Online Data Supplement

Methods

SNP Analysis

Donor and recipient DNA was stored at -80°C; quantity and purity were determined spectrophotometrically. HLA-G SNPs were selected based on previous work[1-4]. We included 4 SNPs from the 5' untranslated region (UTR), 3 from the coding region, and 4 from the 3'-UTR (Table S1).

SNP Genotyping

Agena Assay Designer Suite software (Agena Bioscience, Inc., San Diego, CA) was used to generate polymerase chain reaction (PCR) and sequencing primers. The iPLEX assay reaction relied on a single termination mix and universal reaction conditions for all SNPs. Regions containing SNPs of interest (80-150 bp) were amplified, treated with shrimp alkaline phosphatase (SAP) (Agena Bioscience) to neutralize unincorporated deoxynucleotide triphosphates, and sequenced using extension primers designed with the 3'-end immediately adjacent to the SNP. Extension products were detected by MALDI-TOF mass spectrometry using the Sequenom MassARRAY Analyzer Compact (Agena Bioscience). Data were analyzed using Typer 4.0 Software (Agena Bioscience), which identifies SNP alleles at the expected mass signal peaks according to the molecular weights of the extension products.

BAL Samples

BAL was stored at -80°C and sHLA-G was measured in supernatants using an ELISA kit (Biovendor, Prague, Czech Republic).

RNA Protocol

RNA was extracted from BAL cell pellets stored in TRIZOL (Sigma-Aldrich) at -80°C in the TLTP biobank and reverse transcribed into complementary DNA (cDNA) using a kit (Qiagen). The cDNA was subjected to quantitative real-time PCR using the PrimePCR SYBR green HLA-G Assay (Bio-Rad) with results referenced to amplification of peptidyl prolyl isomerase A (PPIA) as a housekeeping gene.

TBBx Preparation and Antibodies

Paraffin sections underwent antigen retrieval and staining with DAPI, anti-HLA-G (clone MEM-G/2, Thermo Fisher Scientific; anti-mouse Alexa Fluor 647 secondary antibody, Invitrogen), and either anti-CD45 (Abcam polyclonal rabbit antibody; anti-rabbit Alexa Fluor 555 secondary antibody, Invitrogen) to identify leukocytes or anti-pan-cytokeratin (Abcam polyclonal rabbit antibody; anti-rabbit Alexa Fluor 555 secondary antibody) to identify epithelial cells. Placental tissue (Novus Biologicals) was used as a positive control for HLA-G expression.

All of the slides were stained together under the same conditions. The 10 randomly selected fields were chosen using the DAPI image, so that HLA-G, pancytokeratin and CD45 staining did not influence image selection. The observer collecting and analyzing the images was blinded to HLA-G genotype. Once images were collected, the same blinded observer applied a semi-quantitative score to epithelial and leukocyte HLA-G staining in the 10 fields. The reported data for each patient are the average staining intensity scores for the 10 images taken per biopsy.

Intensity was scored as absent (0), mildly positive (1), or strongly positive (2). The 22 patients whose transbronchial biopsies were examined were selected to have no evidence of rejection (ISHLT score A0B0) at 6 months post-transplant. These criteria were applied in an attempt to select patients in whom HLA-G expression in the graft was not altered by concurrent acute rejection, as has been reported previously[5, 6].

SNP effect definition and exclusion

Recipient and donor genotypes were analyzed according to recessive effect, dominant effect, allele effect, and pairing effects to examine associations with death or CLAD. To assess for a recessive effect, the major and heterozygous genotypes were combined and examined against the minor homozygous genotype. To assess for a dominant effect, the minor and heterozygous genotypes were compared against the major genotype. For the allelic effect, homozygous major, heterozygous, and homozygous minor genotypes were considered individually. Donor-recipient genotype interaction was assessed by considering specific donor-recipient combinations and by examining whether HLA-G SNP genotype was matched or mismatched between donor and recipient. SNPs in which allelic variants were present at low frequency (<10 patients) were excluded from further analysis.

