

Table S1. List of abbreviations

Abbreviation	Definition
AA	archetypal analysis
ABMR	antibody-mediated rejection
AMATs	alternative macrophage activation transcripts
ATAGC	Alberta Transplant Applied Genomics Centre
AUC	area under the curve
BAT	B cell-associated transcripts
CAV	cardiac allograft vasculopathy
clRIT	cardiac injury and repair transcripts
DAMP	damage-associated molecular pattern transcripts
DSAST	DSA-selective transcripts
EMB	endomyocardial biopsy
gmlnet	a package that fits a generalized linear model via penalized maximum likelihood
GRIT	Gamma interferon and rejection induced transcripts
HT	heart parenchymal transcripts
HT1	heart parenchymal transcripts set 1
HT2	heart parenchymal transcripts set 2
IGT	immunoglobulin transcripts
INTERHEART	Diagnostic and Therapeutic Applications of Microarrays in Heart Transplantation, a Multicenter Study (ClinicalTrials.gov Identifier: NCT02670408)
Injury archetype	archetypal model for assessing cardiac injury
IRRAT	injury-and-repair transcripts
IRITD3	injury-repair induced transcripts day 3
IRITD5	injury-repair induced transcripts day 5
LVEF	left ventricular ejection fraction
LoLVEFprob	dysfunction probability classifier
MCAT	mast cell transcripts
MMDx	Molecular Microscope [®] Diagnostic System
NKB	natural killer cell burden
NRI	net classification indices
PBTs	pathogenesis-based transcript sets
PCA	principal component analysis
PC1	principal component 1
PC2	principal component 2
PC3	principal component 3
QCAT	Quantitative cytotoxic T cell-associated transcripts
QCMAT	Quantitative constitutive macrophage-associated transcripts
RATs	rejection-associated transcripts
RF	Random forest
TCMR	T cell-mediated rejection
TxBx	Time post-transplant

Table S2. INTERHEART centers and investigators (ClinicalTrials.gov #NCT02670408)

Location	Principal Investigator	Number of biopsies
Edmonton, AB, Canada	Daniel Kim	128
Los Angeles, CA, USA	Jon Kobashigawa	80
Salt Lake City, UT, USA	Josef Stehlik	47
Dallas, TX, USA	Shelley Hall	126
Los Angeles, CA, USA	Mario Deng, Eugene Depasquale, and Martin Cadeiras (currently in Davis, CA, USA)	13
Richmond, VA, USA	Keyur Shah	24
Bologna, Italy	Luciano Potena	384
A Coruña, Spain	Marisa Crespo	276
Vienna, Austria	Andreas Zuckermann, Arezu Zuckermann-Aliabadi, and Johannes Gökler	196
Sydney, Australia	Peter Macdonald	153

Table S3. Histologic and molecular archetype diagnoses in 3230 endomyocardial biopsies	
Histology diagnosis¹	N (% of recorded)
No Rejection	639 (44.5)
TCMR	121 (8.4)
ABMR	77 (5.4)
Mixed (ABMR + TCMR)	17 (1.2)
Possible TCMR	426 (29.7)
Possible ABMR	155 (10.8)
<i>Incomplete</i>	1795 (55.6)
Rejection archetypes (RAT-based)^{2,4}	N (% of 3230)
No rejection	735 (22.8%)
TCMR/mixed	274 (8.5%)
Early Injury	213 (6.6%)
ABMR	497 (15.4%)
Minor	1511 (46.8%)
Injury archetypes^{3,4}	N (% of 3230)
No injury	1141 (35.3)
Severe injury	149 (4.6)
Late atrophy-fibrosis	621 (19.2)
Moderate injury	525 (16.3)
Mild injury	794 (24.6)

1. Histology diagnoses as reported by the collaborating centers per ISHLT guidelines.
2. Halloran, P. F., et al. (2021). "Many heart transplant biopsies currently diagnosed as no rejection have mild molecular antibody-mediated rejection-related changes." *J. Heart and Lung Transpl* 41(3): 334-344
3. Madill-Thomsen, K. S., et al. (2022). "Assessing the relationship between molecular rejection and parenchymal injury in heart transplant biopsies." *Transplantation* 106(11): 2205-2216.
4. Halloran, P. F. and K. S. Madill-Thomsen (2023). "The Molecular Microscope Diagnostic System: Assessment of Rejection and Injury in Heart Transplant Biopsies." *Transplantation* 107(1): 27-44.

