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Supplementary Table 1: Top 50 complement-activating donor-specific anti-HLA antibody-related annotated transcripts in the prospective cohort study.

Probeset ID	Name	GENE	FDR adjusted P.Val	Fold change (C1q+ *vs.* C1q-)	BIOLOGICAL ASSOCIATION
11749245_a_at	Chemokine (C-X-C motif) ligand 11	**CXCL11**	0.009575164	2.48	ENDOTHELIAL IFNG RESPONSIVE
11732466_a_at	Chemokine (C-X-C motif) ligand 11	**CXCL11**	0.006574825	2.44	ENDOTHELIAL IFNG RESPONSIVE
11732467_x_at	Chemokine (C-X-C motif) ligand 11	**CXCL11**	0.011176333	2.37	ENDOTHELIAL IFNG RESPONSIVE
11731422_s_at	Fc fragment of IgG, low affinity IIIa, receptor (CD16a)	**FCGR3A**	0.001899275	2.27	NK CELL
11752930_a_at	Guanylate-binding protein 1, interferon-inducible	**GBP1**	0.001586593	2.13	IFNG RESPONSE
11728679_a_at	CD163 molecule	**CD163**	0.001693452	2.07	MONOCYTE/MACROPHAGE
11733439_a_at	Guanylate-binding protein 5	**GBP5**	0.007150959	2.04	IFNG RESPONSE
11745114_a_at	Egf-like module containing, mucin-like, hormone receptor-like 2	**EMR2**	0.001656958	1.96	MONOCYTE/MACROPHAGE
11718983_x_at	Chemokine (C-C motif) ligand 4	**CCL4**	0.010983178	1.94	NK CELL CD16-ENGAGEMENT/MACROPHAGE IFNG RESPONSIVE
11733004_s_at	Fc fragment of IgG, low-affinity IIIa, receptor (CD16a) ///Fc fragment of IgG, low-affinity IIIb, receptor (CD16b)	**FCGR3A**	0.001918375	1.92	NK CELL
11716846_a_at	Membrane-spanning 4 domains, subfamily A, member 6A	**MS4A6A**	0.001656958	1.89	MONOCYTE/MACROPHAGE
11719465_a_at	Complement component 1, q subcomponent, B chain	**C1QB**	0.002472295	1.88	MACROPHAGE IFNG RESPONSIVE
11756780_a_at	Membrane-spanning 4 domains, subfamily A, member 7	**MS4A7**	0.001656958	1.87	MONOCYTE/MACROPHAGE
11720388_s_at	Complement component 1, q subcomponent, C chain	**C1QC**	0.004195709	1.86	MACROPHAGE IFNG RESPONSIVE
11740871_a_at	Membrane-spanning 4 domains, subfamily A, member 7	**MS4A7**	0.001656958	1.81	MONOCYTE/MACROPHAGE
11746087_a_at	CD84 molecule	**CD84**	0.004070185	1.80	MONOCYTE/MACROPHAGE
11736311_x_at	Fc fragment of IgG, high-affinity Ia, receptor (CD64) ///Fc fragment of IgG, high-affinity Ib, receptor (CD64) ///Fc fragment of IgG, high-affinity Ic, receptor (CD64), pseudogene	**FCGR1A ///FCGR1B ///FCGR1C**	0.00405367	1.79	MONOCYTE/MACROPHAGE
11749293_x_at	Membrane-spanning 4 domains, subfamily A, member 6A	**MS4A6A**	0.002275958	1.78	MONOCYTE/MACROPHAGE
11740873_x_at	Membrane-spanning 4 domains, subfamily A, member 7	**MS4A7**	0.001518221	1.76	MONOCYTE/MACROPHAGE
11743560_a_at	Protein tyrosine phosphatase, receptor type, C	**PTPRC**	0.008003269	1.74	NK CELL/MONOCYTE
11730637_a_at	Cytotoxic T-lymphocyte-associated protein 4	**CTLA4**	0.005534756	1.74	EFFECTOR T CELL
11719466_s_at	Complement component 1, q subcomponent, B chain	**C1QB**	0.005534756	1.74	MACROPHAGE IFNG RESPONSIVE
11723849_a_at	Membrane-spanning 4 domains, subfamily A, member 6A	**MS4A6A**	0.001918375	1.73	MONOCYTE/MACROPHAGE
11744567_a_at	CD72 molecule	**CD72**	0.004535173	1.73	NK CELL CD16-ENGAGEMENT
11728266_a_at	Leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 2	**LILRB2**	0.009998633	1.73	MONOCYTE/MACROPHAGE
11728265_a_at	Leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 2	**LILRB2**	0.001797393	1.73	MONOCYTE/MACROPHAGE
11743917_a_at	FK506-binding protein 5	**FKBP5**	0.01016574	1.72	RENAL EPITHELIUM
11752095_a_at	Protein tyrosine phosphatase, receptor type, C	**PTPRC**	0.010186561	1.72	NK CELL/MONOCYTE
11730372_a_at	FYN binding protein	**FYB**	0.001656958	1.69	MONOCYTE/MACROPHAGE
11749589_x_at	Cathepsin S	**CTSS**	0.006875177	1.69	MONOCYTE/MACROPHAGE
11721923_a_at	Protein kinase C, beta	**PRKCB**	0.008086817	1.69	NK CELL
11756806_a_at	Interferon-stimulated exonuclease gene 20 kDa	**ISG20**	0.002969744	1.68	IFNG RESPONSE
11754649_s_at	IL2-inducible T-cell kinase	**ITK**	0.010810255	1.68	T CELL/NK CELL
11751570_a_at	Membrane-spanning 4 domains, subfamily A, member 4A	**MS4A4A**	0.002602523	1.68	MONOCYTE/MACROPHAGE
11725024_a_at	Uncharacterized LOC100129518 ///superoxide dismutase 2, mitochondrial	**SOD2**	0.001355415	1.68	MACROPHAGE IFNG RESPONSIVE
11728944_a_at	Leukocyte-specific transcript 1	**LST1**	0.005757252	1.67	MONOCYTE/MACROPHAGE
11721099_at	Complement component 3a receptor 1	**C3AR1**	0.004306127	1.67	MONOCYTE/MACROPHAGE
11755759_a_at	Multiple EGF-like-domains 11	**MEGF11**	0.001918375	1.67	IFNG RESPONSE
11751647_a_at	Interleukin 7 receptor	**IL7R**	0.008222882	1.67	T CELL/MACROPHAGE
11733353_at	Cytotoxic and regulatory T cell molecule	**CRTAM**	0.009787677	1.66	NK CELL CD16-ENGAGEMENT
11716416_at	Complement component 1, q subcomponent, A chain	**C1QA**	0.002969744	1.65	MACROPHAGE IFNG RESPONSIVE
11733841_a_at	Ecotropic viral integration site 2A	**EVI2A**	0.00826557	1.65	MONOCYTE/MACROPHAGE
11724004_a_at	FYN-binding protein	**FYB**	0.004535173	1.65	MONOCYTE/MACROPHAGE
11732927_x_at	Killer cell lectin-like receptor subfamily C, member 1	**KLRC1**	0.008682772	1.65	NK CELL
11730457_a_at	Absent in melanoma 2	**AIM2**	0.005534756	1.64	MACROPHAGE IFNG RESPONSIVE
11760710_a_at	Membrane-spanning 4 domains, subfamily A, member 6A	**MS4A6A**	0.001693452	1.64	MONOCYTE/MACROPHAGE
11727876_at	Cytochrome b-245, beta polypeptide	**CYBB**	0.006837264	1.64	MONOCYTE/MACROPHAGE
11724997_a_at	CD86 molecule	**CD86**	0.002450958	1.64	MONOCYTE/MACROPHAGE
11743561_a_at	Protein tyrosine phosphatase, receptor type, C	**PTPRC**	0.009998633	1.63	NK CELL/MONOCYTE
11749587_x_at	Fc fragment of IgG, low-affinity IIa, receptor (CD32)	**FCGR2A**	0.004310792	1.48	NK CELL

Supplementary Table 2: Top canonical pathways overrepresented with complement-activating donor-specific anti-HLA antibody-associated transcripts aligned by adjusted P value.