Statistical Analysis

Survival and competing risk analyses were performed using R version 3.5.1 with packages: "survival" [3], "survminer" [4] and "cmprsk " [5].

Supplementary Tables

Table S1: HLA-G SNPs frequency, minor allele frequency, and Hardy-Weinberg equilibrium for donors and recipient.

SNP	SNP ID		Major allele	Minor allele	Major allele frequency	Heterozygous allele frequency	Minor allele frequency	MAF	HWE
5' UTR -725	RS1233334	Donor	CC	GG/TT	204 (68.69%)	84 (28.28%)	9 (3.03%)	17.17%	NA
		Recipient	CC	GG/TT	243 (70.64%)	98 (28.49%)	3 (0.87%)	15.12%	NA
5' UTR -716	RS2249863	Donor	TT	GG	73 (24.58%)	151 (50.84%)	73 (24.58%)	50%	0.77
		Recipient	TT	GG	94 (27.25%)	184 (53.33%)	67 (19.42%)	46.09%	0.17
5' UTR -201	RS1233333	Donor	CC	TT	73 (24.58%)	151 (50.84%)	73 (24.58%)	50%	0.77
		Recipient	CC	TT	94 (27.25%)	184 (53.33%)	67 (19.42%)	46.09%	0.17
5' UTR -56	RS17875397	Donor	CC	TT	273 (91.92%)	24 (8.08%)	0 (0.00%)	4.04%	0.47
		Recipient	CC	TT	318 (92.17%)	26 (7.54%)	1 (0.29%)	4.06%	0.55
3' UTR +3142	RS1063320	Donor	GG	CC	87 (29.39%)	145 (49.32%)	63 (21.28%)	45.95%	0.9

		Recipient	GG	CC	84 (24.42%)	178 (51.74%)	82 (23.84%)	49.71%	0.52
3' UTR +3187	RS9380142	Donor	AA	GG	153 (51.69%)	121 (40.88%)	22 (7.43%)	27.87%	0.77
		Recipient	AA	GG	157 (45.64%)	144 (41.86%)	43 (12.50%)	33.43%	0.27
3' UTR +3196	RS1610696	Donor	CC	GG	141 (48.62%)	115 (39.66%)	34 (11.72%)	31.55%	0.16
		Recipient	CC	GG	161 (47.21%)	158 (46.33%)	22 (6.45%)	29.62%	0.04
14BP INDEL	RS66554220	Donor	DEL	INS	99 (33.33%)	141 (47.47%)	57 (19.19%)	42.93%	0.59
		Recipient	DEL	INS	114 (33.05%)	182 (52.75%)	49 (14.20%)	40.58%	0.08
G*01:03	RS41551813	Donor	AA	TT	272 (91.58%)	25 (8.42%)	0 (0.00%)	4.21%	0.45
		Recipient	AA	TT	317 (92.42%)	26 (7.58%)	0 (0.00%)	3.79%	0.47
G*01:04	RS12722477	Donor	CC	AA	234 (78.79%)	62 (20.88%)	1 (0.34%)	10.77%	0.14
		Recipient	CC	AA	281 (81.45%)	64 (18.55%)	0 (0.00%)	9.28%	0.058
G*01:05N	RS41557518	Donor	CC	CDEL	293 (98.65%)	4 (1.35%)	0 (0.00%)	0.67%	0.91

		Recipient	CC	CDEL	333 (96.80%)	11 (3.20%)	0 (0.00%)	1.60%	0.76

Data are described as frequencies and p-value given, as appropriate.

Legend: SNP: single nucleotide polymorphism, MAF: minor allele frequency, HWE: Hardy-Weinberg equilibrium, UTR: untranslated region, NA: not applicable.

