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Complement-Activating Anti-HLA Antibodies in Kidney Transplantation: Allograft Gene Expression Profiling and Response to Treatment

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ABSTRACT

Complement-activating anti-HLA donor-specific antibodies (DSAs) are associated with impaired kidney transplant outcome; however, whether these antibodies induce a specific rejection phenotype and influence response to therapy remains undetermined. We prospectively screened 931 kidney recipients for complement-activating DSAs and used histopathology, immunostaining, and allograft gene expression to assess rejection phenotypes. Effector cells were evaluated using *in vitro* human cell cultures. Additionally, we assessed the effect of complement inhibition on kidney allograft rejection phenotype and the clinical response to complement inhibition in 116 independent kidney recipients with DSAs at transplant receiving rejection prophylaxis with eculizumab or standard of care (plasma exchange and intravenous Ig) at ten international centers. The histomolecular rejection phenotype associated with complement-activating DSA was characterized by complement deposition and accumulation of natural killer cells and monocytes/macrophages in capillaries and increased expression of five biologically relevant genes (*CXCL11*, *CCL4*, *MS4A7*, *MS4A6A*, and *FCGR3A*) indicative of endothelial activation, IFNγ response, CD16-mediated natural killer cell activation, and monocyte/macrophage activation. Compared with standard of care, eculizumab specifically abrogated this histomolecular rejection phenotype and associated with a decreased 3-month rejection incidence rate in patients with complement-activating DSAs (56%; 95% confidence interval [95% CI], 38% to 74% versus 19%; 95% CI, 8% to 35%; P=0.001) but not in those with noncomplement-activating DSAs (9%; 95% CI, 2% to 25% versus 13%; 95% CI, 2% to 40%; P=0.65). In conclusion, circulating complement-activating anti-HLA DSAs are associated with a specific histomolecular kidney allograft rejection phenotype that can be abrogated by complement inhibition.

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Despite extraordinary advances in the field of transplant medicine, the long-term survival of kidney allografts has not improved in recent decades and remains insufficient.[1] Anti-HLA antibody-mediated rejection has been identified as the main reason for the failure of kidney transplants.[2,3] Various antibody-mediated rejection phenotypes have been recognized, allowing capture of the clinical scope of the disease, including acute, chronic, C4d-negative, subclinical, and vascular antibody–mediated rejection.[4,5] However, because the phenotypes are on the basis of clinical and histologic presentation of the disease and because the underlying biologic mechanisms are not integrated, the level of phenotyping of these antibody-mediated rejection subtypes is rather

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low.[4,5] Nonetheless, addressing the heterogeneity of antibody-mediated rejection by identifying phenotypes on the basis of pathophysiology is critical for improving the longevity of allografts. Indeed, the importance of precise disease phenotyping for personalized care and improving outcomes has been shown in many conditions, such as cancer, cardiovascular disease, obesity, diabetes mellitus, and infectious diseases.[6] Our current inability to accurately identify antibody-mediated rejection phenotypes within this heterogeneous and overlapping condition forces clinicians to use a less than optimal approach as a guide for therapeutic decisions.[7]

We recently found that the presence of complement-activating anti-HLA donor-specific antibodies (DSAs) after transplantation is a strong determinant of kidney allograft loss.[8] This finding has been validated in different cohorts of patients with kidney transplants in the United States and Europe[9–13] as well as in other solid transplant organs.[14–16] However, the specific effects of complement-activating anti-HLA DSAs on the pathogenesis of antibody-mediated rejection have not been identified among the various effects of anti-HLA antibodies.[17] An understanding of these effects has major therapeutic consequences and may provide insight into conflicting results regarding the use of complement inhibitor therapies[18–25] (despite their potential rationale) in the field of antibody-mediated rejection.

The aim of this prospective study was to identify the specific biologic effect of complement-activating anti-HLA DSAs in the kidney allograft and whether antibody complement-activating capacity influences the response to complement inhibition therapy. First, we addressed in a prospective cohort study the specific allograft rejection phenotype associated with circulating complement-activating anti-HLA DSAs in kidney transplant recipients by combining histopathology, immunochemistry, and gene expression evaluation in the allograft. Second, we evaluated in a multicenter, international study the effect of complement inhibition therapy with anti-C5 mAb on the complement-activating donor-specific anti-HLA antibody-mediated histomolecular kidney allograft rejection phenotype and the clinical response to complement inhibition according to the complement-activating capacity of circulating donor-specific anti-HLA antibodies.

