

SUPPLEMENTAL MATERIAL

Impact of older donor age in kidney transplants in a biopsy-based observational study

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INTERCOMEX Study: ClinicalTrials.gov #NCT01299168; **Trifecta-Kidney Study:** ClinicalTrials.gov #NCT04239703

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Supplemental Table 1. Patient demographics and biopsy data in the kidney biopsy 4502 population^A				
Patient Demographics		All patients (N=3611)	Patients with known donor age≥50 (N=671)	Patients with known donor age<50 (N=894)
Mean recipient age (range)		50 (3 – 93)	55 (16 – 81)	46 (10 – 93)
Recipient gender (% known)	Male	1129 (62%)	202 (65%)	308 (60%)
	Female	694 (38%)	110 (35%)	204 (40%)
Ethnicity	Caucasian	698	154	269
	Black	260	50	102
	Other ^B	504	23	56
	Not available ^C	931	89	90
Primary Disease	Diabetic nephropathy	305	52	98
	Hypertension / large vessel disease	140	28	37
	Glomerulonephritis / vasculitis	616	66	152
	Interstitial nephritis / pyelonephritis	34	5	4
	Polycystic kidney disease	188	45	36
	Others	432	67	114
	Unknown etiology	317	50	63
Not available		361	3	13
Mean, median donor age (range)		43, 45 (1– 85)	59, 57 (50 – 85)	33, 35 (1 – 49)
Donor gender (% known)	Male	551 (50%)	119 (47%)	220 (48%)
	Female	546 (50%)	135 (53%)	242 (52%)
Donor type (% deceased donor transplants)		1461 (72%)	215 (68%)	317 (63%)
Latest kidney status (% of total)	Graft failed	208 (16%)	44 (16%)	95 (21%)
	Graft functioning/censored	1078 (84%)	236 (84%)	347 (78%)
	Missing/No graft status available	2325	36	75
	Mean (median) follow-up (functioning grafts) in days	617 (282)	789 (265)	875 (415)
Biopsy features		All biopsies (N=4502)	Biopsies with known donor age≥50 (N=810)	Biopsies with known donor age<50 (N=1103)
Mean, median time of biopsy posttransplant (TxBx) (range)		1182, 367 days (1 day – 45 years)	839, 270 days (1 – 26 years)	1307, 531 (1 – 32 years)
Mean, median days to most recent follow-up after biopsy (range)		858, 564 days (1 – 10.5 years)	821, 437 days (2 – 10.5 years)	918, 630 days (1 – 11 years)
Early biopsies (< 1 year)		2093 (49%)	426 (55%)	419 (41%)
Late biopsies (≥ 1 year)		2208 (51%)	348 (45%)	609 (59%)
Indication for biopsy	For Cause	2047 (82%)	642 (80%)	886 (82%)
	Surveillance	439 (18%)	157 (20%)	195 (18%)
	Not recorded	2016	11	22
Local DSA status at biopsy, if tested	DSA-positive	842 (48%)	251 (44%)	375 (49%)
	DSA-negative	919 (52%)	314 (56%)	391 (51%)
	Not done	2741 (61%)	245	337
Histology diagnoses	ABMR	397 (9%)	107 (13%)	171 (16%)
	AKI	114 (3%)	60 (7%)	40 (4%)
	BK	47 (1%)	19 (2%)	19 (2%)
	Borderline	133 (3%)	50 (6%)	55 (5%)
	IFTA	197 (4%)	89 (11%)	71 (6%)
	Mixed	82 (2%)	20 (2%)	46 (4%)
	NOMOA	383 (9%)	114 (14%)	181 (16%)
	pABMR	116 (3%)	30 (4%)	60 (5%)
	TCMR	152 (3%)	43 (5%)	68 (6%)
Other	2881 (64%)	278 (34%)	392 (36%)	
MMDx archetypes	NR	2479 (55%)	505 (62%)	590 (53%)
	TCMR1	231 (5%)	25 (3%)	50 (5%)
	TCMR2	345 (8%)	56 (7%)	96 (9%)
	EABMR	598 (13%)	98 (12%)	148 (13%)
	FABMR	524 (12%)	74 (9%)	148 (13%)
	LABMR	232 (5%)	33 (4%)	55 (5%)
	Minor ABMR	93 (2%)	19 (2%)	16 (1%)