Table S4. Description of pathogenesis-based transcript sets (PBTs)^A

Category	Abbreviation	Description
Rejection-related	GRIT3	Interferon gamma-inducible (1, 2)
	Rej-RAT	Rejection-associated (3)
TCMR-related	TCMR-RAT	TCMR-associated (3)
	QCAT	Cytotoxic T cell associated (2, 4)
ABMR-related	ABMR-RAT	ABMR-associated (1, 3)
	NKB	NK cell burden (5)
	DSAST	DSA selective transcripts (6)
Recent injury-related	IRITD3	Injury-repair induced, day 3 (IRITD3) (7)
	IRRAT30	Injury-repair associated (IRRAT30) (8)
Late injury-related	IGT	Immunoglobulin transcripts (IGT) (9)
	MCAT	Mast cell-associated transcripts (10)
Normal heart transcripts	HT1	Normal heart transcripts-set 1 (heart-selective compared with kidneys) (11, 12)
	HT2	Normal heart transcripts-set 2 (heart-selective compared with kidneys) (11, 12)
Macrophage-related	AMAT1	Alternatively activated macrophage (AMAT1) (13)
	QCMAT	Constitutive macrophage (QCMAT) (13)

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

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Table S5. Comparing function and future graft loss in protocol vs indication biopsies (excluding biopsies for which the protocol vs indication status was not designated)

	Protocol	Indications (For cause)	Row Totals
LVEF≤55 at time of biopsy (998)			
All biopsies^A (N=998 with known LVEF and Indication status, % of biopsies with low LVEF within that indication category)	125/820 (15%)	83/178 (47%)	998
One random biopsy per transplant^B (N=539 with known LVEF and Indication status, % of biopsies with low LVEF within that indication category)	56/416 (13%)	57/123 (46%)	539
3-year postbiopsy graft failure (1119 with known graft status at three years and known indication)			
All biopsies^C (N=1119 with known graft status and indication, % of biopsies with 3-year failure and within that indication category)	51/914 (6%)	39/205 (19%)	1119
One random biopsy per transplant^D (N=744 with known graft status and indication, % of biopsies with 3-year failure and within that indication category)	38/588 (6%)	36/156 (23%)	744
<p>A. Chi squared P<2.2E-16 B. Chi squared P=9.6E-15 C. Chi squared P=3.9E-10 D. Chi squared P=1.8E-9</p>			

Table S6. GO Terms for top 200 genes increased in hearts with dysfunction (1013 EMBs)

