Telomere Length and Immunosuppression in Non-Idiopathic Pulmonary Fibrosis Interstitial Lung Disease

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Supplemental Methods:

Study Populations:

Patients with fibrotic hypersensitivity pneumonitis (fHP), unclassifiable ILD (uILD), and connective tissue disease ILD (CTD-ILD) were enrolled into registries across the five centers. Each registry included clinical information for patients with ILD evaluated at the respective centers. The institutional review boards approved the study at each participating centre (UTSW: 082017-127, 092017-007; UCSF: 10-01592, 10-00198; UCD 585448-7, 875917-2; Chicago 14163A; and CUMC AAAS0753).

The five cohorts were divided into discovery and replication cohorts according to the location of leukocyte telomere length (LTL) measurement. Patients in the discovery cohort underwent research LTL measurement at UTSW/CUMC while patients in the replication cohort had LTL measured at UCSF. The discovery cohort consisted of 328 patients from UTSW, 85 from UCSF, 188 from Chicago, and 12 from CUMC. The replication cohort consisted of 170 patients from UCSF, 70 from Chicago, and 85 from UCD. There were 40 patients, 19 from Chicago and 21 from UCSF, that had research LTL measured at both sites; these patients were included in the replication cohort given its smaller sample size and the UCSF generated LTL data was used.

Immunosuppressant Exposure:

Dates of exposure to individual ILD-directed therapies including mycophenolate, azathioprine, cyclophosphamide, rituximab, prednisone, pirfenidone, and nintedanib were abstracted from the medical records from each site. For patients treated sequentially with mycophenolate and azathioprine, the first drug was considered primary. In general, the dosages of each medication followed accepted practices, such as mycophenolate 1000-3000 mg per day and azathioprine 50-200 mg per day. Prednisone indication, duration, and dose was expected to be highly variable across institutions but largely coincided with exposure to steroid-sparing immunosuppressants. Therefore, prednisone was included as a covariate in the primary propensity score model. Medication-related adverse events were unable to be systematically assessed.

Leukocyte Telomere Length

The qPCR protocol used at UTSW and CUMC was identical and performed by the Garcia laboratory at each site. Using this protocol, the UTSW/CUMC raw LTL were represented by the log transformed ratio of telomere to single copy gene (ln[T/S]); age-adjusted LTL values were calculated by subtracting the ln[T/S] from the expected ln[T/S] generated from a reference population.[1,2] At UCSF, raw LTL were compared to terminal restriction fragment length data from a reference population to transform LTL into kilobase pair units (kb).[3,4] To calculate age-adjusted LTL for samples measured at UCSF, the age expected LTL ln[T/S] values were transformed into kilobase pairs then subtracted from observed kilobase pairs.[5]

Propensity Scores and Inverse Probability of Treatment Weighting

Given that we were interested in estimating the interaction between LTL strata and immunosuppression exposure, we chose to account for indication bias by utilizing inverse probability of treatment weighting (IPTW). This procedure is able to balance potential confounder variables across treatment and control groups, allows for retention of the entire study cohort, and estimates the effect of treatment if all patients in the cohort were treated.[6]

The IPTW procedure followed a two-step process.[6] First, conditional probability of immunosuppressant (mycophenolate or azathioprine) exposure was estimated by calculating a propensity score for each patient. This score was generated via multivariable logistic regression to determine the odds of immunosuppressant (either mycophenolate or azathioprine) exposure within two years from blood collection adjusted for age, sex, race/ethnicity, family history of ILD, smoking status, baseline FVC percent predicted, DLCO percent predicted, radiographic usual interstitial pneumonia (UIP), prednisone exposure, non-IPF ILD diagnosis and ILD centre. Each covariate in the propensity score model was chosen based on their potential association with either exposure status, outcome, or LTL strata. Propensity scores were generated in the discovery and validation

cohorts separately. Second, patient weights were calculated by taking the inverse of their propensity score for the exposed patients and the inverse of one minus the propensity score for the unexposed patients. The weights then determined the extent to which each patient contributed to a new pseudo-population, that differs in sample size from the original population but has improved covariate balance.

To assess balance, standardized mean difference (SMD) between exposure groups were calculated before and after weighting. Post weighting variables with SMD > 0.15 were considered imbalanced. The weights generated from IPTW were then incorporated into the survival and joint models to assess influence of immunosuppression exposure on outcomes. Given that the weights are estimated based on the covariates included in the propensity score model and the pseudo-population sample size is artificially inflated, we included robust variance estimation in the models.[7]

In the secondary analyses evaluating the individual effects of mycophenolate or azathioprine, we performed similar IPTW procedures for each drug separately. Patients exposed to mycophenolate were excluded from the azathioprine analysis, and vice versa. For patients exposed to both drugs sequentially, the first immunosuppressant medication used was considered primary. The effect of prednisone monotherapy was also examined, excluding patients exposed to mycophenolate or azathioprine, using similar methods. However, the prednisone exposure covariate was removed from the multivariable logistic regression propensity score model for this analysis.

Survival Models

Weighted Cox proportional hazards regression was used to assess the time to death or transplant from blood collection date. Those that were still alive without transplant at two years were censored. The models included immunosuppressant exposure as a time-dependent variable as well as LTL group, radiographic honeycombing, non-IPF ILD diagnosis, and centre as time-independent covariates. The covariates in the

survival models were included given residual imbalance after weighting (centre) as well expected and observed influence on two-year transplant free survival (honeycombing, non-IPF ILD diagnosis).[8,9] The model also included an interaction term for immunosuppressant exposure and LTL group. In addition, we performed robust variance estimation given that weights were estimated as above. For the primary analysis, the model was applied to the discovery cohort and replication cohort separately, then combined through random-effect meta-analysis. For the secondary survival analyses, the models were restricted to each non-IPF ILD diagnosis, each immunosuppressant medication, or timing of immunosuppression initiation, where appropriate, and applied to the discovery and replication cohorts separately, then combined through meta-analysis.