RESULTS

Characteristics of Patients with Post-Transplant Circulating Complement-Activating Donor-Specific Anti-HLA Antibodies in the Prospective Cohort Study

Among the 931 patients undergoing renal transplantation (550 at Necker Hospital and 381 at Saint-Louis Hospital), we prospectively identified 157 (17%) patients with circulating anti-HLA DSAs detected in the first year after transplantation, 44 (28%) patients with complement-activating anti-HLA DSAs, and 113 (72%) patients with noncomplement-activating anti-HLA DSAs. Table 1 shows the characteristics of the donors and recipients at the time of transplantation as well as the characteristics of patients at the time of the detection of post-transplant anti-HLA DSAs. Complement-activating anti-HLA DSAs were preexisting to transplantation in 28 (64%) patients, and 16 (36%) patients developed *de novo* DSAs. Complement-activating anti-HLA DSAs had a mean fluorescence intensity (MFI) of 9483 (748), and all were composed of IgG1 and/or IgG3 subclasses, which were also associated with IgG2 and/or IgG4 in 20 (45%) patients. The characteristics of post-transplant anti-HLA DSAs according to their complement-activating capacity are detailed in Table 1.

Patients with complement-activating anti-HLA DSAs had a lower eGFR (31.0 [13.7] ml/min per 1.73 m^2) and a higher rate of proteinuria (1.1 [1.1] g/g) at the time of post-transplant anti-HLA DSA detection compared with patients with noncomplement-activating anti-HLA DSAs (eGFR of 44.2 [17.8] ml/min per 1.73 m^2 and proteinuria of 0.3 [0.5] g/g; $P<0.001$ for both comparisons). Patients with complement-activating anti-HLA DSAs experienced decreased allograft survival at 3 years post-transplantation compared with that of patients with noncomplement-activating anti-HLA DSAs (64%; 95% confidence interval [95% CI], 48 to 77 versus 95%; 95% CI, 88 to 98, respectively; $P<0.001$).

Histopathology and Immunohistochemical Analyses in the Prospective Cohort Study

Patients with complement-activating anti-HLA DSAs had (*1*) increased microvascular inflammation (glomerulitis score of 1.68±0.14 versus 1.09±0.10 and peritubular capillaritis score of 1.77±0.14 versus 1.05±0.09, respectively; $P=0.002$ and $P<0.001$, respectively); (*2*) a higher rate of peritubular capillary C4d deposition (64% versus 18%, respectively; $P<0.001$); (*3*) more endarteritis lesions (0.45±0.12 versus 0.11±0.04; $P=0.001$); and (*4*) higher scores of transplant glomerulopathy (0.73±0.16 versus 0.34±0.07; $P=0.01$) compared with patients with noncomplement-activating anti-HLA DSAs (Supplemental Figure 1).

Immunostaining revealed extensive CD68+ monocyte/macrophage infiltration in peritubular and glomerular capillaries in patients with complement-activating anti-HLA DSAs (5.8 [2.7] monocytes/macrophages per peritubular capillary and 2.2 [1.5] monocytes/macrophages per glomeruli) compared

Significance Statement

Complement-activating anti-HLA donor-specific antibodies (DSAs) are associated with an increased risk of kidney allograft loss, but their specific effects on kidney allograft injury are unknown. This study uses gene expression analysis as well as histopathology and immunostaining to characterize circulating complement-activating anti-HLA DSA-mediated rejection in kidney allografts and in *in vitro* human cell cultures. The specific phenotype defined, when applied in a stratified analysis, predicted the response of antirejection treatment with eculizumab, the anti-C5 mAb; benefit was restricted to patients with pretransplant complement-activating anti-HLA DSAs. Complement-activating anti-HLA DSAs may help to define the population of kidney recipients for whom complement-targeting intervention will provide the greatest benefit.

Table 1. Characteristics of patients with post-transplant donor-specific anti-HLA antibodies according to complement-activating capacity in the prospective cohort study