A. Percentages shown are percentage of known data

B. Center specified ethnicity as 'Other'

C. Some centers preferred not to identify ethnicity

Abbreviations: TxBx – time of biopsy posttransplant

Modified from Halloran PF, Chang J, Mackova M, Madill-Thomsen KS, Akalin E, Alhamad T, et al. A cross-sectional study of the role of epithelial cell injury in kidney transplant outcomes. *JCI Insight*. 2025;10(10):e188658.

Supplemental Table 2. Linear relationships between recipient age and rejection archetypal analysis (AA) scores including time posttransplant as a covariate (N=2669 with available recipient age and time posttransplant)					
Rejection AA score	Value	Estimate	Standard error	t	P value
No Rejection Score	Intercept	0.52	0.03	15.0	<2E-16
	recipient age	0.002	0.0004	4.6	5.1E-6
	time posttransplant	-0.07	0.008	-8.0	1.7E-15
TCMR1 score	Intercept	0.14	0.01	10.2	<2E-16
	recipient age	-0.001	0.0002	-5.5	3.4E-8
	time posttransplant	-0.02	0.003	-5.1	3.9E-7
TCMR2 score	Intercept	0.11	0.02	6.8	1.4E-11
	recipient age	-0.0006	0.0002	-2.8	0.005
	time posttransplant	-0.0002	0.004	-0.6	0.95
EABMR score	Intercept	0.23	0.02	12.1	<2E-16
	recipient age	0.0005	0.0002	2.2	0.03
	time posttransplant	-0.04	0.005	-8.4	<2E-16
FABMR score	Intercept	0.02	0.02	0.8	0.40
	recipient age	-0.0007	0.0003	-2.5	0.01
	time posttransplant	0.05	0.005	9.4	<2E-16
LABMR score	Intercept	-0.07	0.01	-5.1	3.6E-7
	recipient age	-0.0003	0.0002	-1.7	0.09
	time posttransplant	0.07	0.0003	22.1	<2E-16
Minor score	Intercept	0.05	0.01	5.6	2.3E-8
	recipient age	-3.1E-5	0.0001	-0.3	0.8
	time posttransplant	3.2E-3	0.002	1.3	0.18