Joint Models

To assess the estimated change in FVC over two years while accounting for informative dropout due to death or transplant, we constructed joint models that included weighted survival and linear mixed-effects submodels.[10,11] The survival submodel was fit similar to the primary analysis with immunosuppressant exposure was treated as a time-dependent covariate and LTL, radiographic honeycombing, non-IPF ILD diagnosis, and centre as time-independent covariates. The linear mixed-effects model included additional terms for time, immunosuppressant exposure at time of FVC measurement (yes or no), forced vital capacity, and LTL group as well as an interaction term for time x immunosuppressant x LTL group. The model was adjusted for radiographic honeycombing, non-IPF ILD diagnosis, and centre and included random intercepts and slopes. For this analysis, patients were included if they had ≥2 FVC measurements while exposed to immunosuppressant medications (for the exposed group) within 2 years of blood collection or if they had ≥2 FVC measurements within two years of blood collection (for the unexposed group). The time span of FVC measurements for each patient across exposure groups was also assessed.

Supplemental References:

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Table E1: Distribution of age-adjusted leukocyte telomere length across radiographic usual interstitial pneumonia and honeycombing.

	Radiographic UIP	Not Radiographic UIP	P-value	Honey-combing	No Honey-combing	P-value
CTD-ILD	*N=73*	*N=196*		*N=84*	*N=185*	
LTL <10th percentile, n (%)	17 (45)	56 (24)	0.02	20 (53)	64 (28)	0.004
fHP	*N=28*	*N=254*		*N=70*	*N=212*	
LTL <10th percentile, n (%)	12 (17)	16 (8)	0.03	30 (43)	40 (19)	<0.001
uILD	*N=77*	*N=310*		*N=120*	*N=267*	
LTL <10th percentile, n (%)	22 (22)	55 (19)	0.60	35 (35)	85 (30)	0.34
All non-IPF ILD	*N=178*	*N=760*		*N=274*	*N=664*	
LTL <10th percentile, n (%)	51 (25)	127 (17)	0.02	85 (41)	189 (26)	<0.001

Abbreviations: UIP, usual interstitial pneumonia; CTD-ILD, connective tissue disease interstitial lung disease; fHP, fibrotic hypersensitivity pneumonitis; uILD, unclassifiable interstitial lung disease; LTL, leukocyte telomere length

Table E2: Association between radiographic variables and two-year transplant free survival in meta-analysis of discovery and replication cohorts of non-IPF ILD patients

	HR (95% CI)	p-value	p-interaction
Model 1*			
UIP	1.54 (0.95-2.49)	0.083	--
Model 2†			
UIP	1.39 (0.85-2.28)	0.20	0.25
LTL <10th %	2.46 (1.59-3.80)	<0.001	
Model 3#			
UIP with IS exposure	1.85 (1.13-3.03)	0.015	0.89
No UIP with IS exposure	1.98 (0.55-7.17)	0.30	
Model 1*			
Honeycombing	1.80 (1.16-2.81)	0.009	
Model 2†			
Honeycombing	1.61 (1.02-2.53)	0.04	0.28
LTL <10th %	2.37 (1.52-3.70)	<0.001	
Model 3#			
Honeycombing with IS exposure	2.73 (1.48-5.05)	<0.001	0.50
No Honeycombing with IS exposure	3.64 (0.94-14.1)	0.06	

Meta-analysis of Discovery and Replication cohort, two-year transplant free survival.

*Cox model with covariates: radiographic variable, centre, and non-IPF ILD diagnosis

†Cox model with covariates: radiographic variable, LTL <10th percentile, centre, diagnosis, and interaction between radiographic variable and LTL <10th percentile

#Cox model with covariates: radiographic variable, immunosuppression exposure, centre, diagnosis, and interaction between radiographic variable and immunosuppression exposure

Abbreviations: UIP, usual interstitial pneumonia; LTL, leukocyte telomere length; IS, immunosuppression

Table E3: Association between prednisone monotherapy compared to no prednisone, mycophenolate, or azathioprine exposure and two-year transplant free survival stratified by LTL above and below the 10th percentile.

	Prednisone N (event)	No Prednisone/ MMF/AZA N (event)	HR (95% CI)	P-value	P-int
Meta-analysis					
LTL <10th	27 (6)	101 (24)	1.29 (0.45-3.73)	0.63	0.80
LTL ≥10th	71 (6)	356 (45)	1.06 (0.36-3.09)	0.92	

Weighted Cox proportional hazards regression model with robust variance estimation and adjustment for non-IPF ILD diagnosis, radiographic honeycombing, and ILD centre. Abbreviations: MMF, mycophenolate; AZA, azathioprine; LTL, leukocyte telomere length; HR, hazard ratio; CI, confidence interval; P-int, p-interaction.

Table E4: Annualized change in forced vital capacity stratified by cohort and non-IPF ILD diagnosis

	N (N FVC)	ΔFVC, ml/year (95% CI) *	P-value†
Cohorts			
Discovery	391 (1539)	-66 (-92, -39)	0.45
Replication	148 (502)	-85 (-127, -42)	
Non-IPF ILD Diagnoses			
fHP	162 (642)	-89 (-130, -49)	
CTD-ILD	182 (688)	-50 (-88, -12)	0.67
uILD	194 (711)	-76 (-114, -38)	

*****Joint-model incorporating time-to-event and linear mixed-effects submodels, adjusted for ILD diagnosis, radiographic honeycombing, and ILD centre. Restricted to patients with ≥2 FVC while on immunosuppression within two-years of blood collection for exposed patients, and ≥2 FVC measurements within two-years of blood collection for unexposed patients

†p-value indicates comparison across cohorts or non-IPF ILD diagnoses

Abbreviations: FVC, forced vital capacity, CI, confidence interval; fHP, fibrotic hypersensitivity pneumonitis; CTD-ILD, connective tissue disease interstitial lung disease; uILD, unclassifiable interstitial lung disease

Table E5: Annualized change in forced vital capacity for non-IPF ILD patients stratified by immunosuppressant exposure (mycophenolate or azathioprine) and age-adjusted LTL above and below the 10th percentile of normal.

