of Colorado	Aurora, CO	Central IRB Advarra Protocol ID Pro00037470	-
13	Richard Fatica (MD)	Cleveland Clinic	Cleveland, OH	-	Cleveland Clinic IRB # 19-1610
14	Henry Randall (MD)	Saint Louis University Hospital	Saint Louis, MO	Central IRB Advarra Protocol ID Pro00037470	-
15	Muna Alnimri (MBBS)	University of California Davis	Sacramento, CA	Central IRB Advarra Protocol ID Pro00037470	-
16	Anil Chandraker (MD, MBChB)	Brigham and Women's Hospital	Chestnut Hill, MA	Central IRB Advarra Protocol ID Pro00037470	-
17	Lewis Teperman (MD)	Northwell Health	Manhasset, NY	Central IRB Advarra Protocol ID Pro00037470	-
18	Sanjeev Akkina (MD)	Loyola University	Maywood, IL	-	Loyola University Chicago IRB# 212638
19	Layla Kamal (MD)	Virginia Commonwealth University	Richmond, VA	WCG IRB Study 1282067 IRB # 20193133	-
20	Thomas Diflo (MD)	Westchester Medical Center	Westchester County, NY	-	New York Medical College IRB Protocol # 14099
21	Sanjiv Anand (MD)	Intermountain Health Center	Salt Lake City, UT	-	Intermountain Healthcare Research IRB # 1051418
22	Neerja Agrawal (MD)	Cleveland Clinic Florida (Weston)	Weston, FL	-	Cleveland Clinic IRB # 19-1640

23	David Leeser (MD)	East Carolina University	Greenville, NC	Central IRB Advarra Protocol ID Pro00037470	East Carolina University, University & Medical Center IRB, Study # UMCIRB 20-000295
24	Fuad Shihab (MD)	University of Utah	Salt Lake City, UT	-	University of Utah IRB# 00129786
25	Edward Walshe (MD)	Lahey Clinic	Burlington, MA	Central IRB Advarra Protocol ID Pro00037470	-
26	Martin Aldana-Campos (MD)	Tampa General Hospital	Fort Myers, FL	Central IRB Advarra Protocol ID Pro00037470	-
27	Silas Norman (MD, MPH)	University of Michigan	Ann Arbor, MI	-	Office of Human Research Protection IRB # IRB00001995
28	Robert Stratta (MD)	Wake Forest University Health Sciences	Winston-Salem, NC	Central IRB Advarra Protocol ID Pro00037470	-
29	Nicolae Leca (MD)	University of Washington Medical Center	Seattle, WA	WCG IRB Study # 1281959 IRB # 20193133	-
30	Ayoola Adekile (MD)	University Medical Center of South Nevada	Las Vegas, NV	-	University Medical Center of Southern Nevada IRB # UMC-2020-292
31	Suphamai Bunnapradist (MD, MS)	University of California Los Angeles	Los Angeles, CA	-	University of California Los Angeles Office of Human Research Protection Program IRB # 20-000511-AM-00012
32	Ekamol Tantisattamo (MD)	University of California, Irvine Health	Irvine, CA	Central IRB Advarra Protocol ID Pro00037470	-
33	Obi Ekwenna (MD)	University of Toledo	Toledo, OH	Central IRB Advarra Protocol ID Pro00037470	-
34	Arman Faravardeh (MD)	Balboa United	San Diego, CA	Central IRB Advarra Protocol ID Pro00037470	-

35	James Sondheimer (MD, FACP, FASN)	Wayne State University	Detroit, MA	WCG IRB Study # 1297778 IRB # 20193133	**-**
36	Shikha Mehta (MD)	University of Alabama	Birmingham, AL	Central IRB Advarra Protocol ID Pro00037470	**-**
37	Venkatesh Ariyamuthu (MD)	University of Arizona, Banner University Medical Center	Phoenix, AZ	Central IRB Advarra Protocol ID Pro00037470	**-**
38	Joseph Tremaglio (MD) Caroline Rochon (MD)	Hartford Hospital	Hartford, CT	Central IRB Advarra Protocol ID Pro00037470	**-**
39	Scott Westphal (MD)	University of Nebraska Medical Center	Omaha, NE	Central IRB Advarra Protocol ID Pro00037470	**-**
40	Mallika Gupta (MD, FASN)	University of Kansas Medical Center	Lawrence, KS	**-**	University of Kansas Medical Center, IRB # IRB00000161

Table S2: Median dd-cfDNA% (IQR; (25% -75%), in clinically indicated and surveillance biopsies in the month prior to biopsy. P Values from Wilcoxon rank-sum test with FDR correction for multiple testing.

Biopsy Indication	Biopsy Type Group1	AR (N)	Median / IQR dd-cfDNA (%) AR	Non Rej Comparison	Non-rejection (N)	Median / IQR dd-cfDNA (%) Non-rejection	p.adj	P .adj signif
Clinically Indicated	**TCMR**	46	2.625 (1.015-4.405)	**No Rejection**	121	0.31 (0.14-0.93)	<0.001	****
	ABMR	15	2.91 (2.7-5.89)	**No Rejection**	121	0.31 (0.14-0.93)	<0.001	****
Surveillance	**ABMR**	3	3.42 (2.55-3.63)	**No Rejection**	65	0.18 (0.08-0.3)	0.005	**

Table S3: Performance metrics indicating Sensitivity, Specificity, NPV, PPV with 95% Clopper Pearson confidence intervals for active rejection within 14 days of biopsy (n = 249 biopsies).

Group	Sensitivity (%)	Specificity (%)	PPV* (%)	NPV* (%)
ABMR (n= 19)	94.7 (74.0, 99.9)	85.3 (79.5, 90.0)	36.6 (22.7, 52.3)	99.4 (96.8, 1.00)
TCMR (n= 39)	71.8 (55.1, 85.0)	85.3 (79.5, 90.0)	30.4 (17.6, 46.0)	97.1 (93.6, 99.0)
All rejections (n= 58)	79.3 (66.6, 88.8)	85.3 (79.5, 90.0)	32.6 (20.0, 47.3)	97.9 (94.8, 99.4)

*Calculated using a prevalence of 8.22% based upon AKP 1631 patient cohort.