Supplemental Table 3. Correlations between injury features and donor age in all kidney biopsies (N=4502)									
Scores for gene sets, classifiers, and injury archetypes ^A		All biopsies (N=4502)		Biopsies ≤42 days (N=639)		Biopsies >42 days and ≤1y (N=1504)		Biopsies >1 year (N=2158)	
		Spearman correlation coefficient	P value	Spearman correlation coefficient	P value	Spearman correlation coefficient	P value	Spearman correlation coefficient	P value
Recent injury/failed repair gene sets	IRITD3	0.12	6E-08	0.01	0.88	0.22	6E-08	0.05	0.11
	IRRAT	0.14	1E-09	0.02	0.70	0.22	3.5E-08	0.06	0.06
	TAL_New4	0.15	8E-11	0.12	0.05	0.24	1.7E-09	0.08	0.02
Mitosis	MKI67 ^B	-0.06	0.007	-0.15	0.01	-0.12	0.003	-0.07	0.05
Epithelial dedifferentiation gene set	KT1	-0.06	0.007	0.02	0.79	-0.13	0.001	-0.02	0.63
	KT2	-0.09	4E-05	-0.05	0.43	-0.16	7.2E-05	-0.05	0.16
Chronic injury (atrophy-fibrosis) related scores	IGT	-0.12	7E-08	0.004	0.94	0.05	0.3	-0.12	0.0003
	CXCL6	0.16	2E-12	0.25	4E-05	0.29	4E-13	0.13	5E-05
	Classifier ci>1 _{Prob}	0.12	1E-07	0.37	2E-10	0.33	5.2E-17	0.13	0.0001
	Classifier ct>1 _{Prob}	0.14	8E-10	0.37	2E-10	0.34	4.3E-18	0.14	3E-05
Injury classifiers	Classifier lowGFR _{Prob}	0.18	5E-16	0.10	0.10	0.26	8.6E-11	0.11	0.001
	Classifier Prot _{Prob}	0.08	0.0005	0.09	0.15	0.22	3.5E-08	0.03	0.44
Injury PCA scores	Injury PC1	0.10	1E-05	0.07	0.23	0.25	6.1E-10	0.05	0.11
	Injury PC2	-0.10	2E-05	0.10	0.09	-0.03	0.5	0.05	0.15
	Injury PC3	0.20	1E-18	0.25	3E-05	0.15	0.0002	0.22	1E-11
Injury AA scores	Normal	-0.07	0.002	-0.11	0.08	-0.21	2.00E-07	0.002	0.96
	AKI1	0.13	2.00E-08	0.17	4.00E-03	0.07	0.07	0.09	0.01
	AKI2	0.06	0.01	-0.12	0.04	0.06	0.11	-0.1	0.003
	Mild CKD	-0.18	1.00E-14	-0.03	0.67	-0.09	0.03	-0.16	2.00E-06
	CKD AKI	0.16	4.00E-12	0.3	7.00E-07	0.31	5.00E-15	0.15	4.00E-06

^A PBTs described at <https://www.ualberta.ca/en/medicine/institutes-centres-groups/atagc/research/gene-lists.html>

^B Mean of MKI67 probesets: 11721145_s_at; 11721143_a_at; 11721146_a_at.

NOTE. Bolding signifies Spearman correlation coefficient>0.10 or <-0.10 and P<0.01.

Supplemental Table 4. Correlations between injury features and donor age in kidney biopsies called molecular No rejection (N=2479)

Scores for gene sets, classifiers, and injury archetypes ^A		All biopsies (N=2479)		Early: Biopsies ≤42 days (N=459)		Intermediate: Biopsies >42 days and ≤1y (N=887)		Late: Biopsies >1 year (N=996)	
		Spearman correlation coefficient	P value	Spearman correlation coefficient	P value	Spearman correlation coefficient	P value	Spearman correlation coefficient	P value
Recent injury/failed repair gene sets	IRITD3	0.16	4E-08	0.07	0.32	0.29	2E-08	0.06	0.19
	IRRAT	0.19	9E-11	0.11	0.13	0.30	2E-09	0.09	0.06
	TAL_New4	0.19	1E-10	0.17	0.02	0.30	5E-09	0.10	0.04
Mitosis	MKI67 ^B	0.0003	0.99	-0.05	0.48	-0.12	0.02	0.01	0.86
Epithelial dedifferentiation gene set	KT1	-0.13	2E-05	-0.08	0.29	-0.19	2E-04	-0.07	0.16
	KT2	-0.15	5E-07	-0.13	0.08	-0.20	8E-05	-0.10	0.03
Chronic injury (atrophy-fibrosis) related scores	IGT	-0.07	0.03	0.02	0.80	0.14	0.01	-0.08	0.08
	CXCL6	0.18	2E-09	0.30	2E-05	0.34	8E-12	0.09	0.06
	Classifier ci>1 _{Prob}	0.15	4E-07	0.39	1E-08	0.41	3E-16	0.11	0.02
	Classifier ct>1 _{Prob}	0.16	1E-07	0.37	1E-07	0.39	2E-15	0.12	0.01
Injury classifiers	Classifier lowGFR _{Prob}	0.22	3E-13	0.13	7E-02	0.30	4E-09	0.13	6E-03
	Classifier Prot _{Prob}	0.13	2E-05	0.15	0.03	0.24	3E-06	0.04	0.44
Injury PCA scores	Injury PC1	0.15	4E-07	0.13	0.07	0.33	3E-11	0.07	0.16
	Injury PC2	-0.10	6E-04	0.05	0.48	-0.01	0.90	0.03	0.55
	Injury PC3	0.19	8E-11	0.26	2E-04	0.13	0.01	0.19	8E-05
Injury AA scores	Normal	-0.14	6.00E-06	-0.17	0.02	-0.26	2.00E-07	-0.02	0.63
	AKI1	0.13	8.00E-06	0.2	4.00E-03	0.06	0.24	0.04	0.38
	AKI2	0.09	0.004	-0.08	0.26	0.07	0.19	-0.08	0.09
	Mild CKD	-0.13	1.00E-05	-0.04	0.54	-0.02	0.75	-0.13	0.01
	CKD AKI	0.17	4.00E-08	0.29	3.00E-05	0.35	3.00E-12	0.12	8.00E-03