Ingenuity Canonical Pathways	Adjusted P value	Molecules
Natural Killer Cell Signaling	5.8E-09	CD300A, FCER1G, FCGR2A FCGR3A/FCGR3B, HCST, KLRC1, KLRD1, LAIR1, LCP2, LILRB1, PIK3R5, PRKCB, VAV1
Phagosome Formation	5.8E-09	CLEC7A, FCER1G, FCGR1A, FCGR1B, FCGR2A, FCGR2C, FCGR3A/FCGR3B, MRC1, MSR1, PIK3R5, PRKCB, TLR2, TLR8
Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	2.8E-07	C1QA, C1QB, C1QC, C3AR1, C5AR1, CASP1 CLEC7A, OAS3, PIK3R5, PRKCB, TLR2, TLR8
Leukocyte Extravasation Signaling	4.8E-07	ARHGAP9, CXCR4, CYBB, ITGAL, ITGAM, ITK, NCF1, NCF2, NCF4, PIK3R5, PRKCB, TIMP1, VAV1, WIPF1
Fc γ Receptor-mediated Phagocytosis in Macrophages and Monocytes	1.1E-06	FCGR1A, FCGR2A, FCGR3A/FCGR3B, FYB, HCK, LCP2, LYN, NCF1, PRKCB, VAV1
Complement System	2.5E-06	C1QB, C1QA, C1QC, C3AR1, C5AR1, CFB, ITGA
Role of NFAT in Regulation of the Immune Response	3.1E-05	CD86, FCER1G, FCGR1A, FCGR1B, FCGR2A FCGR2C, FCGR3A/FCGR3B, ITK, LCP2, LYN, PIK3R5
CD28 Signaling in T Helper Cells	4.6E-04	CD86, CTLA4, FCER1G, ITK, LCP2, PIK3R5, PTPRC, VAV1
GM-CSF Signaling	6.6E-04	CSF2RB, HCK, LYN, PIK3R5, PRKCB, STAT1
Production of Nitric Oxide and Reactive Oxygen Species in Macrophages	1.1E-03	CYBB, JAK3, NCF1, NCF2, NCF4, PIK3R5, PRKCB, STAT1, TLR2
T Helper Cell Differentiation	1.1E-03	BCL6, CD86, FCER1G, IL10RA, STAT1, STAT4
T Cell Receptor Signaling	5.4E-03	CTLA4, ITK, LCP2, PIK3R5, PTPRC, VAV1
Phospholipase C Signaling	6.6E-03	ADCY7, FCER1G, FCGR2A, FCGR2C, ITK, LCP2, LYN, PRKCB, TGM2
Role of Macrophages, Fibroblasts and Endothelial Cells in Rheumatoid Arthritis	6.8E-03	C5AR1, CEBPB, FCGR1A FCGR3A/FCGR3B, IRAK3, MYC, PIK3R5, PRKCB, TLR2, TLR8
Fc Epsilon RI Signaling	6.8E-03	FCER1G, LCP2, LYN, PIK3R5, PRKCB, VAV1
JAK/Stat Signaling	6.9E-03	CEBPB, JAK3, PIK3R5, STAT1, STAT4
NF-κB Signaling	1.3E-02	FCER1G, IRAK3, PIK3R5, PRKCB, TLR2, TLR8, TNFAIP3
Tumoricidal Function of Hepatic Natural Killer Cells	1.4E-02	GZMB, ITGAL, SRGN
CTLA4 Signaling in Cytotoxic T Lymphocytes	1.4E-02	CD86, CTLA4, FCER1G, LCP2, PIK3R5
Granulocyte Adhesion and Diapedesis	1.4E-02	C5AR1, CCL4L1/CCL4L2, CXCL11, CXCR4, FPR2, ITGAL, ITGAM
IL-12 Signaling and Production in Macrophages	1.5E-02	CEBPB, PIK3R5, PRKCB, STAT1, STAT4, TLR2
Interferon Signaling	3.4E-02	PSMB8, STAT1, TAP1
IL-17 Signaling	3.4E-02	CEBPB, CXCL11, PIK3R5, TIMP1
PKC θ Signaling in T Lymphocytes	3.5E-02	CD86, FCER1G, LCP2, PIK3R5, VAV1
Toll-like Receptor Signaling	3.5E-02	IRAK3, TLR2, TLR8, TNFAIP3

Supplementary Table 3: Bivariate analysis of donor-specific anti-HLA antibody complement-activating capacity and MFI level for antibody-mediated allograft histological lesions.

	Number of patients	Number of events	OR	95% CI	P
Univariate analysis					
g+ptc Banff score (≤3 *vs.* >3)					
MFI (continuous)	157	47	1.00	1.00-1.00	0.18
C1q binding					
No	113	27	1		
Yes	44	20	2.65	[1.27-5.53]	0.009
v Banff score (0 *vs.* >0)					
MFI (continuous)	155	23	1.00	1.00-1.00	0.52
C1q binding					
No	111	10	1		
Yes	44	13	4.24	[1.69-10.60]	0.002
cg Banff score (0 *vs.* >0)					
MFI (continuous)	156	39	1.00	[1.00-1.00]	0.038
C1q binding					
No	112	22	1		
Yes	44	17	2.58	[1.20-5.54]	0.015
C4d Banff score (0 *vs.* >0)					
MFI (continuous)	157	48	1.00	[1.00-1.00]	<0.001
C1q binding					
No	113	21	1		
Yes	44	27	6.96	[3.22-15.03]	<0.001
Multivariable analysis					
g+ptc Banff score (≤3 *vs.* >3)					
MFI (continuous)	157	47	1.00	[1.00-1.00]	0.68
C1q binding					
No	113	27	1		
Yes	44	20	3.02	[1.15-7.89]	0.024
v Banff score (0 *vs.* >0)					
MFI (continuous)	155	23	1.00	[1.00-1.00]	0.10
C1q binding					
No	111	10	1		
Yes	44	13	8.39	[2.48-28.39]	0.001
cg Banff score (0 *vs.* >0)					
MFI (continuous)	156	39	1.00	[1.00-1.00]	0.48
C1q binding					
No	112	22	1		
Yes	44	17	2.06	[0.77-5.54]	0.15
C4d Banff score (0 *vs.* >0)					
MFI (continuous)	157	48	1.00	[1.00-1.00]	0.37
C1q binding					
No	113	21	1		
Yes	44	27	5.26	[2.00-13.84]	0.001

MFI, mean fluorescence intensity; OR, odds ratio

Supplementary Table 4: Bivariate analysis of donor-specific anti-HLA antibody complement-activating capacity and MFI level for gene expression levels.

	Number of patients	β	s.e.	P value
Univariate analysis				
CXCL11 (log2 optical density)				
MFI (continuous)	157	.0001455	.000035	<0.001
C1q binding				
No	113	2.002154	.3458602	<0.001
Yes	44			
CCL4 (log2 optical density)				
MFI (continuous)	157			
C1q binding		.0000823	.0000264	0.002
No	113	1.226063	.2630621	<0.001
Yes	44			
MS4A6A (log2 optical density)				
MFI (continuous)	157	.0000611	.0000197	0.002
C1q binding				
No	113	.9167953	.1965654	<0.001
Yes	44			
MS4A7 (log2 optical density)				
MFI (continuous)	157	.0000615	.0000164	<0.001
C1q binding				
No	113	.8144332	.1647329	<0.001
Yes	44			
FCGR3A (log2 optical density)				
MFI (continuous)	157	.0000768	.0000224	0.001
C1q binding				
No	113	1.007242	.2262078	<0.001
Yes	44			
Multivariable analysis				
CXCL11 (log2 optical density)				
MFI (continuous)	157	.0000388	.0000431	0.37
C1q binding				
No	113	1.750014	.4455718	<0.001
Yes	44			
CCL4 (log2 optical density)				
MFI (continuous)	157	.0000125	.0000125	0.70
C1q binding				
No	113	1.144546	.3396299	0.001
Yes	44			
MS4A6A (log2 optical density)				
MFI (continuous)	157	8.63e-06	.0000246	0.73
C1q binding				
No	113	.8606551	.2537964	0.001
Yes	44			

Supplementary Table 4: Bivariate analysis of donor-specific anti-HLA antibody complement-activating capacity and MFI level for gene expression levels (continued).

	Number of patients	β	s.e.	P value
MS4A7 (log2 optical density)				
MFI (continuous)	157	.0000196	.0000205	0.34
C1q binding				
No	113	.6866938	.2121524	0.001
Yes	44			
FCGR3A (log2 optical density)				
MFI (continuous)	157	.0000255	.0000282	0.37
C1q binding				
No	113	.8414578	.2914154	0.004
Yes	44			

MFI, mean fluorescence intensity; s.e., standard error

Supplementary Table 5: Bivariate analysis of donor-specific anti-HLA antibody complement-activating capacity and time between transplantation and donor-specific anti-HLA antibody detection for antibody-mediated allograft histological lesions.