ONTOLOGY ¹	ID	Description	P value	Q value	Gene ID ¹	Count
BP	GO:0002460	adaptive immune response based on somatic recombination of immune receptors	1.3E-11	2.9E-08	CXCL13/ARID5A/IGKC/CD27/IGLL5/IGHM/KLHL6/IGHG1/IL18/IGHG3/HLA-DOA/SMAD7/CD274/SLA2/LEF1/RAB27A/C4A/SLAMF6/CD8A/HLA-A/HLA-F/SEMA4A	22
BP	GO:0002449	lymphocyte mediated immunity	4.8E-10	5.4E-07	ARID5A/IGKC/CD27/IGLL5/IGHM/CD96/IGHG1/IL18/IGHG3/HLA-DOA/SMAD7/PLEKHM2/SLAMF7/SLA2/RAB27A/C4A/SLAMF6/CD8A/HLA-A/HLA-F	20
BP	GO:0002443	leukocyte mediated immunity	7.1E-10	5.4E-07	ARID5A/IGKC/CD27/IGLL5/IGHM/CD96/CD84/IGHG1/IL18/IGHG3/HLA-DOA/SMAD7/PLEKHM2/SLAMF7/SLA2/RAB27A/C4A/SLAMF6/IRAK4/CD8A/HLA-A/HLA-F	22
MF	GO:0003823	antigen binding	2.2E-08	8.4E-06	IGKV3-20/IGKC/IGLL5/IGHM/IGHG1/SLC7A5/IGKV1-5/IGKV1-39/IGHG3/JCHAIN/HLA-A/HLA-F/TAP1	13
BP	GO:0051251	positive regulation of lymphocyte activation	2.5E-08	1.4E-05	<u>IGFBP2</u> /ICOS/IGKC/SIRPG/CD27/IGLL5/IGHM/IGHG1/IL18/IGHG3/HLA-DOA/CD274/PTPN22/LEF1/CDKN1A/IL7/HLA-A/HLA-F	18
CC	GO:0009897	external side of plasma membrane	6.1E-08	1.6E-05	CXCR6/CTLA4/SDC1/IGKC/CD27/IGLL5/IGHM/CD84/IGHG1/CXCR4/SLC7A5/IGHG3/CD274/SLAMF7/SLAMF6/TNFRSF9/CD8A/ITGAD/HLA-F	19
BP	GO:0002440	production of molecular mediator of immune response	7.2E-08	3.3E-05	IGKV3-20/ARID5A/IGKC/IGKV2-28/CD96/IGKV1-27/SLC7A5/IL18/IGKV1-5/IGKV1-39/HLA-DOA/SMAD7/MZB1/PTPN22/HLA-A/HLA-F	16
BP	GO:0022407	regulation of cell-cell adhesion	1.1E-07	4.0E-05	<u>IGFBP2</u> /CXCL13/ARG2/LAX1/FSTL3/ICOS/CTLA4/SIRPG/CD27/IL18/HLA-DOA/SMAD7/TIGIT/CD274/PTPN22/LEF1/SPINT2/IL7/HLA-A	19
BP	GO:0050851	antigen receptor-mediated signaling pathway	1.2E-07	4.0E-05	LAX1/TXK/CTLA4/IGKC/IGLL5/IGHM/KLHL6/CD8B/IGHG1/IGHG3/PTPN22/SLA2/CD8A/HLA-A	14