^A PBTs described at <https://www.ualberta.ca/en/medicine/institutes-centres-groups/atagc/research/gene-lists.html>

^B Mean of MKI67 probesets: 11721145_s_at; 11721143_a_at; 11721146_a_at.

NOTE. Bolding signifies SCC>0.10 or <-0.10 and p<0.01.

Supplemental Table 5. Description of pathogenesis-based transcript sets (PBTs)^A used in analyses or as input in the injury PCA

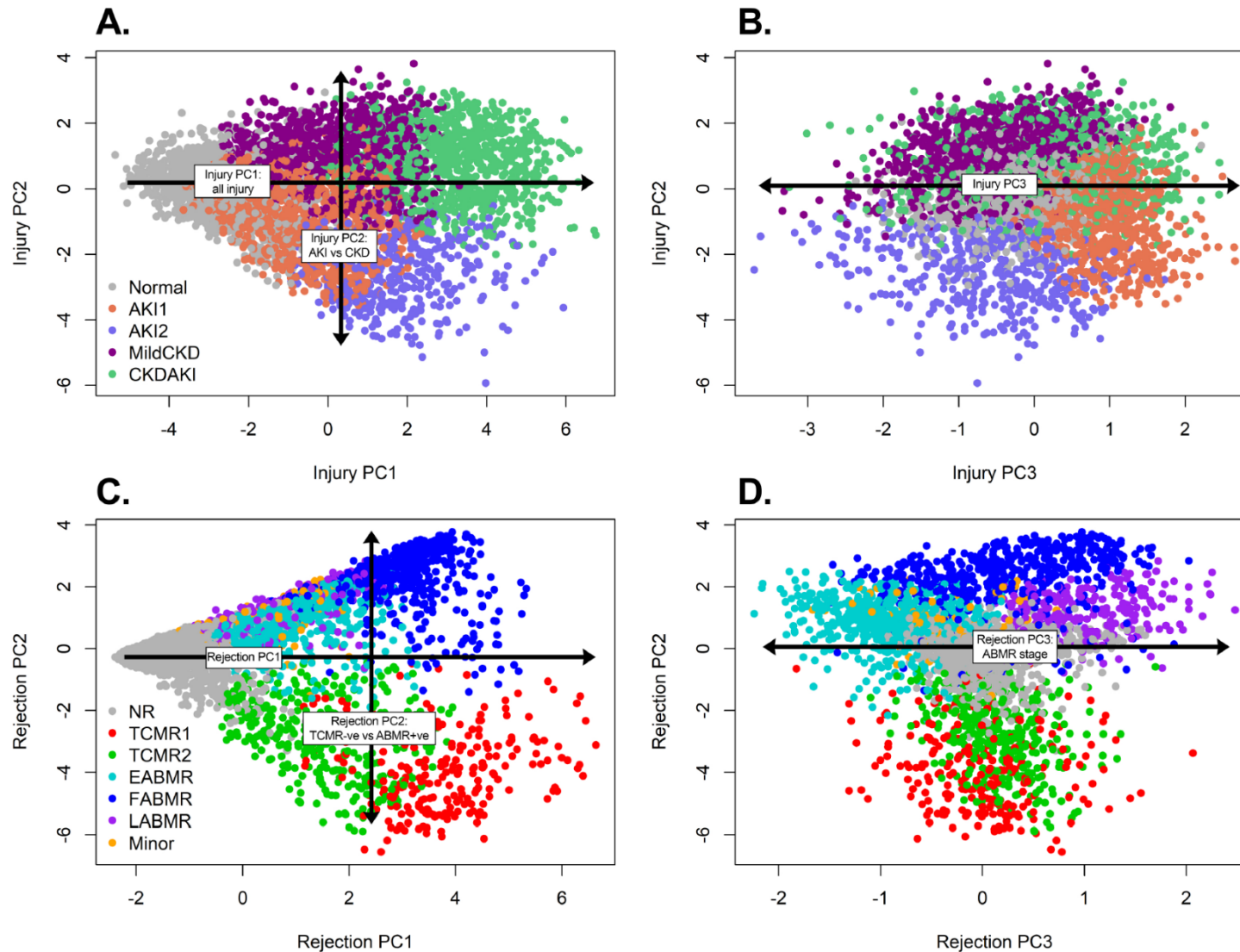
Category	Abbreviation	Description
Recent injury-related	IRITD3 ^B	Injury-repair induced, day 3 (IRITD3) (1)
	IRITD5 ^B	Injury-repair induced, day 5 (IRITD3) (1)
	IRRAT ^B	Injury-repair associated (IRRAT) (2)
Injury classifiers	lowGFR _{Prob}	Classifier predicting GFR ≤30 (3)
	Prot _{Prob}	Classifier predicting proteinuria (3)
Late injury (atrophy-fibrosis)-related	IGT ^B	Immunoglobulin transcripts (IGT) (4)
	ci>1 _{Prob}	Fibrosis score (ci classifier – ci>1 vs ≤1) (5)
	ct>1 _{Prob}	Tubular atrophy score (ci classifier – ci>1 vs ≤1) (5)
Normal kidney transcripts	KT1	Normal kidney transcripts-set 1 (6)
	KT2	Normal kidney transcripts-set 2 (6)
Macrophage-related	AMAT1	Alternatively activated macrophage (AMAT1) (7)
	QCMAT	Constitutive macrophage (QCMAT) (8)
Damage-associated	DAMPs ^B	Damage associated molecular patterns (9, 10)
Inflammation	MCAT ^B	Mast cell transcripts (11)
“New” injury gene sets	TAL_New4 EMT	Thick ascending limb epithelial mesenchymal transition (12, 13)
	PT_New4	Proximal tubule mesenchymal transition (12-14)
	DCT_New4	Distal convoluted tubule mesenchymal transition (12-14)
Injury PCA scores	PC1	Principal component 1 (14)
	PC2	Principal component 2 (14)
	PC3	Principal component 3 (14)
Injury archetypal score (Injury AAs)	Normal	Normal injury archetypal group (14)
	AKI1	Acute kidney injury 1 archetypal group (14)
	AKI2	Acute kidney injury 2 archetypal group (14)
	Mild CKD	Mild chronic kidney diseases archetypal group (14)
	CKDAKI	Chronic kidney disease with acute kidney injury group (14)

^A <https://www.ualberta.ca/medicine/institutes-centres-groups/ataqc/research/gene-lists>