	Number of patients	Number of events	OR	95% CI	P value
Univariate analysis					
g+ptc Banff score (≤3 *vs.* >3)					
Time since Tx (continuous)	157	47	1.00	0.99-1.00	0.017
C1q binding					
No	113	27	1		
Yes	44	20	2.65	[1.27-5.53]	0.009
v Banff score (0 *vs.* >0)					
Time since Tx (continuous)	155	23	0.99	0.99-1.00	0.002
C1q binding					
No	111	10	1		
Yes	44	13	4.24	[1.69-10.60]	0.002
cg Banff score (0 *vs.* >0)					
Time since Tx (continuous)	156	39	1.00	[1.00-1.00]	0.66
C1q binding					
No	112	22	1		
Yes	44	17	2.58	[1.20-5.54]	0.015
C4d Banff score (0 *vs.* >0)					
Time since Tx (continuous)	157	48	0.99	[0.99-1.00]	<0.001
C1q binding					
No	113	21	1		
Yes	44	27	6.96	[3.22-15.03]	<0.001
Multivariable analysis					
g+ptc Banff score (≤3 *vs.* >3)					
Time since Tx (continuous)	157	47	1.00	[1.00-1.00]	0.073
C1q binding					
No	113	27	1		
Yes	44	20	2.22	[1.04-4.76]	0.040
v Banff score (0 *vs.* >0)					
Time since Tx (continuous)	155	23	1.00	[0.99-1.00]	0.011
C1q binding					
No	111	10	1		
Yes	44	13	3.08	[1.18-8.02]	0.022
cg Banff score (0 *vs.* >0)					
Time since Tx (continuous)	156	39	1.00	[1.00-1.00]	0.24
C1q binding					
No	112	22	1		
Yes	44	17	3.01	[1.33-6.82]	0.008
C4d Banff score (0 *vs.* >0)					
Time since Tx (continuous)	157	48	0.99	[0.99-1.00]	<0.001
C1q binding					
No	113	21	1		
Yes	44	27	5.46	[2.41-12.40]	<0.001

OR, odds ratio; Tx, transplantation

Supplementary Table 6: Bivariate analysis of donor-specific anti-HLA antibody complement-activating capacity and time between transplantation and donor-specific anti-HLA antibody detection for gene expression levels.

	Number of patients	β	s.e.	P
Univariate analysis				
CXCL11 (log2 optical density)				
Time since Tx (continuous)	157	-.004089	.001136	<0.001
C1q binding				
No	113	2.002154	.3458602	<0.001
Yes	44			
CCL4 (log2 optical density)				
Time since Tx (continuous)	157	-.0031573	.000845	<0.001
C1q binding				
No	113	1.226063	.2630621	<0.001
Yes	44			
MS4A6A (log2 optical density)				
Time since Tx (continuous)	157	-.0027301	.0006127	<0.001
C1q binding				
No	113	.9167953	.1965654	<0.001
Yes	44			
MS4A7 (log2 optical density)				
Time since Tx (continuous)	157	-.0025622	.0005095	<0.001
C1q binding				
No	113	.8144332	.1647329	<0.001
Yes	44			
FCGR3A (log2 optical density)				
Time since Tx (continuous)	157	-.0029445	.0006946	<0.001
C1q binding				
No	113	1.007242	.2262078	<0.001
Yes	44			
Multivariable analysis				
CXCL11 (log2 optical density)				
Time since Tx (continuous)	157	-.0026119	.0010967	0.018
C1q binding				
No	113	1.776177	.3537253	<0.001
Yes	44			
CCL4 (log2 optical density)				
Time since Tx (continuous)	157	-.0023154	.0008429	0.007
C1q binding				
No	113	1.012399	.2718608	<0.001
Yes	44			
MS4A6A (log2 optical density)				
Time since Tx (continuous)	157	-.0021203	.0006112	0.001
C1q binding				
No	113	.7333533	.1971468	<0.001
Yes	44			

s.e., standard error; Tx, transplantation

Supplementary Table 6: Bivariate analysis of donor-specific anti-HLA antibody complement-activating capacity and time between transplantation and donor-specific anti-HLA antibody detection for gene expression levels (continued).

	Number of patients	β	s.e.	P
MS4A7 (log2 optical density)				
Time since Tx (continuous)	157	-.002031	.0005061	<0.001
C1q binding				
No	113	.6387101	.1632297	<0.001
Yes	44			
FCGR3A (log2 optical density)				
Time since Tx (continuous)	157	-.0022444	.0006921	0.001
C1q binding				
No	113	.8419087	.2232457	<0.001
Yes	44			

s.e., standard error; Tx, transplantation

Supplementary Table 7: Bivariate analysis of donor-specific anti-HLA antibody complement-activating capacity and C4d positivity for antibody-mediated allograft histological lesions.

	Number of patients	Number of events	OR	95% CI	P value
Univariate analysis					
g+ptc Banff score (≤3 *vs.* >3)					
C4d deposition					
No	109	28	1		
Yes	48	19	1.90	[0.92-3.90]	0.082
C1q binding					
No	113	27	1		
Yes	44	20	2.65	[1.27-5.53]	0.009
v Banff score (0 *vs.* >0)					
C4d deposition					
No	107	11	1		
Yes	48	12	2.91	[1.18-7.18]	0.021
C1q binding					
No	111	10	1		
Yes	44	13	4.24	[1.69-10.60]	0.002
cg Banff score (0 *vs.* >0)					
C4d deposition					
No	108	26	1		
Yes	48	13	1.17	[0.54-2.54]	0.69
C1q binding					
No	112	22	1		
Yes	44	17	2.58	[1.20-5.54]	0.015
Multivariable analysis					
g+ptc Banff score (≤3 *vs.* >3)					
C4d deposition					
No	109	28	1		
Yes	48	19	1.34	[0.60-3.00]	0.47
C1q binding					
No	113	27	1		
Yes	44	20	2.35	[1.05-5.26]	0.038
v Banff score (0 *vs.* >0)					
C4d deposition					
No	107	11	1		
Yes	48	12	1.78	[0.65-4.89]	0.26
C1q binding					
No	111	10	1		
Yes	44	13	3.33	[1.22-9.13]	0.019
cg Banff score (0 *vs.* >0)					
C4d deposition					
No	108	26	1		
Yes	48	13	0.73	[0.30-1.78]	0.49
C1q binding					
No	112	22	1		
Yes	44	17	2.95	[1.25-6.96]	0.014

OR, odds ratio

Supplementary Table 8: Bivariate analysis of donor-specific anti-HLA antibody complement-activating capacity and C4d positivity for gene expression levels.

	Number of patients	β	s.e.	P value
Univariate analysis				
CXCL11 (log2 optical density)				
C4d deposition				
No	109	1.548057	.3504149	<0.001
Yes	48			
C1q binding				
No	113	2.002154	.3458602	<0.001
Yes	44			
CCL4 (log2 optical density)				
C4d deposition				
No	109	1.110178	.2588967	<0.001
Yes	48			
C1q binding				
No	113	1.226063	.2630621	<0.001
Yes	44			
MS4A6A (log2 optical density)				
C4d deposition				
No	109	.6667462	.1974917	0.001
Yes	48			
C1q binding				
No	113	.9167953	.1965654	<0.001
Yes	44			
MS4A7 (log2 optical density)				
C4d deposition				
No	109	.7606548	.1616237	<0.001
Yes	48			
C1q binding				
No	113	.8144332	.1647329	<0.001
Yes	44			
FCGR3A (log2 optical density)				
C4d deposition				
No	109	.8572055	.223845	<0.001
Yes	48			
C1q binding				
No	113	1.007242	.2262078	<0.001
Yes	44			
Multivariable analysis				
CXCL11 (log2 optical density)				
C4d deposition				
No	109	.8886587	.3652017	0.015
Yes	48			
C1q binding				
No	113	1.62199	.3746287	<0.001
Yes	44			

s.e., standard error

Supplementary Table 8: Bivariate analysis of donor-specific anti-HLA antibody complement-activating capacity and C4d positivity for gene expression levels (continued)

	Number of patients	β	s.e.	P value
CCL4 (log2 optical density)				
C4d deposition				
No	109	.7405266	.2767015	0.007
Yes	48			
C1q binding				
No	113	.9092689	.283844	0.001
Yes	44			
MS4A6A (log2 optical density)				
C4d deposition				
No	109	.3559381	.2095566	0.089
Yes	48			
C1q binding				
No	113	.7645266	.2149659	<0.001
Yes	44			
MS4A7 (log2 optical density)				
C4d deposition				
No	109	.5199921	.1722339	0.003
Yes	48			
C1q binding				
No	113	.5919829	.1766798	0.001
Yes	44			
FCGR3A (log2 optical density)				
C4d deposition				
No	109	.5419836	.2394563	0.024
Yes	48			
C1q binding				
No	113	.7753836	.2456374	0.002
Yes	44			

s.e., standard error

Supplementary Table 9: Complement-activating anti-HLA antibody histo-molecular rejection phenotype according to complement-activating donor-specific anti-HLA antibody preformed/de novo status.