Characteristics	All Patients, *n*=931	C1q-Negative Anti-HLA DSAs, *n*=113	C1q-Positive Anti-HLA DSAs, *n*=44	*P* Value
Recipient baseline characteristics				
Age, yr, mean (SD)	47.3 (13.5)	45.8 (12.9)	50.2 (15.3)	0.07
Men, no. (%)	503 (54)	61 (54)	24 (55)	0.95
Retransplantation, no. (%)	177 (19)	37 (33)	14 (32)	0.91
Time since dialysis, yr, mean (SD)	5.6 (5.9)	7.2 (6.7)	7.2 (6.7)	0.94
Blood type, no. (%)				
A	410 (44)	46 (41)	17 (39)	0.74
B	102 (11)	13 (11)	5 (11)	
AB	47 (5)	7 (6)	1 (2)	
O	372 (40)	47 (42)	21 (48)	
CKD, no. (%)				
Glomerulopathy	260 (28)	29 (26)	13 (30)	0.43
Vascular nephropathy	74 (8)	8 (7)	5 (11)	
Chronic interstitial nephropathy	112 (12)	16 (14)	5 (11)	
Malformative uropathy	37 (4)	7 (6)	0	
Diabetes	94 (10)	8 (7)	3 (7)	
Other/not determined	354 (38)	45 (40)	18 (41)	
Donor characteristics				
Age, yr, mean (SD)	51.2 (14.3)	50.8 (13.7)	51.4 (16.0)	0.81
Men, no. (%)	512 (55)	66 (58)	27 (61)	0.74
Deceased, no. (%)	746 (80)	102 (90)	41 (93)	0.57
Transplant characteristics				
Cold ischemia time, h, mean (SD)	18.1 (9.0)	18.2 (8.9)	21.0 (8.8)	0.08
HLA mismatch, mean (SD)				
A	0.8 (0.7)	1.0 (0.7)	0.8 (0.8)	0.26
B	1.0 (0.7)	1.2 (0.7)	1.3 (0.7)	0.52
DR	0.9 (0.6)	1.0 (0.7)	1.0 (0.6)	0.67
Clinical characteristics at the time of post-transplant DSA detection				
No. of patients with post-transplant DSAs (%)	157 (17)	113 (100)	44 (100)	
Time to detection, d, median (IQR)	237 (84–365)	302 (90–365)	97 (17–293)	0.001
eGFR, ml/min per 1.73 m^2, mean (SD)	40.5 (17.7)	44.2 (17.8)	31.0 (13.7)	<0.001
Proteinuria, g/g, mean (SD)	0.6 (0.8)	0.3 (0.5)	1.1 (1.1)	<0.001
Characteristics of all post-transplant DSAs				
No. of HLA specificities, mean (SD)	2.0 (1.2)	1.8 (1.0)	2.6 (1.4)	<0.001
HLA class, no. (%)				
I	39 (25)	31 (28)	8 (18)	<0.001
II	71 (45)	59 (52)	12 (27)	
I and II	47 (30)	23 (20)	24 (55)	
Characteristics of post-transplant dominant DSAs				
HLA class, no. (%)				
I	60 (38)	44 (39)	16 (36)	0.77
II	97 (62)	69 (61)	28 (64)	
Preformed DSA	80 (51)	52 (46)	28 (64)	0.05
MFI, mean (SEM)	4801 (371)	2979 (278)	9483 (748)	<0.001
IgG subclasses, no. (%)				
IgG1	112 (71)	70 (62)	42 (95)	<0.001
IgG2	61 (39)	43 (38)	18 (41)	0.74
IgG3	44 (28)	19 (17)	25 (57)	<0.001
IgG4	33 (21)	22 (19)	11 (25)	0.45

IQR, interquartile range.

with patients with noncomplement-activating anti-HLA DSAs (2.4 [1.9] monocytes/macrophages per peritubular capillary and 0.9 [0.7] monocytes/macrophages per glomeruli; $P<0.001$ for both comparisons). Immunostaining for NKp46 revealed a greater presence of natural killer (NK) cells in the capillaries (glomeruli and peritubular capillaries) of patients with

complement-activating anti-HLA DSAs (3.9 [1.5] NK cells per ten consecutive high-power fields) compared with patients with noncomplement-activating anti-HLA DSAs (0.4 [0.2] NK cells per ten consecutive high-power fields; $P<0.001$) (Figure 1).

Gene Expression Analyses in the Prospective Cohort Study

Identification of Complement-Activating Anti-HLA DSA-Selective Allograft Gene Expression

We compared the global gene expression changes in biopsies from patients with complement-activating anti-HLA DSAs versus noncomplement-activating anti-HLA DSAs. Among the 9954 interquartile range–filtered probe sets, the transcripts that were most significantly increased in patients with complement-activating anti-HLA DSAs were the following (Figure 2A): NK-selective transcripts (FCGR3A, FCGR3B, and PTPRC) and transcripts reflective of CD16 engagement (CCL4 and CD72), endothelial genes (CXCL11), IFNγ (IFNG)-inducible genes (IFNG-inducible chemokines CXCL11, CXCL10, CXCL13, and GPB5), and macrophage genes (C1QA, C1QB, C1QC, FCGR1A, C3AR1, LILRB2, MS4A6A, and MS4A7). The top 50 annotated genes are shown in Supplemental Table 1.

Complement-Activating Anti-HLA DSA-Selective Transcripts in Human Cultured Cells: CD16-Activated NK Cells, Macrophages, and Endothelial Activation Involvement

The top nonredundant complement-activating anti-HLA DSA-selective transcripts expressed in the kidney allograft tissue (Figure 2A, Supplemental Table 1) were studied in a panel of primary human cells, including effector CD8+ and CD4+ T cells, resting and CD16-stimulated NK cells, B cells, monocytes, and macrophages, and unstimulated and IFNG-treated endothelial cells (human umbilical vein endothelial cells) with and without IFNG treatment (Supplemental Figure 2). On the basis of their highest relative expression in cell cultures (probe set signal *z* score), we determined that the top nonredundant complement-activating anti-HLA DSA-selective transcripts were mostly expressed by (*1*) NK cells (FCGR3A/3B) and activated CD16-stimulated NK cells (CCL4, CD72, CRTAM, FCGR3A/3B, and KLRC1/C2); (*2*) monocytes (CD86, CYBB, EMR2, LST1, MS4A6A, and MS4A7), unstimulated macrophages (CD163, CD84, and MS4A4A), and IFNG-treated macrophages (AIM2, C1QA, C1QB, C1QC, FCGR1A/1B/1C, and GPB5); and (*3*) transcripts reflecting IFNG effects in the endothelium, including CXCL11 and FYB.

