SUPPLEMENTAL MATERIAL

Plasma Cell-free DNA Predicts Survival and Maps Specific Sources of Injury in Pulmonary Arterial Hypertension

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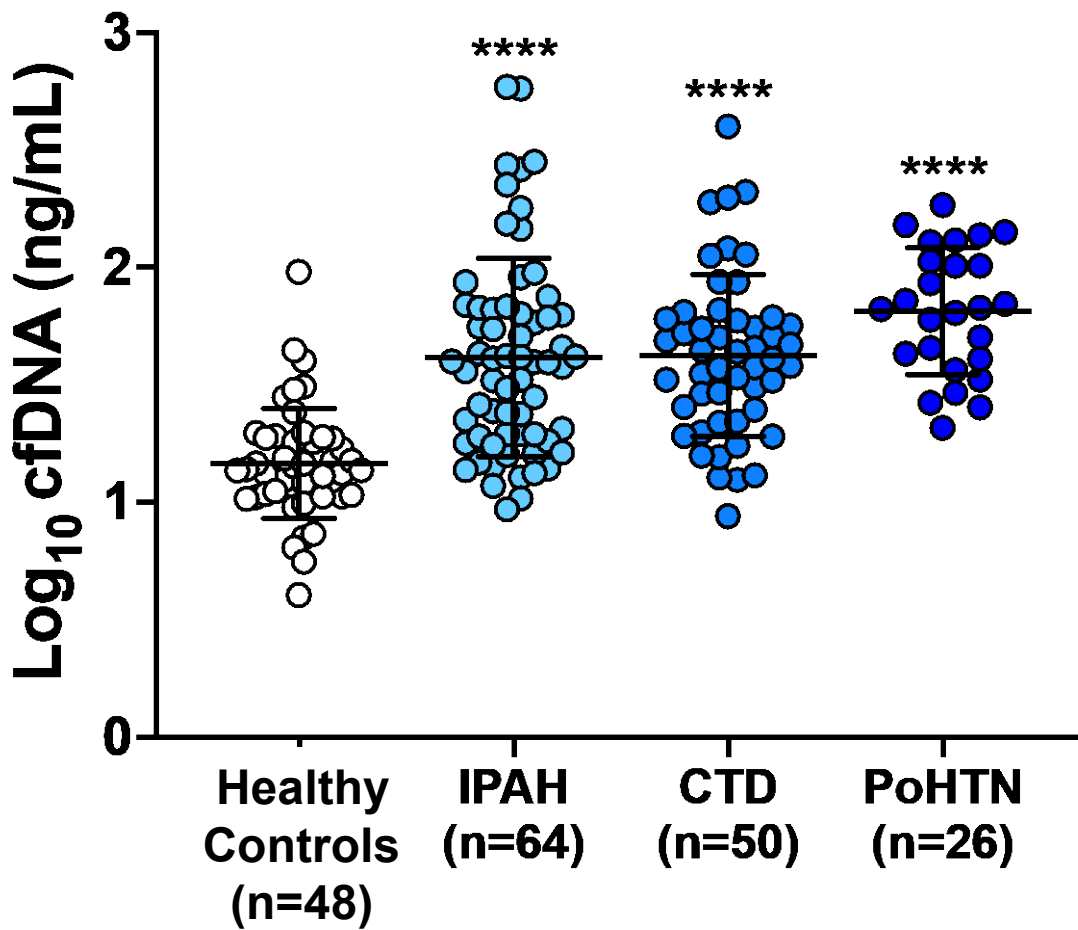Preston, MD,[6] Sean Agbor-Enoh, MD, PhD,*[3,4,11] Michael A Solomon, MD, MBA*[1,12]