	All patients with C1q+ DSA N=44	Preformed DSA N=28	De novo DSA N=16	P value
Histology (Banff scores)				
g score, median (IQR)	2 (1-2)	2 (1-2)	2 (0-2)	0.71
ptc score, median (IQR)	2 (1-2)	2 (1-2)	2 (1-2)	0.64
v score, median (IQR)	0 (0-1)	0 (0-1)	0 (0-0)	0.22
i score, median (IQR)	0 (0-1)	0 (0-1)	0 (0-1)	0.78
t score, median (IQR)	0 (0-2)	0 (0-1)	0 (0-2)	0.57
cg score, median (IQR)	0 (0-1)	0 (0-1)	0 (0-1)	0.87
C4d score, median (IQR)	1 (0-3)	1 (0-3)	1 (0-2)	0.54
Gene expression level (log2 optical density)				
CXCL11, mean (SD)	8.6 (1.6)	8.6 (1.7)	8.6 (1.4)	0.90
CCL4, mean (SD)	8.9 (1.4)	9.0 (1.5)	8.8 (1.2)	0.63
MS4A6A, mean (SD)	10.0 (1.1)	9.9 (1.2)	10.0 (0.8)	0.96
MS4A7, mean (SD)	7.6 (0.9)	7.7 (1.0)	7.6 (0.8)	0.73
FCGR3A, mean (SD)	8.8 (1.5)	8.9 (1.5)	8.7 (1.1)	0.48

DSA, donor-specific antibody

Supplementary Table 10: Patient characteristics according to antibody-mediated rejection prophylaxis and complement-activating donor-specific anti-HLA antibody status in the deceased donor subset of the terminal complement pharmacological blockade study.

	Patients with C1q+ anti-HLA DSAs (N=29)			Patients with C1q- anti-HLA DSAs (N=47)		
	SOC (N=12)	Eculizumab (N=17)	P value	SOC (N=32)	Eculizumab (N=15)	P value
Recipient characteristics						
Age, mean (SD), years	52.4 (10.0)	50.6 (12.4)	0.66	49.3 (13.3)	50.2 (13.1)	0.61
Male gender, No. (%)	6 (50)	7 (41)	0.64	13 (41)	7 (47)	0.70
Retransplantation, No. (%)	8 (67)	9 (53)	0.46	14 (44)	8 (53)	0.54
Time since dialysis, mean (SD), years	5.8 (4.5)	8.2 (7.9)	0.54	7.4 (6.3)	5.3 (3.6)	0.28
Blood type, No. (%)						
A	3 (25)	9 (53)	0.24	18 (56)	10 (67)	0.92
B	3 (25)	1 (6)		2 (6)	1 (7)	
O	6 (50)	7 (41)		11 (34)	4 (26)	
AB	0	0		1 (3)	0	
Chronic kidney disease, No. (%)						
Glomerulopathy	3 (25)	5 (29)	0.87	11 (34)	3 (20)	0.89
Vascular nephropathy	3 (25)	2 (12)		4 (13)	2 (13)	
CIN	3 (25)	3 (18)		4 (13)	2 (13)	
Diabetes	0	1 (6)		3 (9)	2 (13)	
Other	0	2 (12)		2 (6)	2 (13)	
Not determined	3 (25)	4 (23)		8 (25)	4 (27)	
Donor characteristics						
Age, mean (SD), years	51.6 (12.6)	46.8 (16.5)	0.44	47.3 (11.7)	52.1 (13.8)	0.24
Male gender, No. (%)	6 (50)	8 (47)	0.88	17 (53)	9 (60)	0.66
Cause of death, No. (%)						
Cerebrovascular death	7 (58)	11 (65)	>0.99	17 (53)	8 (53)	0.99
Other cause of death	5 (42)	6 (35)		15 (47)	7 (47)	
Serum creatinine, mean (SD), µmol/L	75.7 (27.3)	72.2 (24.2)	0.36	70.4 (24.0)	74.7 (27.0)	0.95
Transplant characteristics						
Cold ischemia time, mean (SD), hours	20.3 (13.2)	21.9 (7.9)	0.67	23.6 (7.9)	25.5 (6.6)	0.13
DGF, No. (%)	5 (42)	3 (18)	0.22	13 (41)	4 (27)	0.35
Immunological characteristics						
Calculated PRA, mean (SD), %	84.3 (21.5)	84.2 (24.6)	>0.99	75.0 (20.3)	81.3 (25.2)	0.11
HLA mismatch, mean (SD)						
A	1.0 (0.7)	1.2 (0.7)	0.38	1.1 (0.7)	1.3 (0.7)	0.33
B	1.1 (0.7)	1.2 (0.7)	0.54	1.1 (0.6)	1.3 (0.7)	0.34
DR	1.0 (0.4)	1.2 (0.6)	0.37	1.1 (0.5)	1.0 (0.5)	0.55
HLA class of DSAs, No. (%)						
I	2 (17)	6 (35)	0.51	11 (34)	4 (27)	0.72
II	3 (25)	5 (29)		12 (38)	4 (27)	
I and II	7 (58)	6 (35)		9 (28)	7 (47)	
MFI max, mean (SEM)	11097 (1311)	10841 (961)	0.96	5027 (227)	5612 (449)	0.28
HLA class of C1q-binding DSAs, No. (%)						
I	4 (33)	7 (41)	0.87	-	-	-
II	7 (58)	8 (47)		-	-	
I and II	1 (8)	2 (12)		-	-	

ATG, anti-thymocyte globulin; CIN, chronic interstitial nephritis; DGF, delayed graft function; DSA, donor-specific antibody; HLA, human leukocyte antigen; IMPDHi, inosine monophosphate dehydrogenase inhibitor; MFI, mean fluorescence intensity; PRA, panel reactive antibody; SOC, standard of care

Supplementary Table 11: Patient characteristics according to antibody-mediated rejection prophylaxis in the living donor subset of the terminal complement pharmacological blockade study.

	SOC (N=20)	Eculizumab (N=20)	P value
Recipient characteristics			
Age, mean (SD), years	43.1 (12.9)	44.1 (14.5)	0.82
Male gender, No. (%)	8 (40)	9 (45)	0.75
Retransplantation, No. (%)	11 (55)	9 (45)	0.53
Time since dialysis, mean (SD), years	6.4 (6.8)	7.2 (7.3)	0.73
Blood type, No. (%)			
A	8 (40)	6 (30)	0.68
B	2 (10)	2 (10)	
O	10 (50)	10 (50)	
AB	0	2 (10)	
Chronic kidney disease, No. (%)			
Glomerulopathy	6 (30)	6 (30)	0.84
Vascular nephropathy	5 (25)	3 (15)	
Diabetes	1 (5)	2 (10)	
Other	4 (20)	3 (15)	
Not determined	4 (20)	6 (30)	
Donor characteristics			
Age, mean (SD), years	48.6 (14.9)	46.9 (12.6)	0.70
Male gender, No. (%)	10 (50)	9 (45)	0.75
Donor type, No. (%)			
Living	20 (100)	20 (100)	-
Serum creatinine, mean (SD), µmol/L	68.5 (11.5)	65.7 (11.6)	0.45
Transplant characteristics			
Cold ischemia time, mean (SD), hours	1.8 (1.2)	2.1 (2.9)	0.60
DGF, No. (%)	1 (5)	1 (5)	>0.99
Immunological characteristics			
Calculated PRA, mean (SD), %	70.1 (24.8)	73.7 (30.4)	0.69
HLA mismatch, mean (SD)			
A	1.1 (0.6)	1.2 (0.7)	0.64
B	1.2 (0.5)	1.3 (0.5)	0.33
DR	1.2 (0.4)	1.1 (0.7)	0.41
HLA class of DSAs, No. (%)			
I	10 (50)	12 (60)	0.82
II	5 (25)	4 (20)	
I and II	5 (25)	4 (20)	
MFI max, mean (SEM)	8585 (1041)	8456 (986)	0.93

CIN, chronic interstitial nephritis; DGF, delayed graft function; DSA, donor-specific antibody; HLA, human leukocyte antigen; MFI, mean fluorescence intensity; PRA, panel reactive antibody; SOC, standard of care

Supplementary Table 12: Clinical and histological characteristics and gene expression in kidney allografts at day 14 after transplantation according to antibody-mediated rejection prophylaxis and complement-activating anti-HLA antibody status in the two terminal complement pharmocological blockade study subsets.