Relationship of Complement-Activating Anti-HLA DSA-Selective Transcripts and Their Biologic Function: NK Cell-CD16A Signaling, Endothelial Injury, and IFNG Effects

Using Ingenuity Pathway Analysis (IPA) analysis, we performed associative testing to identify previously described cellular and pathway gene signatures that were overrepresented in patients with complement-activating anti-HLA DSAs (Supplemental Table 2).

1. NK cell signaling (adjusted $P<0.001$), Fcγ receptor-mediated phagocytosis (adjusted $P<0.001$), and Fcε RI signaling (adjusted $P<0.01$), presumably reflecting shared signal pathway usage with CD16a recognition of endothelial membrane–bound DSAs.

2. Complement system (adjusted $P<0.001$), reflecting complement activation on endothelial cells bound to DSAs and likely reflecting the induction of the complement component by IFNG on macrophages.

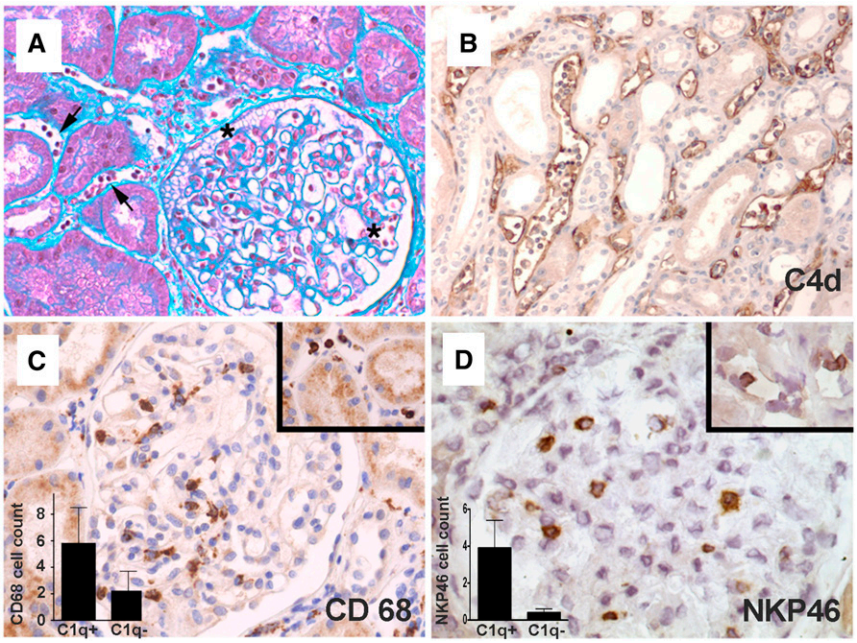

Figure 1. Histology and immunohistopathology analyses showed increased microvascular inflammation, extensive monocyte/macrophage and NK cells infiltration in allograft capillaries in patients with circulating complement-activating donor-specific anti-HLA antibodies in the prospective cohort study. Data are on the basis of 157 kidney allograft biopsies performed within the first year after transplantation that were assessed for immunohistochemistry. (A) Microcirculation inflammation characterized by glomerulitis (asterisks) and peritubular capillaritis (arrows; Masson trichrome stain ×20). (B) C4d deposition in peritubular capillaries (immunoperoxidase ×20). (C) Monocyte/macrophage cells (CD68+) in glomerulitis and peritubular capillaritis (inset; immunoperoxidase method ×40; count per peritubular capillary according to complement-activating antibody status: $P<0.001$). (D) NK cells (NKP46+) in glomerulitis and peritubular capillaritis (inset; immunoperoxidase method ×60; count per ten consecutive high-power fields according to complement-activating antibody status: $P<0.001$).