BP	GO:0002696	positive regulation of leukocyte activation	1.7E-07	4.7E-05	<u>IGFBP2</u> /ICOS/IGKC/SIRPG/CD27/IGLL5/IGHM/IGHG1/IL18/IGHG3/HLA-DOA/CD274/PTPN22/LEF1/CDKN1A/IL7/HLA-A/HLA-F	18
BP	GO:0050863	regulation of T cell activation	2.5E-07	5.7E-05	<u>IGFBP2</u> /ARG2/LAX1/ICOS/CTLA4/SIRPG/CD27/IL18/HLA-DOA/SMAD7/TIGIT/CD274/PTPN22/LEF1/IL7/HLA-A	16
BP	GO:1903037	regulation of leukocyte cell-cell adhesion	2.7E-07	5.7E-05	<u>IGFBP2</u> /ARG2/LAX1/ICOS/CTLA4/SIRPG/CD27/IL18/HLA-DOA/SMAD7/TIGIT/CD274/PTPN22/LEF1/IL7/HLA-A	16
BP	GO:0050867	positive regulation of cell activation	2.8E-07	5.7E-05	<u>IGFBP2</u> /ICOS/IGKC/SIRPG/CD27/IGLL5/IGHM/IGHG1/IL18/IGHG3/HLA-DOA/CD274/PTPN22/LEF1/CDKN1A/IL7/HLA-A/HLA-F	18
CC	GO:0055038	recycling endosome membrane	3.0E-07	3.9E-05	ATP13A3/MCOLN2/SYT11/CD274/RAB11B/PLEKHB2/ARF6/HLA-A/HLA-F	9
CC	GO:0019814	immunoglobulin complex	5.3E-07	4.5E-05	IGKV3-20/IGKC/IGLL5/IGHM/IGKV2-28/IGKV1-27/IGHG1/IGKV1-5/IGKV1-39/IGHG3/JCHAIN	11
BP	GO:0002228	natural killer cell mediated immunity	6.7E-07	1.3E-04	CD96/IL18/PLEKHM2/SLAMF7/RAB27A/SLAMF6/HLA-A/HLA-F	8
BP	GO:0002768	immune response-regulating cell surface receptor signaling pathway	8.0E-07	1.4E-04	LAX1/TXK/CTLA4/IGKC/IGLL5/IGHM/KLHL6/CD8B/IGHG1/IGHG3/BTLA/PTPN22/SLA2/CD8A/HLA-A	15
BP	GO:0022409	positive regulation of cell-cell adhesion	1.0E-06	1.6E-04	<u>IGFBP2</u> /CXCL13/FSTL3/ICOS/SIRPG/CD27/IL18/HLA-DOA/SMAD7/CD274/PTPN22/LEF1/IL7/HLA-A	14
BP	GO:0007159	leukocyte cell-cell adhesion	1.1E-06	1.6E-04	<u>IGFBP2</u> /ARG2/LAX1/ICOS/CTLA4/SIRPG/CD27/IL18/HLA-DOA/SMAD7/TIGIT/CD274/PTPN22/LEF1/IL7/HLA-A	16
BP	GO:0002253	activation of immune response	1.2E-06	1.8E-04	LAX1/TXK/AIM2/CTLA4/IGKC/IGLL5/IGHM/KLHL6/CD8B/IGHG1/IGHG3/PTPN22/SLA2/C4A/CD8A/HLA-A	16