	Mycophenolate Exposed		Azathioprine Exposed		Immunosuppression Unexposed		MMF vs No IS	AZA vs No IS
	N (N FVC)	ΔFVC, ml/year (95% CI)*	N (N FVC)	ΔFVC, ml/year (95% CI)*	N (N FVC)	ΔFVC, ml/year (95% CI)*	P-value	P-value
LTL <10th	41 (165)	-135 (-231, -40)	14 (44)	-188 (-375, 0)	56 (177)	-112 (-191, -34)	0.71	0.48
LTL ≥10th	160 (672)	-58 (-102, -14)	71 (306)	-26 (-90, 39)	195 (673)	-76 (-116, -36)	0.55	0.22

*****Joint-model incorporating time-to-event and linear mixed-effects submodels, adjusted for ILD diagnosis, HRCT UIP pattern, and ILD centre. Restricted to patients with ≥2 FVC measures while on immunosuppression within two-years of blood collection for exposed patients, and >2 FVC measurements within two-years of blood collection for unexposed patients

Abbreviations: FVC, forced vital capacity, CI, confidence interval; LTL, leukocyte telomere length; MMF, mycophenolate; AZA, azathioprine; IS, immunosuppression

Figure E1. Detailed STROBE diagram. Patients may fulfill more than one exclusion criteria. *Abbreviations: ILD, interstitial lung disease; UTSW, University of Texas Southwestern; CUMC, Columbia University Medical Center; UCD, University of California Davis; UCSF, University of California San Francisco; HRCT, high resolution computed tomography; PFT, pulmonary function test; IS, immunosuppression; AF, antifibrotic; fHP, fibrotic hypersensitivity pneumonitis; CTD-ILD, connective tissue disease interstitial lung disease; uILD, unclassifiable interstitial lung disease; IPTW, inverse probability of treatment weighting*

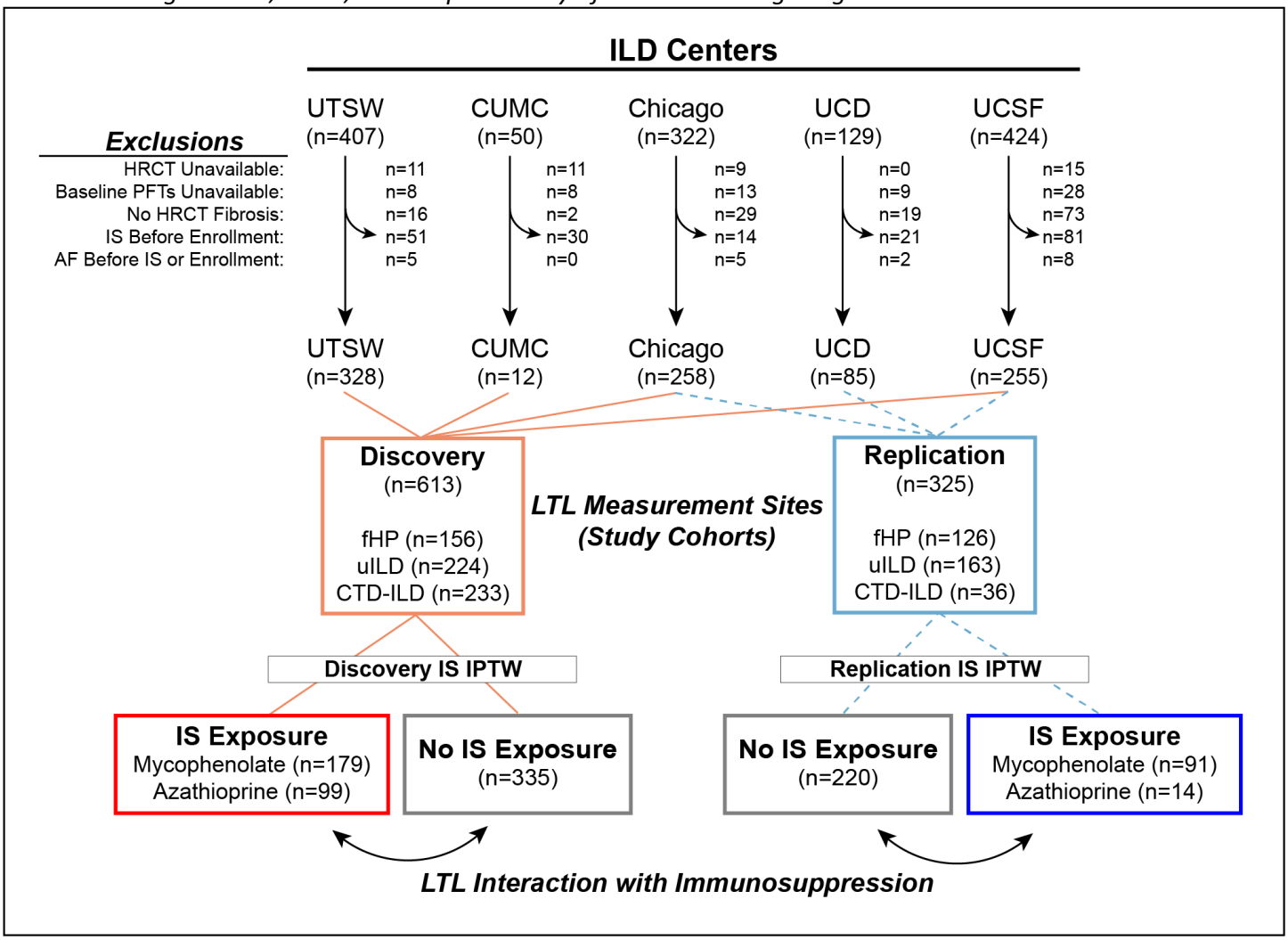

Figure E2: Distribution of radiographic patterns and honeycombing across non-IPF ILD diagnoses.