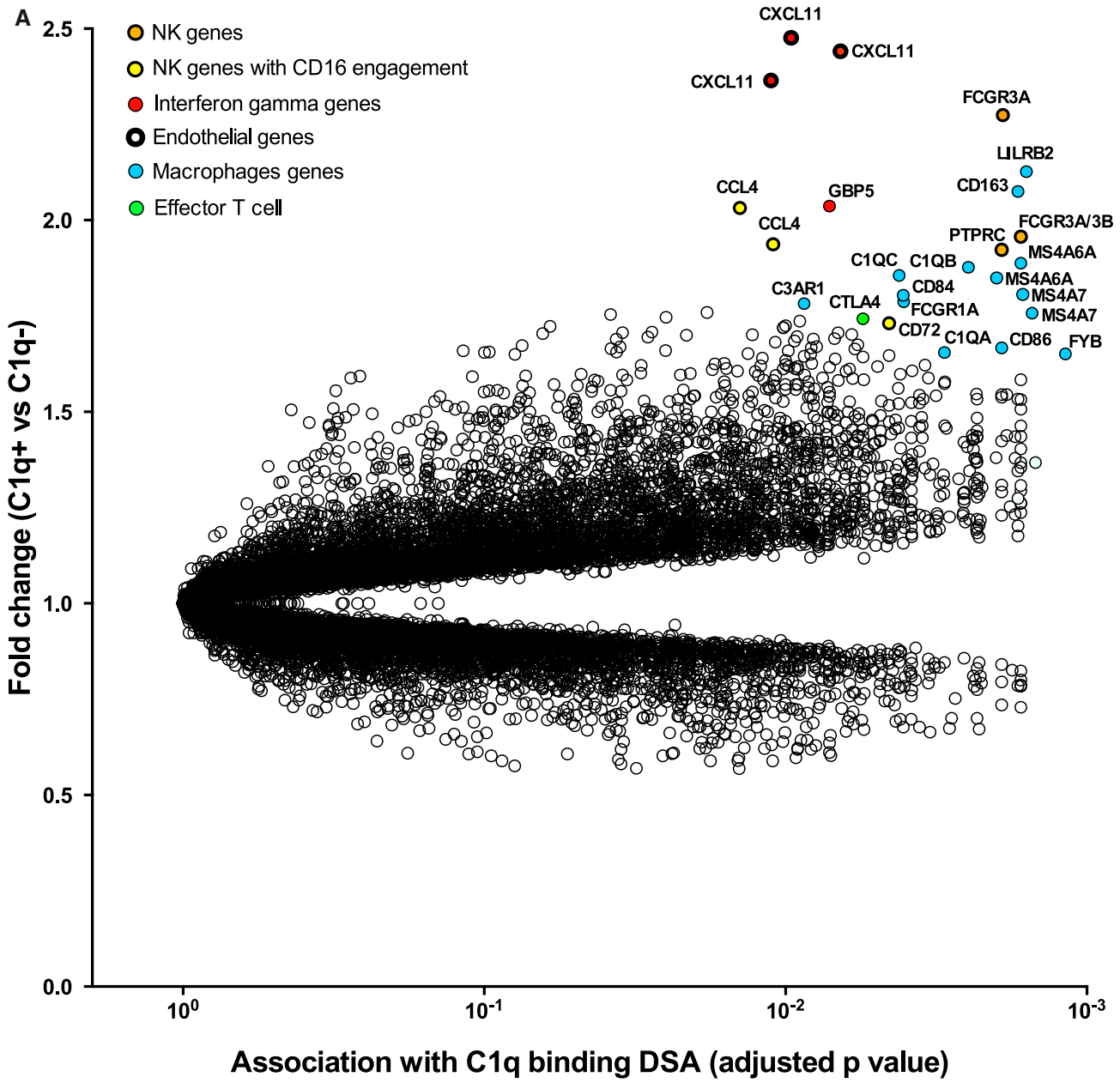

Figure 2. Complement-activating donor-specific anti-HLA antibody molecular landscape in the prospective cohort study, with a hierarchical ranking of probe sets on the basis of the discrimination of complement-activating capacity of donor-specific anti-HLA antibodies demonstrating that complement-activating anti-HLA DSAs are associated with highly selective changes in allograft gene expression. (A) Expression of complement-activating donor-specific anti-HLA antibody transcripts in kidney allografts. Dots represent individual transcripts. The transcripts most associated with complement-activating anti-HLA DSAs are composed primarily of NK-selective transcripts (yellow dots: NK genes with CD16 engagement [CCL4 and CD72] and orange dots: NK genes [FCGR3A, FCGR3B, and PTPRC]); endothelial genes (bold black dots: CXCL11); IFNG genes (red dots: IFNG-inducible genes [CXCL11 and GPB5]); macrophage genes (blue dots: C1QA, C1QB, C1QC, FCGR1A, C3AR1, LILRB2, MS4A6A, MS4A7, FYB, CD86, CD84, and FCGR1A); and effector T cells (green dots: CTLA4). The *x* axis illustrates the false discovery rate–adjusted *P* value for the association of each transcript with the complement-activating capacity of donor-specific anti-HLA antibodies, with the fold change on the *y* axis for complement-activating donor-specific anti-HLA antibodies versus non-complement-activating donor-specific anti-HLA antibodies. (B) Relative importance of complement-activating donor-specific anti-HLA antibody–selective transcripts in determining the complement-activating donor-specific anti-HLA antibody status. Relative importance is shown for the 19 most important annotated genes among the top nonredundant complement-activating donor-specific anti-HLA antibody–selective probe sets. Relative importance was calculated using the random forest method by randomizing the variable values and measuring the resulting decline in model accuracy. The gene set associated with complement-activating donor-specific anti-HLA antibodies included *CXCL11*, *CCL4*, *MS4A7*, *MS4A6A*, and *FCGR3A*, which were more important than histology parameters