Figure S1. Concentrations of cell-free DNA (cfDNA) in patients with idiopathic, connective tissue disease-associated and portal hypertension-associated pulmonary arterial hypertension (PAH) in Cohort B. Patients with idiopathic PAH (IPAH), connective tissue disease-associated (CTD) and portal hypertension-associated (PoHTN) PAH had significantly higher cfDNA concentrations compared to healthy controls (ANOVA P<0.0001; ****P<0.0001 for each pairwise comparison with healthy controls). cfDNA concentrations are displayed as mean +/- SD of $\log_{10}$-transformed values. PAH subtypes with less than 10 patients were not included in the subgroup analysis of cohort B. These included congenital heart disease-associated (n=9), heritable (n=2), drug/toxin-associated (n=4), and HIV-associated (n=3) PAH as well as pulmonary veno-occlusive disease (n=3).
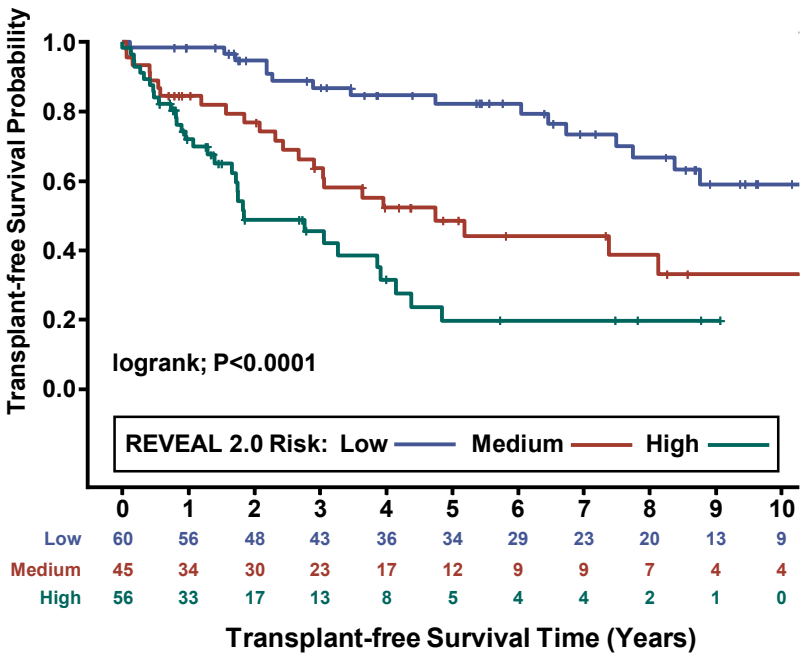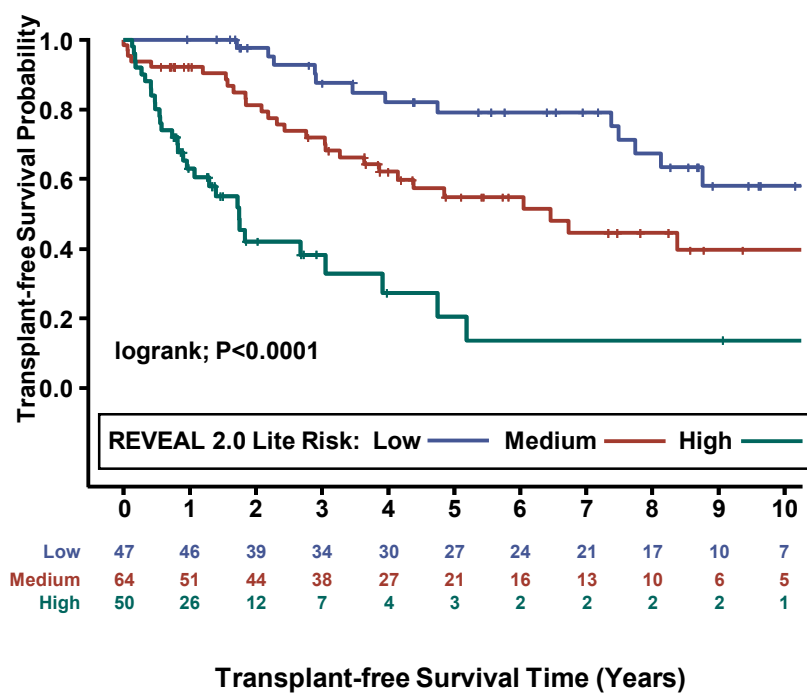
A

B

Figure S2. REVEAL 2.0 and REVEAL Lite 2 risk scores predict transplant-free survival in pulmonary arterial hypertension (PAH) patients. Kaplan-Meier analysis was performed with a median (IQR) follow up time of 2.7 (1.0-5.2) years. Patients were censored at the time of death or transplantation. A, Transplant-free survival was significantly different amongst REVEAL 2.0 risk groups (logrank; P<0.0001). Patients were grouped into low (≤ 6, blue line), medium (7-8, red line), and high risk (≥ 9, green line) categories. B, Transplant-free survival was also significantly different amongst REVEAL Lite 2 risk groups (logrank; P<0.0001). Patients were grouped into low (≤ 5, blue line), medium (6-7, red line), and high risk (≥ 8, green line) categories.