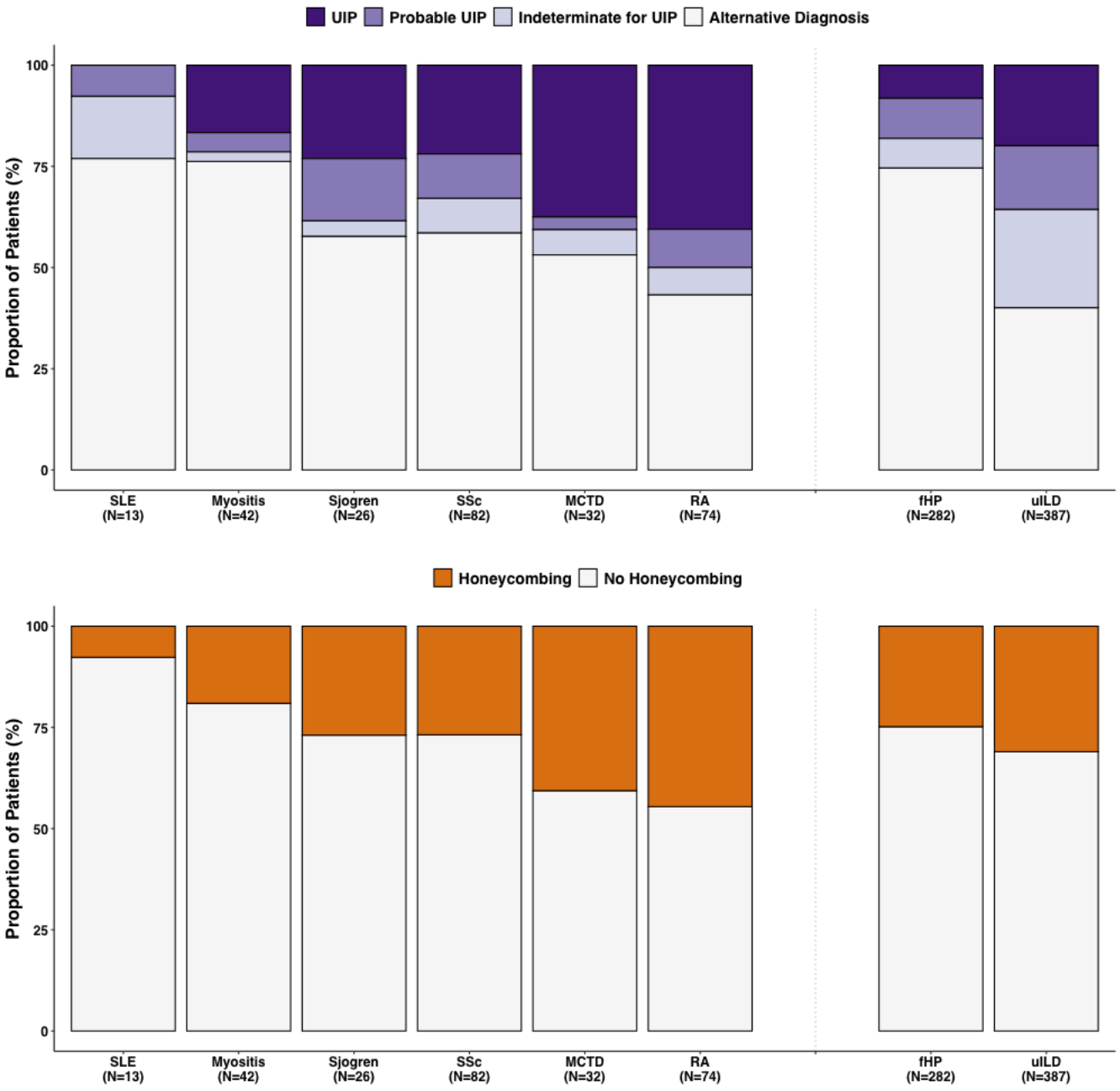

Figure E3: Time from registry enrollment to mycophenolate or azathioprine initiation in the discovery and replication cohorts.