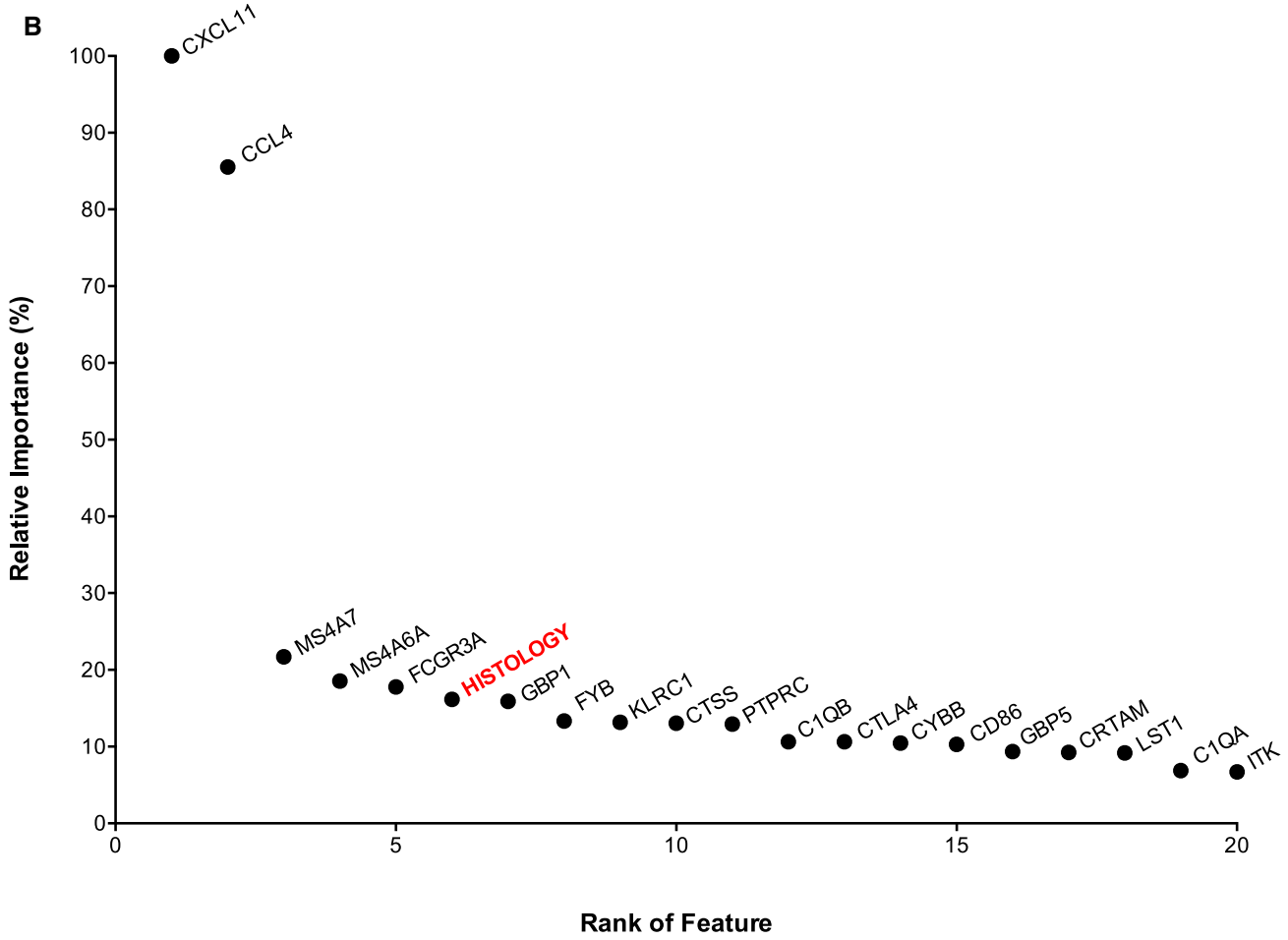

Figure 2. Continued.

3. Antigen presentation (adjusted $P<0.01$) and IFN signaling (adjusted $P=0.03$), reflecting IFNG effects.

4. CD28 signaling (adjusted $P<0.001$), T cell receptor signaling (adjusted $P<0.01$), iCOS-iCOSL signaling (adjusted $P<0.001$), and CTLA4 signaling in T cells (adjusted $P=0.01$), representing T cell receptor triggering and associated costimulation/coinhibition pathways, likely a reflection of concurrent T cell–mediated rejection in some biopsies.

5. Caveolar-mediated endocytosis signaling (adjusted $P=0.03$), endothelin-1 signaling (adjusted $P=0.04$), and iNOS signaling (adjusted $P=0.05$), reflecting the response to wounding in endothelial cells.