Table S2: Univariate analysis for time to mortality using the Cox PH model

Recipient characteristics	**HR**	**LCL**	**UCL**	**p-value**
Age at transplant (years)	1.00	0.99	1.02	0.50
Sex (male)	1.02	0.76	1.39	0.88
Blood group: A	0.76	0.36	1.58	0.46
Blood group: B	0.80	0.35	1.82	0.59
Blood group: O	0.78	0.37	1.61	0.50
Height (cm)	1.00	0.98	1.01	0.80
Weight (kg)	1.00	0.99	1.01	0.81
BMI (kg/m^2)	1.00	0.97	1.03	0.83
Primary diagnosis: Cystic fibrosis	1.03	0.72	1.47	0.88
Pre-transplant PRA	0.74	0.50	1.12	0.15
Pre-transplant DSA positive	0.85	0.50	1.45	0.56
Pre-transplant DSA class I	1.00	0.52	1.92	1.00
Pre-transplant DSA class II	0.93	0.45	1.91	0.83
Pre-transplant PRA +, DSA -	0.72	0.44	1.18	0.19
Pre-transplant PRA +, DSA +	0.78	0.45	1.34	0.37
HLA DQ Mismatch	1.05	0.96	1.15	0.28
Donor characteristics				
Age (years)	1.01	1.00	1.02	0.08
Sex (male)	0.95	0.71	1.29	0.76
Blood group: A	0.74	0.34	1.61	0.44
Blood group: B	0.87	0.37	2.07	0.75
Blood group: O	0.75	0.34	1.62	0.46

Height (cm)	0.99	0.97	1.00	0.10
Weight (kg)	1.00	0.99	1.01	0.74
BMI (kg/m^2)	1.00	0.97	1.03	0.83
Cause of death: Non-heart beating/DCD	1.07	0.63	1.81	0.81
Transplant characteristics				
Ischemic time (minutes)	1.00	0.95	1.06	0.96
CMV (D+, R-)	1.60	1.16	2.21	0.00
Acute Cellular Rejection (ISHLT Grade A1 or greater at any time)	1.70	0.86	3.36	0.13

Legend: HR: Hazard ratio, LCL: lower confidence limit; UCL: upper confidence limit, BMI: body mass index, HLA, human leukocyte antigen, PRA: panel reactive antibodies, DSA: donor specific antibody, DCD: Donor after Cardio-Circulatory Death, CMV: cytomegalovirus.

Table S3: Univariate analysis for time to CLAD using the Cox PH model

Recipient characteristics	**HR**	**LCL**	**UCL**	**p-value**
Age at transplant (years)	1.00	0.99	1.01	0.70
Sex (male)	1.12	0.80	1.55	0.52
Blood group: A	1.32	0.48	3.63	0.60
Blood group: B	2.04	0.70	5.91	0.19
Blood group: O	1.36	0.50	3.75	0.55
Height (cm)	1.01	0.99	1.03	0.24
Weight (kg)	1.01	1.00	1.02	0.10
BMI (kg/m^2)	1.02	0.99	1.06	0.26
Primary diagnosis: Cystic fibrosis	1.16	0.78	1.71	0.47
Pre-transplant PRA	0.74	0.50	1.09	0.13
Pre-transplant DSA positive	0.76	0.45	1.30	0.31
Pre-transplant DSA class I	0.67	0.32	1.37	0.27
Pre-transplant DSA class II	1.03	0.52	2.03	0.94
Pre-transplant PRA +, DSA -	0.77	0.48	1.21	0.26

Pre-transplant PRA +, DSA +	0.70	0.41	1.21	0.21
HLA DQ Mismatch	1.02	0.92	1.13	0.72
Donor characteristics				
Age (years)	1.00	0.99	1.01	0.47
Sex (male)	1.36	0.98	1.89	0.07
Blood group: A	1.55	0.48	4.95	0.46
Blood group: B	2.45	0.73	8.25	0.15
Blood group: O	1.57	0.49	4.98	0.45
Height (cm)	1.00	0.98	1.01	0.86
Weight (kg)	1.00	0.99	1.01	0.93
BMI (kg/m^2)	1.02	0.99	1.06	0.26
Cause of death: Non-heart beating/DCD	1.19	0.68	2.07	0.54
Transplant characteristics				
Ischemic time (minutes)	0.98	0.92	1.05	0.59
CMV (D+, R-)	1.35	0.93	1.96	0.12
Acute Cellular Rejection (ISHLT Grade A1 or greater at any time)	1.57	0.81	3.05	0.18