Table S1. Comparisons of demographics and comorbidities across cfDNA tertiles in Cohort B

Variable	P-value
Gender	0.07[†]
PAH Etiology*	0.01[‡]
Hypertension	0.86[†]
Obstructive sleep apnea	0.84[†]
Chronic obstructive pulmonary disease	0.28[†]
Interstitial lung disease	0.91[‡]
Cancer	0.40[†]
Diabetes	0.83[†]

*Driven by differences in idiopathic, connective tissue disease-associated and portal hypertension-associated pulmonary arterial hypertension (PAH). Idiopathic PAH was enriched in cfDNA tertile 1 (n = 26 vs 19 vs 19), connective tissue disease-associated PAH was enriched in cfDNA tertile 2 (15 vs 22 vs 12), and portal hypertension-associated PAH was enriched in cfDNA tertile 3 (n = 3 vs 9 vs 15)
†Pearson's chi-square test p-value
‡Fisher's Exact test p-value

Table S2. Correlations between cfDNA and PAH clinical variables in Cohort A

Variable	Spearman ρ	P-value
Age	0.15	0.31
Body mass index	-0.05	0.73
REVEAL 2.0	0.39	0.007
REVEAL 2.0 Lite	0.40	0.005
mPAP (mmHg)	-0.01	0.96
RAP (mmHg)	0.21	0.16
PAWP (mmHg)	0.27	0.06
PVR (Wood units)	-0.02	0.91
Heart rate (bpm)	0.44	0.007
SBP (mmHg)	0.16	0.29
DLCO (% predicted)	-0.13	0.39
6MWD (meters)	-0.26	0.09
NYHA	0.37	0.02
NT-proBNP (pg/mL)	0.32	0.03
hs-Troponin T (ng/L)	0.32	0.10
hs-CRP (mg/L)	0.21	0.22
Creatinine (mg/dL)	0.16	0.29

Abbreviations: PAH, pulmonary arterial hypertension; cfDNA, cell-free DNA; REVEAL, Registry to Evaluate Early and Long-Term PAH Disease Management; mPAP, mean pulmonary artery pressure; RAP, right atrial pressure; PAWP, pulmonary artery wedge pressure; PVR, pulmonary vascular resistance; SBP, systolic blood pressure; DLCO, diffusing capacity of the lungs for carbon monoxide; 6MWD, six-minute walk distance; NYHA, New York Heart Association; NT-proBNP, N-terminal pro brain-type natriuretic peptide; hs, high-sensitivity; CRP, C-reactive protein.

Table S3. Correlations between cfDNA and PAH clinical variables in Cohort B

Variable	Spearman ρ	P-value
Age	0.03	0.75
Body mass index	0.15	0.07
REVEAL 2.0	0.35	<0.0001
REVEAL 2.0 Lite	0.22	0.005
mPAP (mmHg)	0.34	<0.0001
RAP (mmHg)	0.18	0.03
PAWP (mmHg)	0.22	0.005
PVR (Wood units)	0.26	0.0008
Heart rate (bpm)	0.01	0.95
SBP (mmHg)	0.12	0.13
6MWD (meters)	-0.25	0.004
NYHA	0.11	0.15
BNP (pg/mL)	0.40	<0.0001
hs-Troponin T (ng/L)	0.39	0.0003
hs-CRP (mg/L)	0.45	<0.0001
Creatinine (mg/dL)	0.06	0.49

Abbreviations: PAH, pulmonary arterial hypertension; cfDNA, cell-free DNA; REVEAL, Registry to Evaluate Early and Long-Term PAH Disease Management; mPAP, mean pulmonary artery pressure; RAP, right atrial pressure; PAWP, pulmonary artery wedge pressure; PVR, pulmonary vascular resistance; SBP, systolic blood pressure; 6MWD, six-minute walk distance; NYHA, New York Heart Association; BNP, brain-type natriuretic peptide; hs, high-sensitivity; CRP, C-reactive protein.