Complement-Activating Anti-HLA DSA Discriminative Gene Set

To determine the most specific gene expression profile of complement-activating anti-HLA DSAs, we assessed the

(glomerulitis, peritubular capillaritis, endarteritis, interstitial inflammation, tubulitis, and C4d complement fraction deposition in peritubular capillaries) in identifying complement-activating donor-specific anti-HLA antibody status. (C) Receiver operating characteristic curves for predicting complement-activating donor-specific anti-HLA antibody status. Receiver operating characteristic curves were plotted for a logistic regression model that included histologic variables (glomerulitis, peritubular capillaritis, endarteritis, interstitial inflammation, tubulitis, and C4d complement fraction deposition in peritubular capillaries; black) and a logistic regression model that included the expression level (log2 OD) of the five-gene set associated with complement-activating donor-specific anti-HLA antibodies (red). The five-gene set model showed a greater performance in discriminating complement-activating donor-specific anti-HLA antibody status than the histologic model (area under the curve [AUC] of 0.87; 95% CI, 0.80 to 0.93; misclassification rate of 20% and AUC of 0.76; 95% CI, 0.68 to 0.85; misclassification rate of 25%, respectively; $P=0.02$). Internal validation using 1000 bootstrap resamplings showed optimism-corrected values of AUC of 0.72 for the histology parameters and 0.84 for the five-gene set.

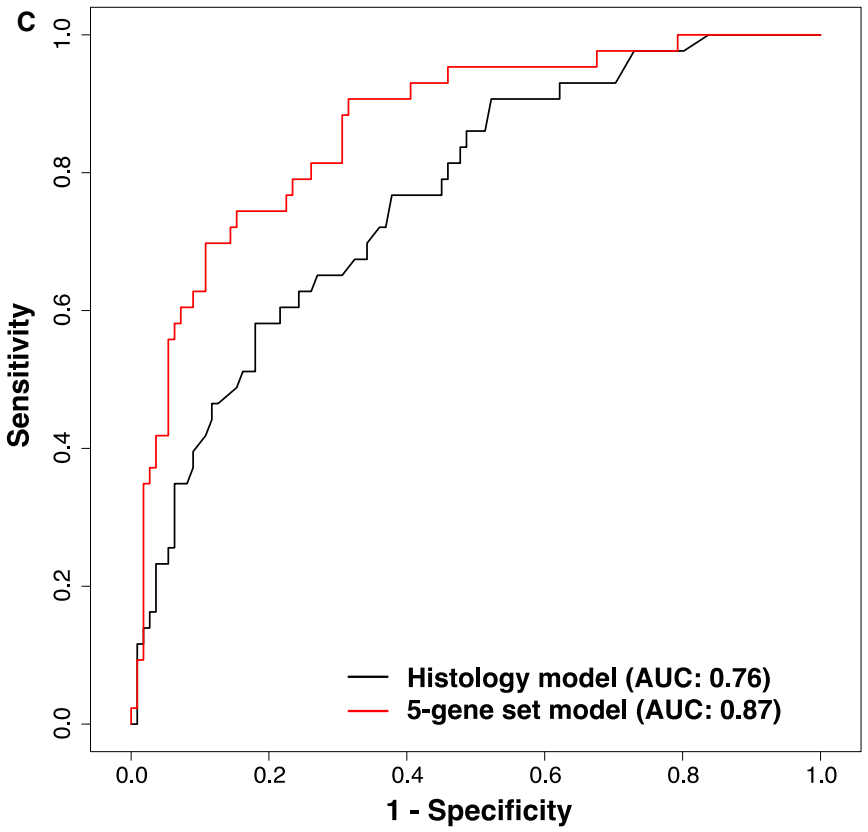

Figure 2. Continued.

relative importance of the top 50 nonredundant genes according to their discriminative performance for complement-activating anti-HLA DSA status using a random forest analysis. We found that, compared with histology (glomerulitis, peritubular capillaritis, endarteritis, interstitial inflammation, tubulitis, and C4d deposition in peritubular capillaries, representing the active lesions defining kidney allograft rejection in the Banff classification[5]), the following set of five individual genes was better able to determine the complement-activating anti-HLA DSA status: *CXCL11*, *CCL4*, *MS4A6A*, *MS4A7*, and *FCGR3A* (Figure 2B). The five-gene set showed a greater performance in discriminating complement-activating antibody status than histology parameters: areas under the curve of 0.87 (95% CI, 0.80 to 0.93) and 0.76 (95% CI, 0.68 to 0.85; P=0.02), respectively (Figure 2C). Internal validation using 1000 bootstrap resamplings showed optimism-corrected values of areas under the curve of 0.72 for the histology parameters and 0.84 for the five-gene set.

Identification of Distinct Allograft Rejection Phenotypes According to Histology and Gene Expression

Principal component analysis integrating histologic parameters of acute injury and the five-gene set associated with complement-activating anti-HLA DSAs identified a distinct allograft rejection pattern in patients with complement-activating anti-HLA DSAs compared with patients with noncomplement-activating anti-HLA DSAs and patients without anti-HLA DSAs (Figure 3). The contribution of the five-gene set and the histologic parameters to the principal component were 71% and 29%, respectively, and they were 31% and 69%, respectively, to the second component. Unsupervised hierarchical clustering showed that histologic parameters of acute injury could identify patients with anti-HLA antibody-mediated rejection. Among this population, the five-gene set distinguished two subtypes of allograft rejection according to the complement-activating capacity of anti-HLA DSAs.