	Deceased donor study (N=76)						Living donor study (N=40)		
	Patients with C1q+ anti-HLA DSAs (N=29)			Patients with C1q- anti-HLA DSAs (N=47)			Patients with C1q+ anti-HLA DSAs (N=40)		
	SOC (N=12)	Eculizumab (N=17)	P	SOC (N=32)	Eculizumab (N=15)	P	SOC (N=20)	Eculizumab (N=20)	P
Clinical parameters									
eGFR, mean (SD), mL/min/1.73 m^2	41.0 (16.4)	44.6 (16.9)	0.59	46.2 (15.6)	48.1 (13.8)	0.63	47.1 (15.2)	49.3 (19.2)	0.75
Proteinuria, mean (SD), g/g	0.5 (0.4)	0.3 (0.2)	0.049	0.3 (0.2)	0.3 (0.2)	0.61	0.6 (0.6)	0.4 (0.4)	0.14
Histology (Banff scores)									
g score, median (IQR)	2 (1-2)	1 (0-2)	0.022	1 (0-2)	1 (0-2)	0.82	2 (1-2)	1 (0-1)	0.016
ptc score, median (IQR)	2 (1-2)	0 (0-1)	0.002	1 (0-1)	0 (0-1)	0.85	2 (1-2)	0 (0-1)	0.010
v score, median (IQR)	0 (0-0)	0 (0-0)	0.36	0 (0-0)	0 (0-0)	0.42	0 (0-0)	0 (0-0)	0.55
i score, median (IQR)	1 (0-1)	0 (0-0)	0.006	0 (0-0)	0 (0-1)	0.88	0 (0-1)	0 (0-0)	0.0044
t score, median (IQR)	0 (0-1)	0 (0-0)	0.018	0 (0-1)	0 (0-1)	0.73	1 (0-2)	0 (0-0)	0.0017
cg score, median (IQR)	0 (0-0)	0 (0-0)	0.36	0 (0-0)	0 (0-0)	0.51	0 (0-0)	0 (0-0)	0.15
C4d score, median (IQR)	2 (1-2)	3 (2-3)	0.33	0 (0-1)	0 (0-2)	0.64	2 (0-2)	2 (0-3)	0.53
Gene expression level (log2 optical density)									
CXCL11, mean (SD)	9.3 (0.6)	4.5 (2.1)	<0.001	4.3 (1.5)	4.1 (1.0)	0.99	8.6 (2.2)	5.2 (2.5)	<0.001
CCL4, mean (SD)	10.0 (0.5)	6.7 (2.2)	<0.001	6.5 (1.6)	6.1 (1.5)	0.52	9.4 (2.2)	6.8 (2.3)	0.0020
MS4A6A, mean (SD)	9.3 (1.0)	7.0 (2.8)	0.014	7.0 (2.4)	6.7 (2.5)	0.78	9.4 (2.6)	6.7 (2.6)	0.0013
MS4A7, mean (SD)	8.2 (0.8)	5.8 (2.8)	0.012	5.2 (2.6)	5.4 (2.5)	0.79	8.0 (2.6)	5.6 (2.5)	0.0027
FCGR3A, mean (SD)	9.4 (0.7)	6.3 (2.3)	<0.001	6.0 (1.8)	5.7 (1.8)	0.66	9.2 (2.2)	6.3 (2.3)	<0.001

DSA, donor-specific antibody; eGFR, estimated glomerular filtration rate; HLA, human leukocyte antigen; SOC, standard of care

Supplementary Table 13: Histological characteristics of ABMR cases according to complement-activating donor-specific anti-HLA antibody status and antibody-mediated rejection prophylaxis in the terminal complement pharmacological blockade study.

	Patients with C1q+ anti-HLA DSAs (N=25)			Patients with C1q- anti-HLA DSAs (N=5)		
	SOC (N=18)	Eculizumab (N=7)	P value	SOC (N=3)	Eculizumab (N=2)	P value
Banff categories						
Acute/active ABMR, No. (%)	15 (83)	5 (71)	0.60	2 (67)	2 (100)	>0.99
Chronic/active ABMR, No. (%)	3 (17)	2 (29)		1 (33)	0	
Acute TCMR, No. (%)	0	0	-	0	0	-
Borderline changes, No. (%)	4 (22)	1 (14)	>0.99	0	1 (50)	0.40
Banff scores						
g score, No. (%)			0.15			>0.99
0	1 (6)	0		0	1 (50)	
1	1 (6)	3 (43)		1 (33)	0	
2	12 (67)	3 (43)		2 (67)	1 (50)	
3	4 (22)	1 (14)		0	0	
ptc score, No. (%)			0.63			0.60
0	0	0		0	0	
1	9 (50)	2 (29)		2 (67)	0	
2	5 (28)	3 (43)		0	1 (50)	
3	4 (22)	2 (28)		1 (33)	1 (50)	
v score, No. (%)			>0.99			0.40
0	16 (89)	7 (100)		3 (100)	1 (50)	
1	2 (11)	0		0	0	
2	0	0		0	1 (50)	
3	0	0		0	0	
i score, No. (%)			0.81			0.40
0	11 (61)	6 (86)		3 (100)	1 (50)	
1	5 (28)	1 (14)		0	1 (50)	
2	1 (6)	0		0	0	
3	1 (6)	0		0	0	
t score, No. (%)			>0.99			0.40
0	14 (78)	6 (86)		3 (100)	1 (50)	
1	3 (17)	1 (14)		0	1 (50)	
2	1 (5)	0		0	0	
3	0	0		0	0	
cg score, No. (%)			0.68			>0.99
0	15 (83)	5 (71)		2 (67)	2 (100)	
1	2 (11)	2 (29)		1 (33)	0	
2	1 (6)	0		0	0	
3	0	0		0	0	

Supplementary Table 13: Histological characteristics of ABMR cases according to complement-activating donor-specific anti-HLA antibody status and antibody-mediated rejection prophylaxis in the terminal complement pharmacological blockade study (continued).

	Patients with C1q+ anti-HLA DSAs (N=25)			Patients with C1q- anti-HLA DSAs (N=5)		
	SOC (N=18)	Eculizumab (N=7)	P value	SOC (N=3)	Eculizumab (N=2)	P value
C4d score, No. (%)			>0.99			>0.99
0	6 (33)	3 (43)		1 (33)	1 (50)	
1	6 (33)	2 (29)		1 (33)	0	
2	4 (22)	1 (14)		0	1 (50)	
3	2 (11)	1 (14)		1 (33)	0	
cv score, No. (%)			0.53			>0.99
0	4 (23)	3 (43)		0	0	
1	9 (50)	4 (67)		2 (67)	1 (50)	
2	2 (12)	0		0	1 (50)	
3	3 (18)	0		1 (33)	0	
ah score, No. (%)			0.49			>0.99
0	8 (44)	3 (43)		1 (33)	0	
1	5 (28)	4 (57)		1 (33)	1 (50)	
2	4 (22)	0		1 (33)	1 (50)	
3	1 (6)	0		0	0	
IF/TA score, No. (%)			0.55			>0.99
0	10 (55)	3 (43)		1 (33)	0	
1	6 (33)	2 (29)		2 (67)	1 (50)	
2	1 (6)	2 (29)		0	1 (50)	
3	1 (6)	0		0	0	

ABMR, antibody-mediated rejection; DSA, donor-specific antibody; HLA, human leukocyte antigen; TCMR, T cell-mediated rejection