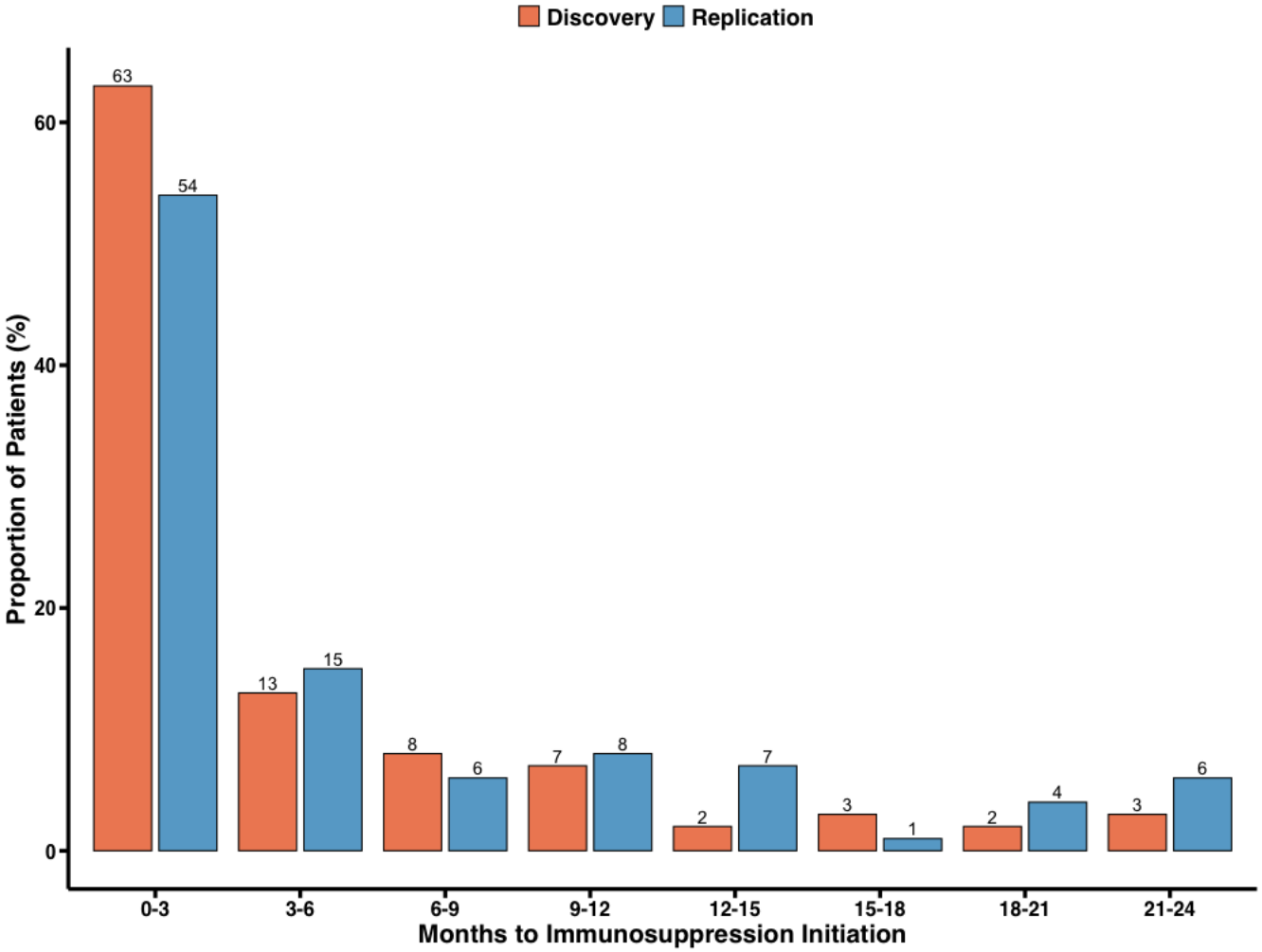

Figure E4: Before and after IPTW balance for immunosuppressant (mycophenolate or azathioprine) exposure in the discovery (A) and replication cohorts (B). Vertical dotted line represents standardized mean difference threshold of 0.15, weighted variables (green dots) with SMD <0.15 were considered balanced. *Abbreviations: FVC, forced vital capacity; DLCO, diffusion capacity of the lung for carbon monoxide; ILD, interstitial lung disease; UIP, usual interstitial pneumonia*