1. BP – Biological Process, CC – Cellular Component, MF – Molecular Function
2. Grey shading indicates terms related to inflammatory processes.
3. Bold and underlined indicates genes that are in the top 20 dysfunction genes in Table 1

Table S7. GO terms associated with the top 200 unique survival genes that are increased in hearts that failed (N=779 EMBs with 74 postbiopsy failures within 3 years)

ONTOLOGY ¹	ID	Description ²	P value	Q value	Gene ID	Count
BP	GO:0070482	response to oxygen levels	1.30E-07	2.01E-04	<u>ERO1A/SLC2A1/EDN1</u> /HK2/CA9/EGLN3/ANGPT2/THBS1/ADM/EGLN1/ENO1/PDK3/KCNMA1/AGTRAP/PGK1/CYB5R4	16
BP	GO:0001666	response to hypoxia	1.50E-07	2.01E-04	<u>ERO1A/SLC2A1/EDN1</u> /HK2/CA9/EGLN3/ANGPT2/THBS1/ADM/EGLN1/ENO1/PDK3/KCNMA1/AGTRAP/PGK1	15
BP	GO:0036293	response to decreased oxygen levels	2.66E-07	2.37E-04	<u>ERO1A/SLC2A1/EDN1</u> /HK2/CA9/EGLN3/ANGPT2/THBS1/ADM/EGLN1/ENO1/PDK3/KCNMA1/AGTRAP/PGK1	15
BP	GO:0043434	response to peptide hormone	5.01E-07	3.35E-04	<u>SLC2A1/EDN1</u> /AREG/SPINK1/ <u>PTPN1</u> /IRS2/ADIPOR1/POR/SESN2/PDK4/ADM/INHBB/MYO5A/AGTRAP/STAT3/TIMP1/CA2	17
CC	GO:0034774	secretory granule lumen	5.75E-07	6.18E-05	VTI1B/VCL/CTSA/MIF/SERPINE1/THBS1/PGAM1/ERP44/CREG1/NEU1/CYB5R3/S100A9/TIMP1/RNASE2/GALNS	15
CC	GO:0060205	cytoplasmic vesicle lumen	6.47E-07	6.18E-05	VTI1B/VCL/CTSA/MIF/SERPINE1/THBS1/PGAM1/ERP44/CREG1/NEU1/CYB5R3/S100A9/TIMP1/RNASE2/GALNS	15
CC	GO:0031983	vesicle lumen	6.99E-07	6.18E-05	VTI1B/VCL/CTSA/MIF/SERPINE1/THBS1/PGAM1/ERP44/CREG1/NEU1/CYB5R3/S100A9/TIMP1/RNASE2/GALNS	15
BP	GO:0006090	pyruvate metabolic process	9.84E-07	5.27E-04	<u>ENO2</u> /LDHA/HK2/PDK4/PGAM1/ENO1/PDK3/PGK1/STAT3	9
BP	GO:0072593	reactive oxygen species metabolic process	3.02E-06	1.35E-03	<u>ARG2</u> /AATF/CDKN1A/ <u>EDN1</u> /HK2/SESN2/PDK4/THBS1/BCR/PDK3/PREX1/CYB5R4	12
BP	GO:1901652	response to peptide	7.00E-06	2.68E-03	<u>SLC2A1/EDN1</u> /AREG/SPINK1/ <u>PTPN1</u> /IRS2/ADIPOR1/POR/SESN2/PDK4/ADM/INHBB/MYO5A/AGTRAP/STAT3/TIMP1/CA2	17
BP	GO:0005996	monosaccharide metabolic process	1.05E-05	3.52E-03	<u>ENO2</u> /SLC2A1/HK2/GSTO1/IRS2/ADIPOR1/SESN2/PDK4/ENO1/PDK3/PGK1/SLC35A2	12
BP	GO:0006096	glycolytic process	1.45E-05	4.17E-03	<u>ENO2</u> /LDHA/HK2/PGAM1/ENO1/PGK1/STAT3	7
CC	GO:0090575	RNA polymerase II transcription regulator complex	1.51E-05	8.85E-04	FOSL2/CEBPD/MAFF/ASCC2/CEBPB/CBFB/NR1H2/GTF2E2/MED8/SUPT3H/STAT3	11
BP	GO:0006757	ATP generation from ADP	1.57E-05	4.17E-03	<u>ENO2</u> /LDHA/HK2/PGAM1/ENO1/PGK1/STAT3	7
BP	GO:2001234	negative regulation of apoptotic signaling pathway	1.71E-05	4.17E-03	AATF/ITPRIP/ <u>PTPN1</u> /BCL2L1/MIF/SERPINE1/THBS1/ENO1/TXNDC12/SIAH2/SLC35F6	11
CC	GO:0005766	primary lysosome	2.00E-05	8.85E-04	CTSA/TOM1/CREG1/CYB5R3/FPR1/DNAJC5/CD63/RNASE2/GALNS	9
CC	GO:0042582	azurophil granule	2.00E-05	8.85E-04	CTSA/TOM1/CREG1/CYB5R3/FPR1/DNAJC5/CD63/RNASE2/GALNS	9
BP	GO:0046031	ADP metabolic process	2.89E-05	6.45E-03	<u>ENO2</u> /LDHA/HK2/PGAM1/ENO1/PGK1/STAT3	7
BP	GO:0071375	cellular response to peptide hormone stimulus	3.41E-05	6.77E-03	<u>EDN1</u> /SPINK1/ <u>PTPN1</u> /IRS2/ADIPOR1/POR/PDK4/INHBB/MYO5A/AGTRAP/STAT3/CA2	12
BP	GO:0040013	negative regulation of locomotion	3.70E-05	6.77E-03	SEMA4B/ABHD2/VCL/ADIPOR1/CXCL13/ANGPT2/MIF/SERPINE1/THBS1/BCR/MIIP/STAT3/ADORA3/TIMP1	14

1. BP – Biological Process, CC – Cellular Component, MF – Molecular Function
2. Shading indicates terms related to oxygen or glycolysis
3. Bold and underlined indicates genes that are in the top 20 dysfunction genes in Table 1 or survival genes in Table 3; EDN1 is also bolded.

Table S8. Concordance statistics between molecular scores and graft survival in all biopsies, biopsies with low LVEF, and biopsies with high LVEF

Variables	Overall (N=779)	Low LVEF (N=116)	High LVEF (N=427)
Survival score based on mean of RF and glmnet scores	0.822	0.843	0.701
Random forest-based score	0.795	0.743	0.732
Glmnet-based score	0.808	0.851	0.684
Dysfunction classifier (LoLVEF_{Prob})	0.731	0.784	0.664