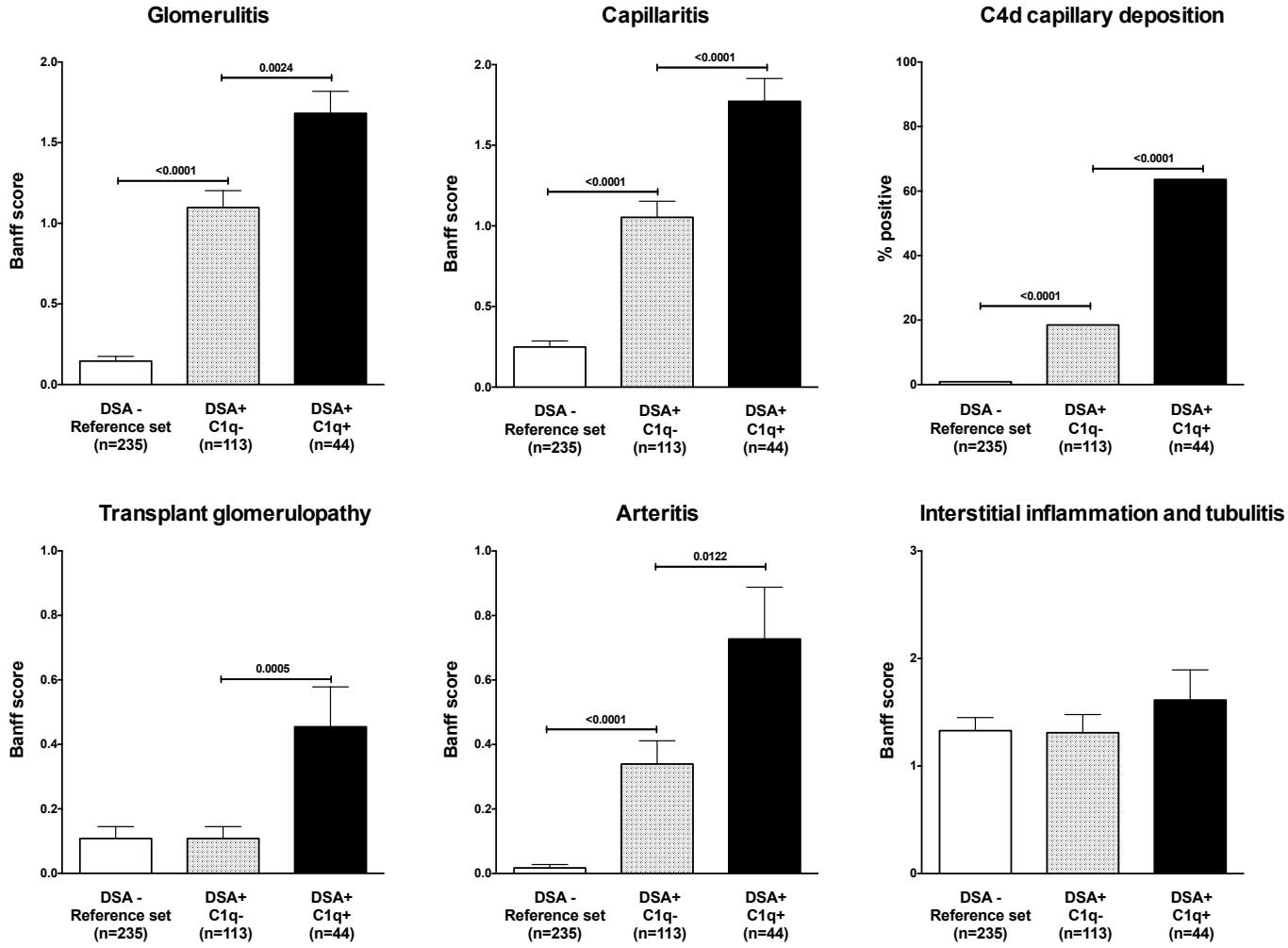
Supplementary Table 14: Baseline characteristics of the Reference Set.

	Reference Set: kidney recipients without anti-HLA DSAs (N=235)
Recipient baseline characteristics	
Age, mean (SD), years	51.4 (14.7)
Male gender, No. (%)	153 (65)
Retransplantation, No. (%)	17 (7)
Donor baseline characteristics	
Age, mean (SD), years	46.9 (15.3)
Male gender, No. (%)	85 (36)
Deceased, No. (%)	153 (65)
Cold ischemia time, mean (SD), hours	8.5 (7.9)
Biopsy characteristics	
Time since transplantation, mean (SD), days	99.2 (90.7)
Serum creatinine at biopsy, mean (SD), µmol/L	189.3 (145.4)
Acute kidney injury, No. (%)	28 (12)
T-cell mediated rejection, No. (%)	22 (9)
Borderline lesions, No. (%)	22 (9)
Recurrent glomerulonephritis, No. (%)	4 (2)
BK virus nephropathy, No. (%)	8 (4)
Isolated interstitial fibrosis - tubular atrophy, No. (%)	7 (3)
No major abnormalities, No. (%)	28 (12)
Other, No. (%)	116 (49)

DSA, donor-specific antibody; HLA, human leukocyte antigen

Supplementary Figure 1: Histopathological injury according to the presence of donor-specific anti-HLA antibodies and their complement-activating capacity in the prospective cohort study.

Data are based on 392 kidney allograft biopsies performed in the first year after transplantation that were assessed for histopathology and immunohistochemistry. The international Banff classification scores for glomerulitis, peritubular capillaritis, endarteritis, transplant glomerulopathy, the sum of Banff scores for interstitial inflammation and tubulitis and percentage of C4d complement fraction deposition in peritubular capillaries are given according to the circulating anti-HLA DSA status (DSA-/DSA+C1q-/DSA+C1q+). Each of the Banff scores ranges from 0 to 3, with higher scores indicating a more severe abnormality.



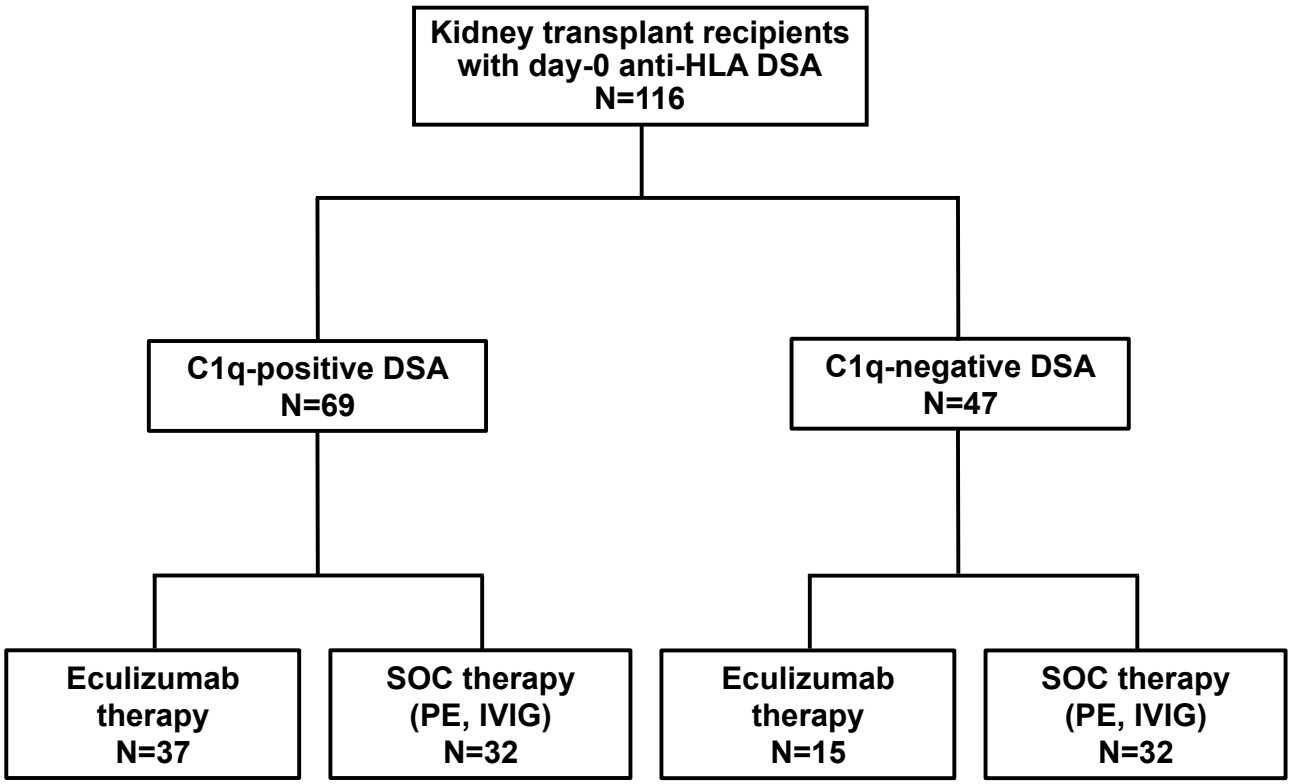
Supplementary Figure 2: Expression of complement-activating donor-specific anti-HLA antibody selective transcripts in a panel of primary human cells, including effector CD8+ and CD4+ T-cells, unstimulated NK cells, CD16-stimulated NK cells, B cells, monocytes, macrophages, IFNG-treated macrophages, and endothelial cells (HUVECs), with and without IFNG treatment. The top non-redundant complement-activating donor-specific anti-HLA antibody selective transcripts are represented. The color is representative of the standardized (z)-score of the probe set signal (red color indicates high expression).