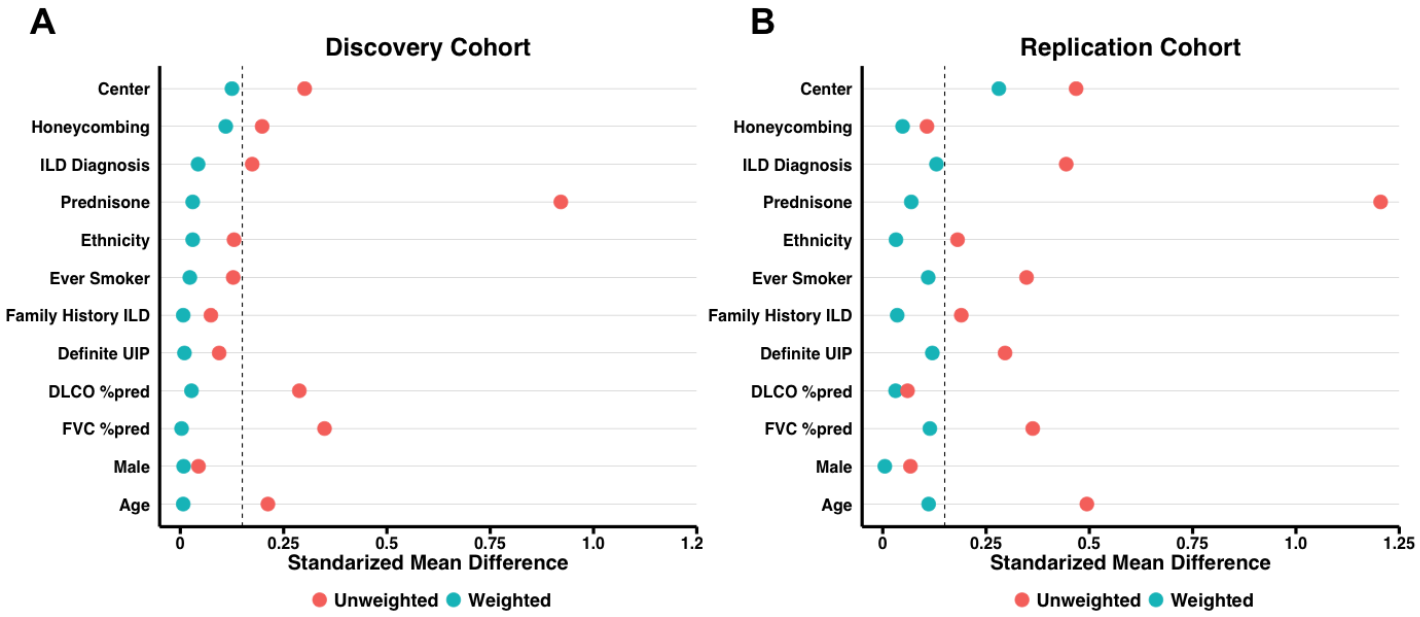

Figure E5: Age-adjusted LTL values for discovery and replication cohort (A). Pairwise comparison between age-adjusted LTL values from UTSW across the other centers using Dunnett's test with multiple-comparisons correction (***p<0.001). *Abbreviations: LTL, leukocyte telomere length; UTSW, University of Texas Southwestern; CUMC, Columbia University Medical Center; UCD, University of California Davis; UCSF, University of California San Francisco*

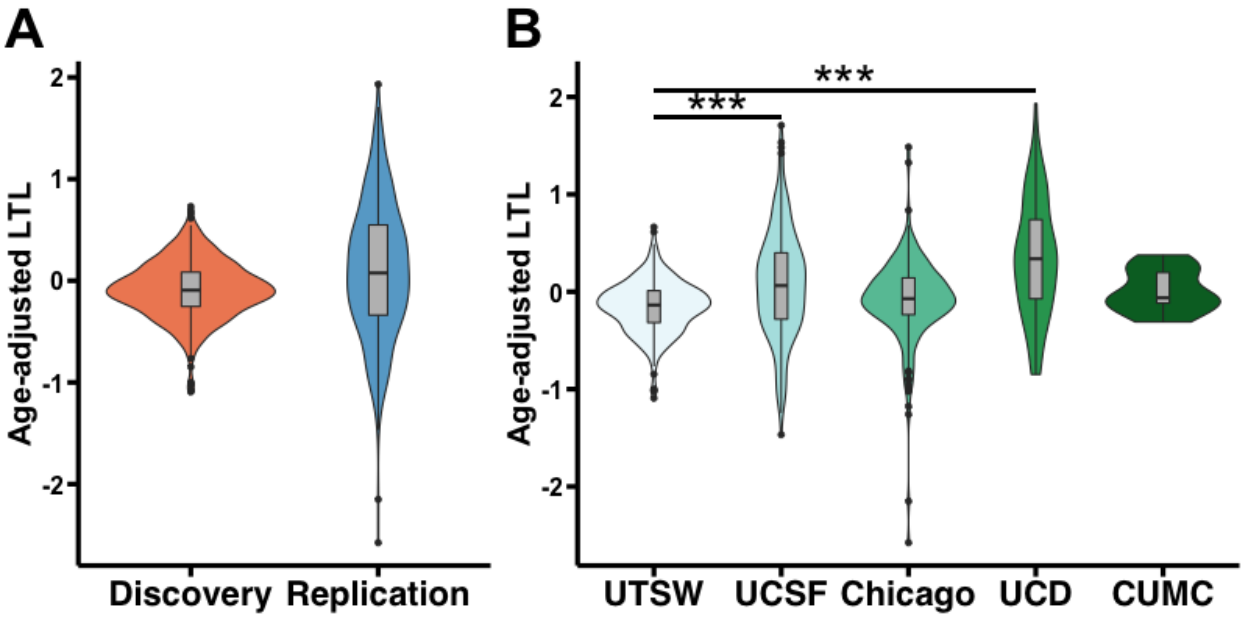

Figure E6: Proportion of non-IPF ILD diagnoses stratified by leukocyte telomere length thresholds
Abbreviations: CTD-ILD, connective tissue disease interstitial lung disease; fHP, fibrotic hypersensitivity pneumonitis; uILD, unclassifiable interstitial lung disease; LTL, leukocyte telomere length

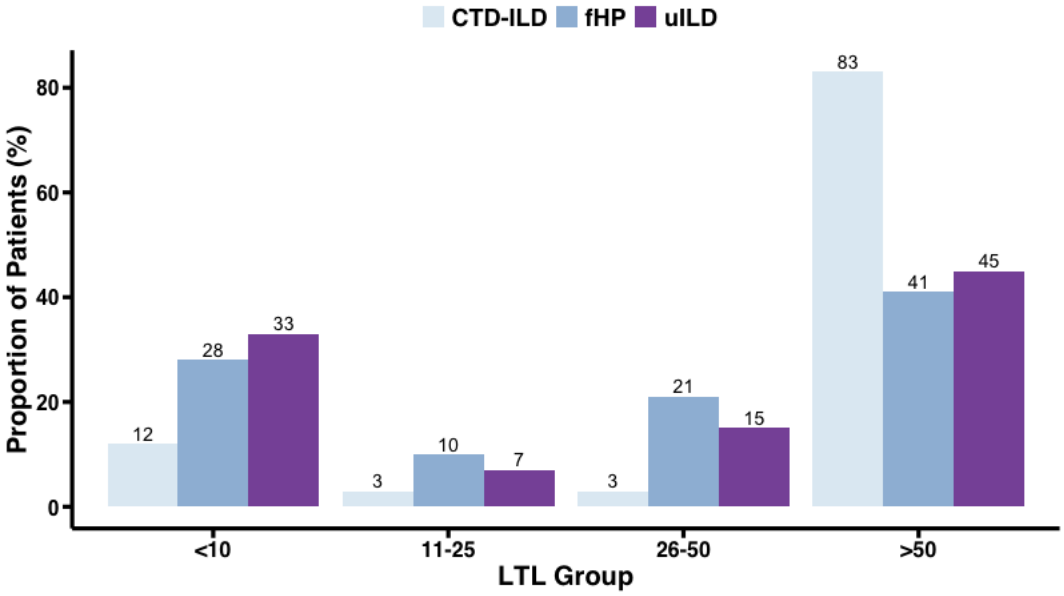

Figure E7: Correlation between common LTL measurements performed at both UTSW/CUMC (discovery cohort) and UCSF (replication cohort). *Abbreviations: LTL, leukocyte telomere length*