Legend: HR: Hazard ratio, LCL: lower confidence limit; UCL: upper confidence limit, BMI: body mass index, HLA, human leukocyte antigen, PRA: panel reactive antibodies, DSA: donor specific antibody, DCD: Donor after Cardio-Circulatory Death, CMV: cytomegalovirus, ISHLT: international society for heart and lung transplantation.

CLAD-free survival analysis

Table S4: Recessive effect of SNPs for CLAD-free survival analysis

	Hazard Ratio	**LCI**	**UCI**	**P-value**
RS1063320GG+CG	1.27	0.88	1.84	0.20
RS1233333GG+AG	0.78	0.56	1.07	0.12
RS1610696GG	1.25	0.82	1.92	0.30

RS2249863TT+GT	0.78	0.56	1.07	0.12
RS66554220INS	1.22	0.86	1.74	0.27
RS9380142GG	0.83	0.47	1.45	0.51

Legend: LCL: lower confidence limit, UCL: upper confidence limit.

Table S5: Dominant effect of SNPs for CLAD-free survival analysis

	Hazard Ratio	**LCI**	**UCI**	**P-value**
RS1063320	1.37	1.01	1.86	0.042
RS1233333	0.77	0.54	1.09	0.14
RS1233334	0.95	0.69	1.31	0.76
RS12722477	0.91	0.64	1.29	0.59
RS1610696	1.21	0.91	1.62	0.20
RS17875397	0.85	0.48	1.50	0.58
RS2249863	0.77	0.54	1.09	0.14
RS41557518	1.52	0.48	4.78	0.47
RS41551813	0.94	0.53	1.65	0.82
RS66554220	0.86	0.63	1.17	0.33
RS9380142	0.73	0.59	1.04	0.092

Legend: LCL: lower confidence limit, UCL: upper confidence limit.

Table S6: Allele effect of SNPs for CLAD-free survival analysis

	Hazard Ratio	**LCI**	**UCI**	**P-value**
RS1063320CG	1.15	0.78	1.70	0.48
RS1063320GG	1.52	1.00	2.30	0.05
RS1233333AG	0.83	0.59	1.16	0.27
RS1233333GG	0.68	0.45	1.02	0.064
RS1233334CT+CG+GT	1.01	0.73	1.39	0.98

RS1233334GG+TT	0.57	0.21	1.54	0.27
RS1610696GC	1.17	0.86	1.61	0.32
RS1610696GG	1.35	0.86	2.12	0.19
RS2249863GT	0.83	0.59	1.16	0.27
RS2249863TT	0.68	0.45	1.02	0.064
RS66554220INS	1.30	0.87	1.95	0.20
RS66554220INSDEL	1.12	0.80	1.56	0.51
RS9380142AG	0.79	0.58	1.07	0.12
RS9380142GG	0.74	0.42	1.32	0.31

Legend: LCL: lower confidence limit, UCL: upper confidence limit.

Competing risk regression analysis

Table S7: Recessive effect of SNPs with competing risk regression

	Hazard Ratio	**LCI**	**UCI**	**P-value**
RS1063320	1.11	0.73	1.69	0.63
RS1233333	0.85	0.56	1.27	0.42
RS1610696	1.31	0.79	2.16	0.29
RS2249863	0.85	0.56	1.27	0.42
RS66554220	1.27	0.82	1.95	0.28
RS9380142	1.05	0.57	1.94	0.87

Legend: LCL: lower confidence limit, UCL: upper confidence limit.