Gene Symbol	Control Kidney	CD4	CD8	NK Unstimulated	NK CD16-Stimulated	B cell	Monocyte	Macrophage unstim	Macrophage + IFNG	HUVEC	HUVEC + IFNG	Avg signal
AIM2	-0.66	0.06	-0.03	-0.55	-0.53	2.00	0.43	-0.33	2.23	-0.67	-0.60	162
C1QA	0.13	-0.44	-0.46	-0.44	-0.42	-0.44	0.01	0.35	3.13	-0.46	-0.43	208
C1QB	-0.17	-0.33	-0.31	-0.32	-0.31	-0.33	-0.25	0.04	3.31	-0.35	-0.33	318
C1QC	-0.18	-0.33	-0.32	-0.33	-0.33	-0.33	-0.32	0.22	3.29	-0.34	-0.34	394
C3AR1	-0.57	-0.34	-0.07	0.02	-0.51	-0.69	1.56	1.53	1.98	-0.71	-0.71	458
CCL4	-0.55	-0.50	0.03	0.60	2.95	-0.56	-0.53	0.13	0.71	-0.57	-0.57	1258
CD163	-0.45	-0.48	-0.48	-0.48	-0.48	-0.48	0.72	2.71	1.36	-0.48	-0.48	641
CD72	-0.40	-0.35	-0.30	-0.33	3.09	0.97	-0.40	-0.32	-0.34	-0.40	-0.41	137
CD84	-0.57	-0.24	-0.23	-0.55	-0.50	-0.21	0.33	2.66	1.58	-0.56	-0.57	706
CD86	-0.54	-0.55	-0.42	-0.57	-0.52	-0.08	2.36	1.19	1.50	-0.58	-0.55	103
CRTAM	-0.29	-0.29	-0.27	-0.27	3.33	-0.29	-0.27	-0.24	-0.22	-0.29	-0.29	85
CTLA4	-0.40	2.85	1.48	-0.38	-0.38	-0.39	-0.40	-0.39	-0.39	-0.40	-0.40	80
CTSS	-0.72	-0.58	-0.61	-0.50	-0.55	-0.16	1.53	1.87	1.75	-0.72	-0.07	3789
CXCL11	-0.34	-0.34	-0.34	-0.35	-0.33	-0.35	-0.34	-0.34	-0.13	-0.34	3.28	708
CYBB	-0.53	-0.54	-0.54	-0.53	-0.53	-0.33	2.28	1.43	1.44	-0.54	-0.54	1260
EMR2	-0.46	-0.45	-0.45	-0.44	-0.45	-0.46	2.93	1.08	0.49	-0.45	-0.46	136
EVI2A	-1.06	0.96	0.48	0.31	1.49	-0.35	1.49	0.63	0.33	-1.05	-1.06	974
FCGR1A///FCGR1B///FCGR1C	-0.34	-0.34	-0.34	-0.34	-0.33	-0.34	0.05	0.05	3.29	-0.34	-0.34	553
FCGR2A	-0.49	-0.56	-0.56	-0.47	-0.50	-0.48	1.66	1.66	1.92	-0.57	-0.57	389
FCGR3A///FCGR3B	-0.49	-0.50	-0.49	2.32	2.12	-0.49	0.06	-0.31	-0.24	-0.50	-0.50	642
FKBP5	-0.39	-0.48	-0.69	-0.64	-0.26	-0.81	0.09	0.26	-0.12	-0.71	-0.46	1494
FYB	-0.85	0.95	1.35	0.26	-0.59	-0.87	1.93	0.41	0.79	-0.84	-0.78	208
GBP1	-0.65	-0.36	-0.56	-0.45	-0.30	-0.70	-0.41	-0.30	1.96	-0.66	2.08	1884
GBP5	-0.78	0.01	-0.26	0.54	0.13	-0.74	-0.63	-0.72	2.72	-0.78	0.88	1037
IL7R	-0.64	2.72	0.33	-0.19	-0.03	-0.63	-0.64	1.29	0.24	-0.60	-0.54	560
ISG20	-1.16	0.59	1.43	-0.18	0.25	1.75	-0.96	-0.65	1.14	-1.08	0.11	832
ITK	-0.63	1.38	1.08	1.24	1.96	-0.63	-0.63	-0.63	-0.63	-0.63	-0.63	455
KLRC1///KLRC2	-0.40	-0.40	-0.24	1.34	2.92	-0.40	-0.40	-0.40	-0.40	-0.40	-0.40	309
LILRB2	-0.52	-0.53	-0.53	-0.52	-0.53	-0.52	1.79	1.60	1.86	-0.53	-0.53	354
LST1	-0.49	-0.25	-0.21	-0.45	-0.46	-0.43	3.02	0.48	0.83	-0.51	-0.50	582
MEGF11	-0.27	-0.29	-0.30	-0.31	-0.31	-0.31	-0.31	-0.30	-0.32	-0.30	-0.31	19
MS4A4A	-0.36	-0.41	-0.41	-0.41	-0.41	-0.41	0.23	3.14	0.67	-0.41	-0.41	137
MS4A6A	-0.39	-0.47	-0.47	-0.45	-0.45	-0.47	2.92	0.84	0.83	-0.46	-0.47	502
MS4A7	-0.44	-0.51	-0.51	-0.51	-0.51	-0.29	2.70	1.29	0.82	-0.51	-0.51	632
PRKCB	-0.66	-0.03	0.40	0.33	-0.32	2.59	1.45	-0.55	-0.53	-0.67	-0.67	178
PTPRC	-1.03	0.44	0.14	1.51	1.63	0.44	1.05	0.07	-0.02	-1.06	-1.06	1473
SOD2	-0.43	-0.97	-1.03	-0.95	-0.76	-0.40	1.54	-0.04	0.41	-0.95	0.62	287

Supplementary Figure 3: Flow diagram of the terminal complement pharmacological blockade study population.

Patients derived from the only two available clinical trials investigating the effect of complement inhibition for rejection prophylaxis in kidney transplant recipients with anti-HLA DSAs at the time of transplantation (NCT01567085 and NCT01399593). In both studies, patients treated with eculizumab received the drug in the first nine weeks post-transplantation (1200 mg one hour prior to transplantation, 900 mg per week for four weeks and 1200 mg every other week for weeks five, seven, and nine); patients treated with standard of care received plasma exchange and intravenous immunoglobulin according to the transplant center‘s standard of care for prophylaxis for antibody-mediated rejection. All patients were screened for the presence of C1q-binding anti-HLA DSAs in sera collected at the time of transplantation.

DSA, donor-speciffic antibody; IVIG, intravenous immunoglobulin; PE, plasma exchange; and SOC, standard of care



SUPPLEMENTARY METHODS

Reference Set

The Reference Set was composed of kidney transplant patients without circulating anti-HLA DSAs who underwent biopsies for clinical indications as the SOC in the first year post-transplantation, with annotated and validated histopathological results and gene allograft expression provided by the Alberta Transplant Applied Genomics Center (ATAGC, Edmonton, Alberta, Canada) Reference Standard. The baseline characteristics of the patients from the Reference Set (N=235) are shown in Supplementary Table 14.

Effects of pharmacological complement blockade by eculizumab on kidney allograft injury

The effects of complement inhibition therapy on kidney allograft injury were studied in kidney transplant recipients who presented anti-HLA DSA before transplantation and received antibody-mediated rejection prophylaxis with eculizumab (Soliris®, Alexion Pharmaceuticals, Cheshire, CT, USA), a humanized monoclonal antibody that is a terminal complement inhibitor, or the SOC of non-complement-directed therapy. The data derived from the only two available clinical trials investigating the effect of complement inhibition for rejection prophylaxis in kidney transplant recipients with anti-HLA DSAs at the time of transplantation (NCT01567085 and NCT01399593).

Patients undergoing kidney transplantation from deceased donors who received eculizumab for the prevention of antibody-mediated rejection came from the open-label, single-arm, multicenter NCT01567085 study conducted to determine the safety and efficacy of eculizumab in the prevention of antibody-mediated rejection in sensitized recipients of a kidney transplant from a deceased donor (N=48, between January 1, 2012 and December 31, 2013). The participating centers were the following: Saint-Louis Hospital, Paris, France (N=13); Necker Hospital, Paris, France (N=6); Centre Hospitalier Universitaire Rangueil, Toulouse, France (N=6); Padua University Hospital, Padua, Italy (N=3); and Bellvitge University Hospital, Barcelona, Spain (N=4). Sixteen patients were excluded for non-available material for gene expression analysis. Inclusion criteria were: male or female patients ≥ 18 years old, patients with stage V chronic kidney disease who will receive a kidney transplant from a deceased donor to whom they are sensitized, history of prior