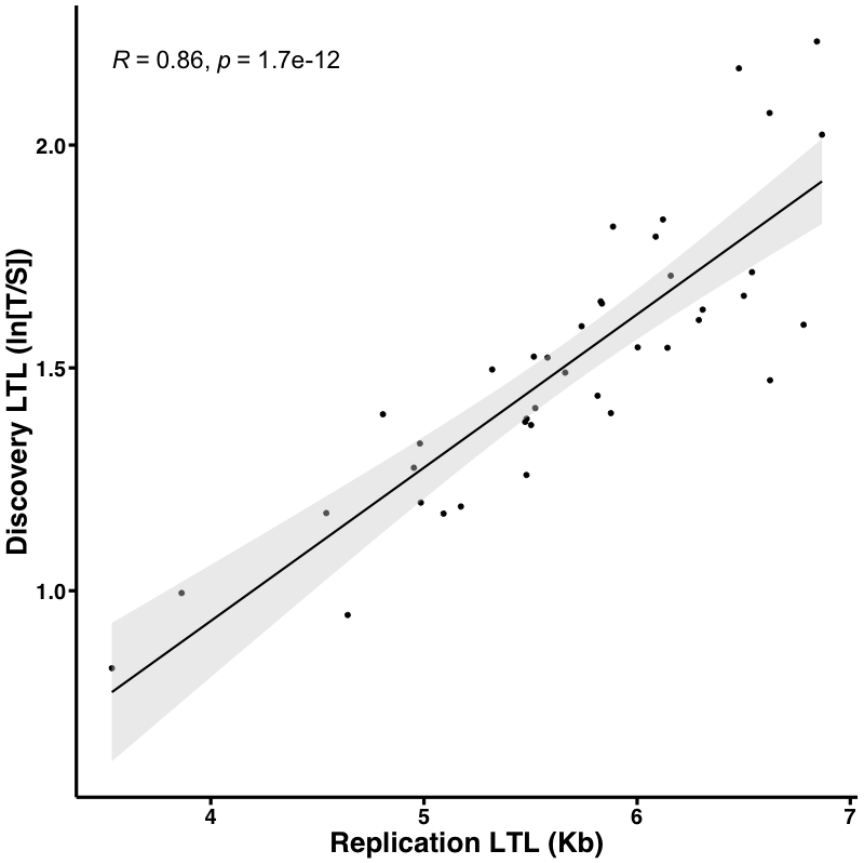

Figure E8: Two-year transplant-free survival between study cohorts (A), centres (B), and non-IPF ILD diagnoses (C).

Abbreviations: CTD-ILD, connective tissue disease interstitial lung disease; fHP, fibrotic hypersensitivity pneumonitis; uILD, unclassifiable interstitial lung disease