Table S8: Dominant effect of SNPs with competing risk regression

	Hazard Ratio	LCI	UCI	P-value
RS1063320	1.25	0.84	1.85	0.27
RS1233333	0.89	0.59	1.35	0.59
RS1233334	0.93	0.63	1.37	0.71
RS12722477	0.96	0.62	1.51	0.87
RS1610696	1.06	0.74	1.52	0.74
RS17875397	0.85	0.39	1.82	0.67
RS2249863	0.89	0.59	1.35	0.59
RS41557518	0.54	0.07	3.9	0.54
RS41551813	0.92	0.43	1.96	0.82
RS66554220	0.97	0.67	1.4	0.86
RS9380142	0.81	0.57	1.15	0.23

Legend: LCL: lower confidence limit, UCL: upper confidence limit.

Table S9: Allele effect of SNPs with competing risk regression

	Hazard Ratio	LCI	UCI	P-value
RS1233333AG	0.86	0.56	1.33	0.50
RS1233333GG	0.81	0.49	1.34	0.41
RS1233334CT+CG+GT	1.03	0.69	1.53	0.88
RS1233334GG+TT	0.21	0.03	1.43	0.11
RS1610696GC	0.99	0.67	1.47	0.96
RS1610696GG	1.30	0.77	2.20	0.32
RS2249863GT	0.86	0.56	1.33	0.50
RS2249863TT	0.81	0.49	1.34	0.41
RS66554220INS	1.23	0.76	1.99	0.39
RS66554220INSDEL	0.95	0.64	1.43	0.82
RS9380142AG	0.78	0.54	1.14	0.20

RS9380142GG	0.94	0.50	1.78	0.86

Legend: LCL: lower confidence limit; UCL: upper confidence limit.

Table S10: Model Validity Tests- Proportional-hazards assumption tests

	Rho	**Chisq**	**P-value**
Recessive effect of RS1063320:			
RS1063320GG+CG	-0.06	0.56	0.46
CMV (D+R-)	0.06	0.48	0.49
GLOBAL	NA	0.95	0.62
Dominant effect of RS1063320:			
RS1063320GG	-0.02	0.08	0.78
CMV (D+R-)	0.06	0.57	0.45
GLOBAL	NA	0.64	0.72
Recessive effect of RS1233333			
RS1233333GG	0.01	0.01	0.93
CMV (D+R-)	0.06	0.47	0.49
GLOBAL	NA	0.47	0.79
Recessive effect of RS17875397			
RS17875397CC	0.07	0.71	0.40
CMV (D+R-)	0.05	0.39	0.53
GLOBAL	NA	1.08	0.58
Recessive effect of RS2249863			
RS2249863TT	0.01	0.01	0.93
CMV (D+R-)	0.06	0.47	0.49
GLOBAL	NA	0.47	0.79
Recessive effect of RS41551813			
RS41551813AA	0.07	0.80	0.37
CMV (D+R-)	0.05	0.39	0.53

GLOBAL	NA	1.19	0.55
Recessive effect of RS66554220			
RS66554220DEL	0.02	0.09	0.76
CMV (D+R-)	0.06	0.52	0.47
GLOBAL	NA	0.59	0.75
Allele effect of RS1063320			
RS1063320CG	-0.05	0.44	0.51
RS1063320GG	-0.06	0.48	0.49
CMV (D+R-)	0.06	0.56	0.45
GLOBAL	NA	1.00	0.80
Allele effect of RS1233333			
RS1233333AG	0.01	0.01	0.92
RS1233333GG	0.01	0.01	0.90
CMV (D+R-)	0.06	0.49	0.48
GLOBAL	NA	0.51	0.92
Allele effect of RS2249863			
RS2249863GT	0.01	0.01	0.92
RS2249863TT	0.01	0.01	0.90
CMV (D+R-)	0.06	0.49	0.48
GLOBAL	NA	0.51	0.92

Legend: CMV: cytomegalovirus, D+: donor positive, R-:recipient negative.

Supplementary Figures

Figure S1: Kaplan-Meier survival probabilities for mortality in the entire study population.