exposure to HLA (prior solid organ or tissue allograft, pregnancy, blood transfusion, prior exposure to specific donor's HLA), historical positive complement-dependent cytotoxicity crossmatch and/or B-cell or T-cell flow cytometric crossmatch ≥300 and ≤500 mean channel shift and/or anti-HLA DSA identified by single antigen bead (SAB) with a single MFI >3000, negative complement-dependent cytotoxicity crossmatch at time of transplantation, able to understand the informed consent form and willing to comply with study procedures, female patients of child-bearing potential had to have a negative pregnancy test (serum beta-hCG) and had to be practicing an effective, reliable and medically approved contraceptive regimen while on eculizumab treatment and for up to 5 months following discontinuation of treatment. Exclusion criteria were: previous treatment with eculizumab at any time prior to enrolling in this study, ABO incompatibility with deceased donor, history of severe cardiac disease, prior splenectomy, known bleeding disorder, active bacterial or other infection which is clinically significant in the opinion of the investigator and is a contraindication to transplantation, participation in any other investigational drug study or exposure to an investigational drug or device within 30 days of screening, treatment with rituximab ≤3 months prior to screening, previous treatment with bortezomib ≤3 months prior to screening, previous treatment with alemtuzumab ≤6 months prior to screening, hypersensitivity to murine proteins or to one of the product excipients, history of illicit drug use or alcohol abuse within the previous year, unresolved meningococcal disease, pregnancy or lactation, current cancer or history of cancer within the 5 years prior to screening with the exception of patients who have successfully treated nonmetastatic basal or squamous cell, any medical condition that, in the opinion of the investigator, might interfere with the patient‘s participation in the study, poses an added risk for the patient, or confounds the assessment of the patient, active infection with hepatitis B (HBV), hepatitis C (HCV) or human immunodeficiency virus (HIV). Patients received eculizumab in the first nine weeks post-transplantation (1200 mg one hour prior to transplantation, 900 mg per week for four weeks and 1200 mg every other week for weeks five, seven, and nine). Patients received induction therapy by thymoglobulin (1.5 mg/kg x4 doses) and maintenance immunosuppression consisting in tacrolimus administered to maintain through levels at 4 to 11 ng/mL, mycophenolate mofetil 1 g BID or enteric-coated mycophenolic acid 720 mg BID and prednisone initially per SOC at the transplant center and tapered to 5 mg daily by 3 months post-

transplantation. Patients were vaccinated against Neisseria meningitidis using tetravalent conjugated vaccines (if not already vaccinated within the time period of active coverage specified by the vaccine manufacturer). Patients undergoing kidney transplantation from deceased donors who received the standard of care (SOC) were represented by all kidney recipients from the same transplant centers (N=44) receiving SOC in the prevention of antibody-mediated rejection, with anti-HLA DSA >3000 MFI detected at the time of transplantation, which was performed between January 1, 2011 and January 1, 2014, and meeting the same inclusion/exclusion criteria as the eculizumab patients. Patients received SOC non-complement-directed therapy consisting of plasma exchanges (four courses: one course on days zero, one, two and three) and intravenous immunoglobulin administered at a dose of two g/kg BW over a 72-hour period of time. The first intravenous immunoglobulin course was started at day three, with subsequent courses given on weeks three, six and nine after kidney transplantation. Patients received induction therapy by thymoglobulin (1.5 mg/kg x4-5 doses) and maintenance immunosuppression consisting in tacrolimus administered to maintain through levels at 4 to 11 ng/mL, mycophenolate mofetil 1 g BID or enteric-coated mycophenolic acid 720 mg BID and prednisone per SOC at the transplant center. All patients were screened for the presence of C1q-binding anti-HLA DSA on the sera collected at the time of transplantation and underwent kidney allograft biopsy at day 14 after transplantation and were assessed for clinical and histological characteristics and allograft gene expression. Additional allograft biopsies were based upon the following criteria: decrease in serum creatinine less than 10% per day in three consecutive days in the first week post-transplantation compared to the Day 0 immediate post-transplantation creatinine; increase in serum creatinine of ≥30% from nadir (nadir was defined as the lowest serum creatinine within the first week post-transplantation); oliguria; clinical suspicion of rejection.

Kidney transplant recipients from living donors with complement-activating anti-HLA DSA-related positive crossmatch came from the open-label, multicenter, randomized, controlled NCT01399593 study conducted to determine the safety and efficacy of eculizumab in the prevention of antibody-mediated rejection in living donor kidney transplant recipients requiring desensitization therapy. Patients were prospectively recruited in nine transplant centers that have accepted to participate to the validation cohort (Oslo University Hospital, Rikshospitalet, Oslo, Norway (N=2); Necker

Hospital, Paris, France (N=4); Saint-Louis Hospital, Paris, France (N=3); Hospital Clínic i Provincial de Barcelona, Barcelona, Spain (N=7); Johns Hopkins Medical Institute, Baltimore, MD, USA (N=9); Centre Hospitalier Régional Universitaire de Tours, Tours, France (N=1); Columbia University Medical Center, New York, NY, USA (N=9); Centre Hospitalier Universitaire Rangueil, Toulouse, France (N=2); Padua University Hospital, Padua, Italy (N=3)). Inclusion criteria were: male or female patients ≥18 years old, patients with stage IV or stage V chronic kidney disease who will receive a kidney transplant from a living donor to whom they are sensitized and require desensitization prior to transplantation, history of prior exposure to HLA (prior solid organ or tissue allograft, pregnancy, blood transfusion, prior exposure to specific donor‘s HLA), presence of anti-HLA DSA by the SAB assay (Luminex LabScreen assay), as described by the manufacturer‘s package insert, positive complement-dependent cytotoxicity (CDC) crossmatch (current or historic) and B-cell flow crossmatch (BFXM) and T-cell flow crossmatch (TFXM) <500 mean channel shift (mcs) or negative CDC crossmatch and BFXM or TFXM >285 and <500 mcs, able to understand the informed consent form and willing to comply with study procedures, female patients of child-bearing potential must have a negative pregnancy test (serum beta-hCG) and must be practicing an effective, reliable and medically approved contraceptive regimen while on eculizumab treatment and for up to 5 months following discontinuation of treatment. Exclusion criteria were: previous treatment with eculizumab at any time prior to enrolling in this study, ABO incompatibility with living donor, history of severe cardiac disease, prior splenectomy, known bleeding disorder, active bacterial or other infection which is clinically significant in the opinion of the investigator and is a contraindication to transplantation, participation in any other investigational drug study or exposure to an investigational drug or device within 30 days of screening, treatment with rituximab ≤3 months prior to screening, previous treatment with bortezomib ≤3 months prior to screening, previous treatment with alemtuzumab ≤6 months prior to screening, hypersensitivity to murine proteins or to one of the product excipients, history of illicit drug use or alcohol abuse within the previous year, unresolved meningococcal disease, pregnancy or lactation, current cancer or history of cancer within the 5 years prior to screening with the exception of patients who have successfully treated nonmetastatic basal or squamous cell, any medical condition that, in the opinion of the investigator, might interfere with the patient‘s participation in the study, poses an

added risk for the patient, or confounds the assessment of the patient, active infection with hepatitis B (HBV), hepatitis C (HCV) or human immunodeficiency virus (HIV). Patients were vaccinated against Neisseria meningitidis using tetravalent conjugated vaccines (if not already vaccinated within the time period of active coverage specified by the vaccine manufacturer). Randomization was performed on a 1:1 basis to either the eculizumab treatment arm or the SOC control arm (two-arm parallel study). The randomization was stratified by the pre-transplant desensitization protocol that was used according to the local transplant center protocol (plasma exchanges and intravenous immunoglobulin, plasma exchanges alone, intravenous immunoglobulin alone). Patients who were randomized in the eculizumab treatment arm received eculizumab in the first nine weeks post-transplantation (1200 mg one hour prior to transplantation, 900 mg per week for four weeks and 1200 mg every other week for weeks five, seven, and nine). Patients who were randomized to the SOC control arm received prophylactic therapy for antibody-mediated rejection after transplantation according to the local transplant center protocol including plasma exchanges and intravenous immunoglobulins. SOC treatments were used uniformly for all patients at a given center on a center-specific basis. All patients in both arms received induction therapy by thymoglobulin (1.5 mg/kg x4 doses) and maintenance immunosuppression consisting in tacrolimus administered to maintain through levels at 4 to 11 ng/mL, mycophenolate mofetil 1 g BID or enteric-coated mycophenolic acid 720 mg BID and prednisone initially per SOC at the transplant center and tapered to 5 mg daily by 3 months post-transplantation. Kidney allograft biopsies were performed at day 14 after transplantation to assess histological characteristics and allograft gene expression. Additional allograft biopsies were based upon the following criteria: decrease in serum creatinine less than 10% per day in three consecutive days in the first week post-transplantation compared to the Day 0 immediate post-transplantation creatinine; increase in serum creatinine of ≥30% from nadir (nadir was defined as the lowest serum creatinine within the first week post-transplantation); oliguria; clinical suspicion of rejection.