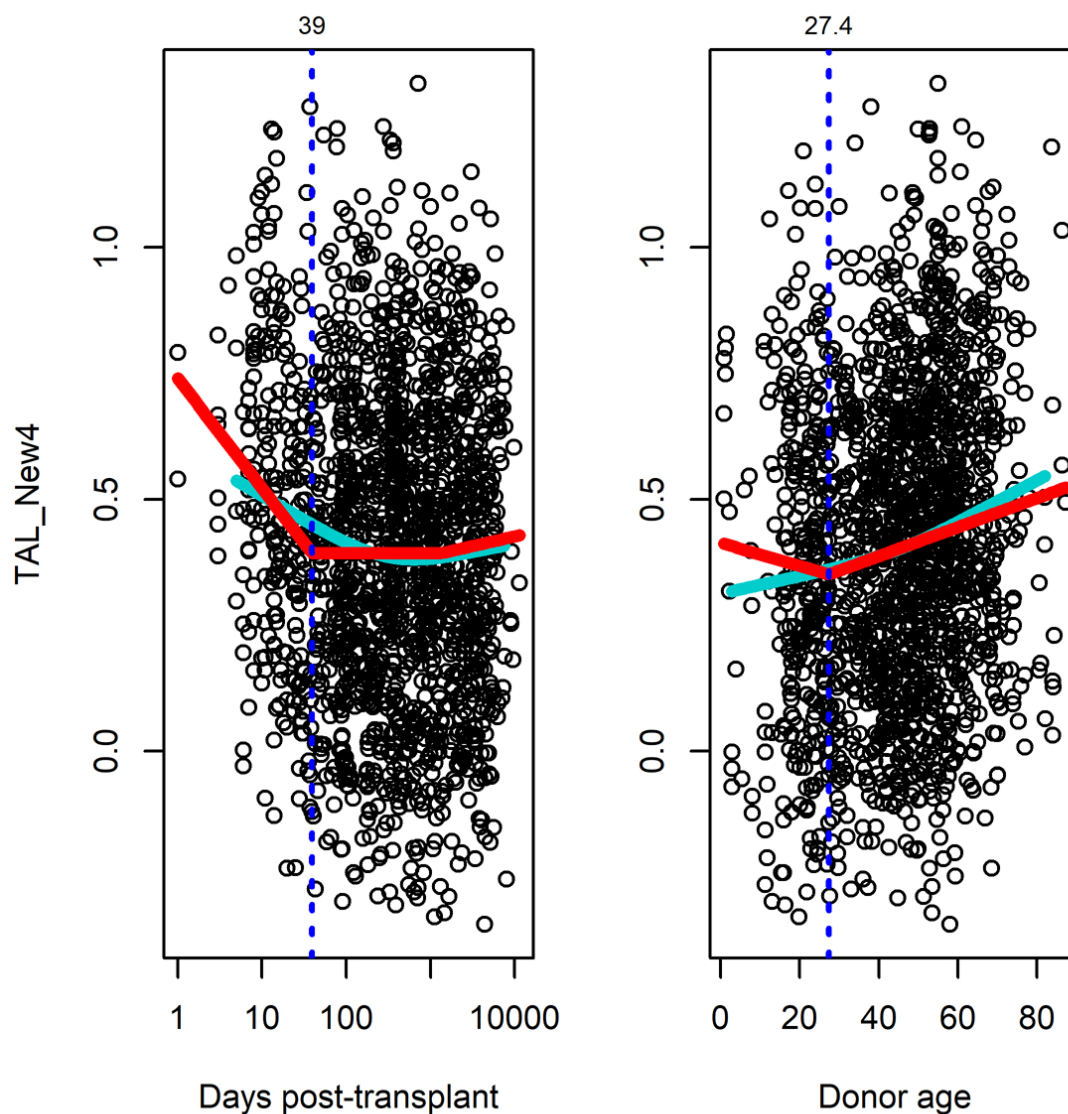
^B PBTs used in injury archetypal analysis

References:

1. Famulski KS, et al. Transcriptome analysis reveals heterogeneity in the injury response of kidney transplants. *Am J Transplant.* 2007;7(11):2483-95.
2. Famulski KS, et al. Molecular phenotypes of acute kidney injury in kidney transplants. *J Am Soc Nephrol.* 2012;23(5):948-58.
3. Einecke G, et al. Factors associated with kidney graft survival in pure antibody-mediated rejection at the time of indication biopsy: Importance of parenchymal injury but not disease activity. *Am J Transplant.* 2021;21(4):1391-401.
4. Einecke G, et al. Expression of B cell and immunoglobulin transcripts is a feature of inflammation in late allografts. *Am J Transplant.* 2008;8(7):1434-43.
5. Halloran PF, et al. Molecular phenotype of kidney transplant indication biopsies with inflammation in scarred areas. *Am J Transplant.* 2019;19(5):1356-70.
6. Einecke G, et al. Early loss of renal transcripts in kidney allografts: relationship to the development of histologic lesions and alloimmune effector mechanisms. *Am J Transplant.* 2007;7(5):1121-30.
7. Famulski KS, et al. Interferon-gamma and donor MHC class I control alternative macrophage activation and activin expression in rejecting kidney allografts: a shift in the Th1-Th2 paradigm. *Am J Transplant.* 2008;8(3):547-56.
8. Famulski KS, et al. Defining the canonical form of T-cell-mediated rejection in human kidney transplants. *Am J Transplant.* 2010;10(4):810-20.
9. Land WG, et al. Transplantation and Damage-Associated Molecular Patterns (DAMPs). *Am J Transplant.* 2016;16(12):3338-61.
10. Heil M, and Land WG. Danger signals - damaged-self recognition across the tree of life. *Front Plant Sci.* 2014;5:578.
11. Mengel M, et al. Molecular correlates of scarring in kidney transplants: the emergence of mast cell transcripts. *Am J Transplant.* 2009;9(1):169-78.
12. Hinze C, et al. Single-cell transcriptomics reveals common epithelial response patterns in human acute kidney injury. *Genome Med.* 2022;14(1):103.
13. Hinze C, et al. Epithelial cell states associated with kidney and allograft injury. *Nat Rev Nephrol.* 2024;20(7):447-59.
14. Halloran PF, et al. A cross-sectional study of the role of epithelial cell injury in kidney transplant outcomes. *Jci Insight.* 2025;10(10):e188658.



Supplemental Figure 1. Principal component analysis (PCA) and archetypal analysis (AA) in the N=4502 kidney transplant biopsy population. We characterized each biopsy for its molecular injury and rejection in principal component analysis (PCA) and used archetypal analysis (AA) scores to assign groups (23, 36, 47, 48). The 4502 population is distributed using the injury PCAs and colored by the injury archetypal analysis groups, showing A) PC2 vs. PC1, and B) PC2 vs. PC3. Injury principal component 1 (PC1) captures acute injury, failed repair, and chronic kidney disease (CKD) changes; injury PC2 captures time-dependent changes; and injury PC3 is a novel dimension correlating with epithelial development and remodeling genes. We also distributed the 4502 biopsies using the rejection PCAs, colored by the rejection archetypal analysis groups, showing C) PC2 vs. PC1, D) PC2 vs. PC3. Rejection PC1 captures pan-rejection changes; PC2 separates antibody-mediated rejection (ABMR) from T cell-mediated rejection (TCMR); and PC3 represents the stages of ABMR.



Supplemental Figure 2. Partial dependence plots of MARS (multivariate adaptive regression spline) models predicting TAL_New4 with log(days posttransplant) and donor age as predictors shown in the left and right panels respectively. N = 1802 samples with complete data. Points represent individual biopsies. Red lines are the fitted MARS partial dependence segments. Teal lines represent restricted cubic splines (3 knots). Blue dashed vertical lines indicate segment boundaries selected by MARS, with corresponding values shown above each panel. Predictor variables used in the models were log₁₀(time posttransplant), donor age, recipient age, and living vs deceased donors (0/1). Abbreviations: TAL_New4 – thick ascending limb mesenchymal transition.