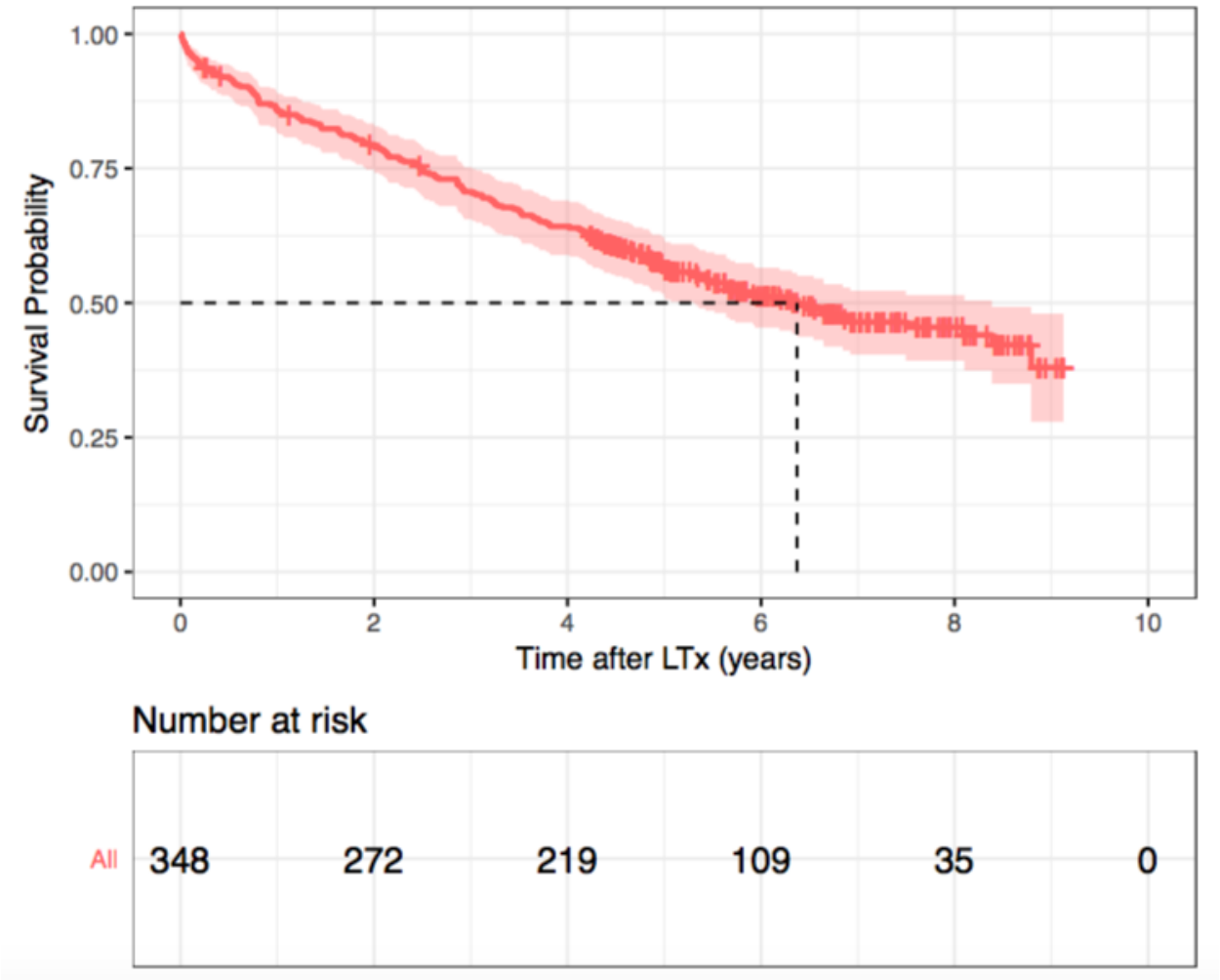
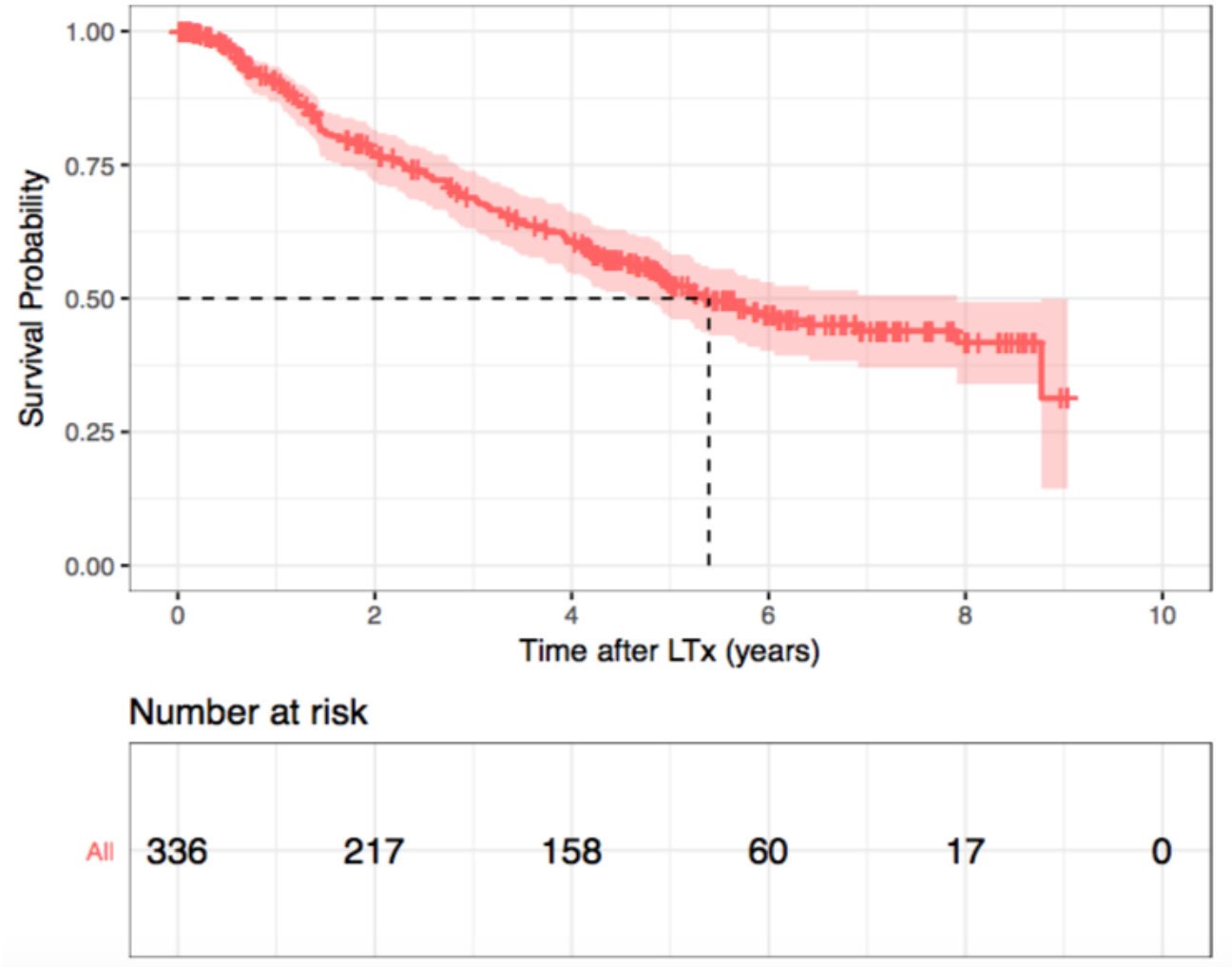


Figure S2: Kaplan-Meier survival probabilities for CLAD in the entire study population.



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