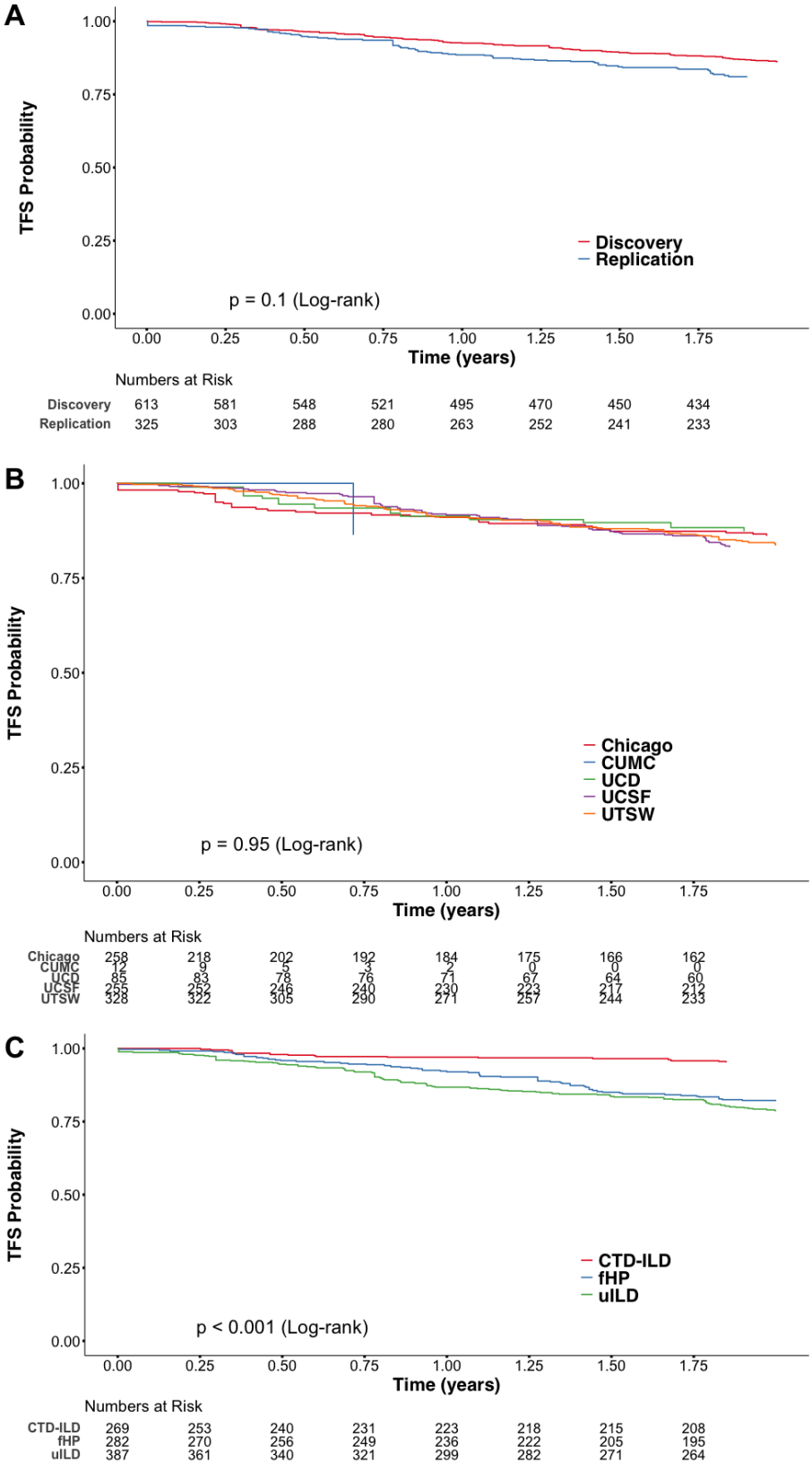

Figure E9. Association between immunosuppression exposure compared to no exposure and two-year transplant free survival stratified by LTL above and below the 10th percentile in fHP and uILD patients. Immunosuppression exposed patients restricted to those initiated on mycophenolate or azathioprine within 1 year of blood draw and had at least three months of exposure. *Weighted Cox proportional hazards regression model with robust variance estimation and adjustment for non-IPF ILD diagnosis, radiographic UIP pattern, and ILD center. Abbreviations: LTL, leukocyte telomere length; IS, immunosuppression; HR, hazard ratio; CI, confidence interval; P-int, p-interaction.*

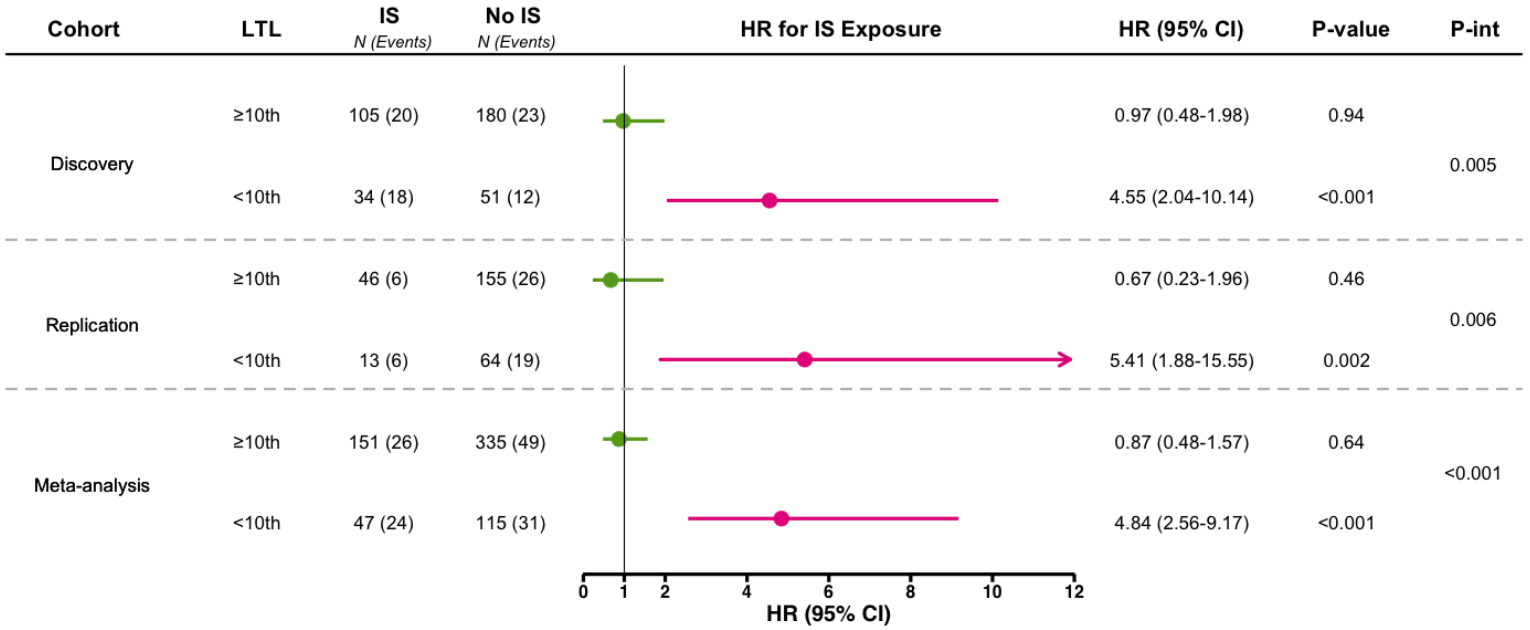

Figure E10. Association between individual immunosuppression medication exposure compared to no exposure and two-year transplant free survival in fHP and uILD patients stratified by age-adjusted LTL above and below the 10th percentile of or normal. *Weighted Cox proportional hazards regression model with robust variance estimation and adjustment for non-IPF ILD diagnosis, radiographic UIP pattern, and ILD center. Abbreviations: LTL, leukocyte telomere length; IS, immunosuppression; HR, hazard ratio; CI, confidence interval; P-int, p-interaction; MMF, mycophenolate; AZA, azathioprine.*