The associations between donor-specific anti-HLA antibody complement-activating status and each component of the histomolecular rejection phenotype associated with complement-activating anti-HLA DSAs were independent of donor-specific anti-HLA antibody MFI level (Supplemental Tables 3 and 4), time to post-transplant anti-HLA DSA detection (Supplemental Tables 5 and 6), and the presence of C4d deposition in peritubular capillaries (Supplemental Tables 7 and 8). The complement-activating anti-HLA DSA histomolecular rejection phenotype was similar between patients with preformed complement-activating anti-HLA DSAs and those with *de novo* complement-activating anti-HLA DSAs (Supplemental Table 9).

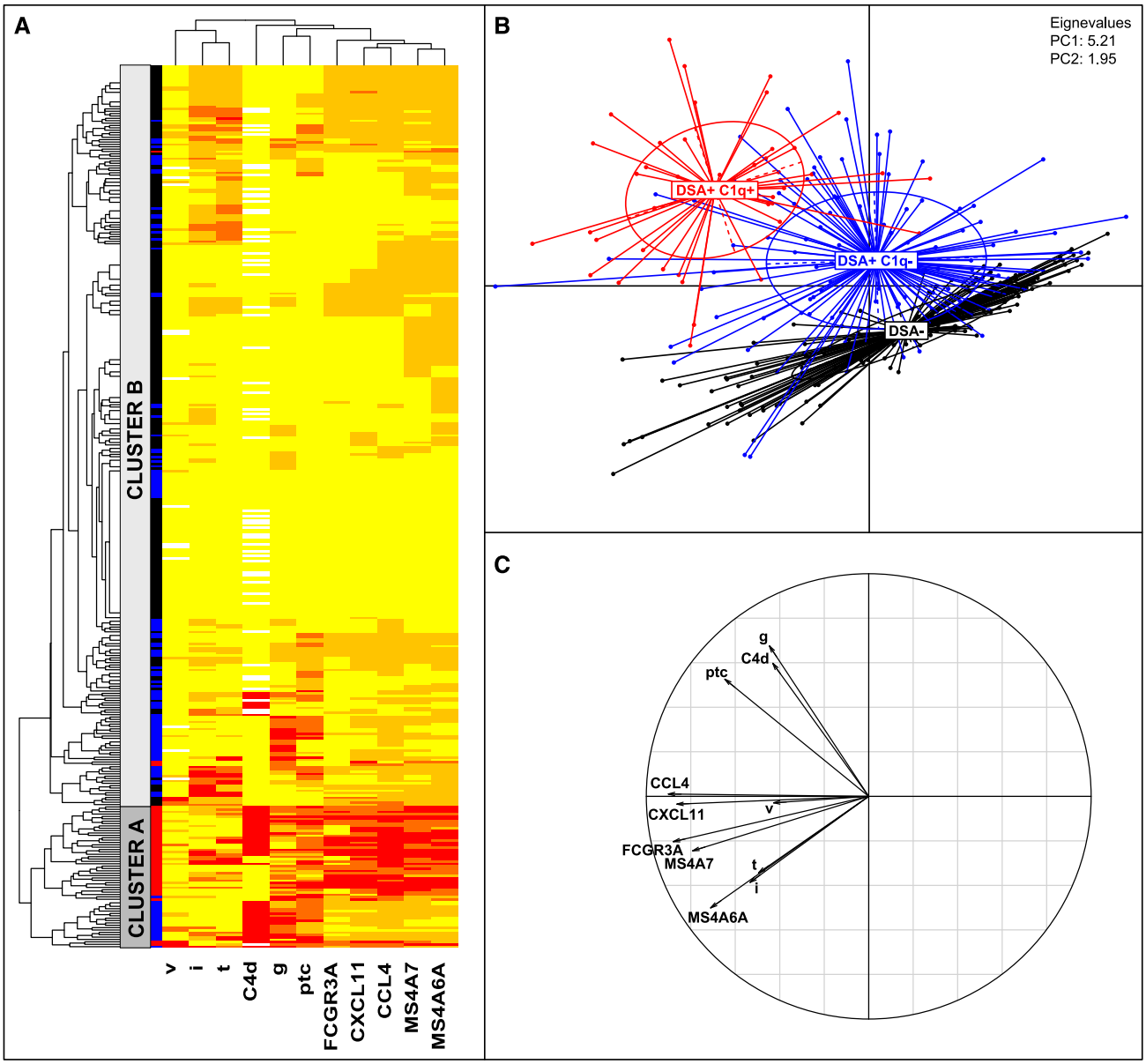

Figure 3. Complement-activating anti-HLA DSAs are associated with a specific histomolecular phenotype of allograft rejection. Segregation of allograft rejection phenotypes according to histology and gene expression levels. Variables considered in these analyses were histologic (glomerulitis, peritubular capillaritis, endarteritis, interstitial inflammation, tubulitis, and C4d complement fraction deposition in peritubular capillaries) and molecular (*i.e.*, intragraft expression of the five genes associated with complement-activating donor-specific anti-HLA antibodies [*CXCL11*, *CCL4*, *MS4A7*, *MS4A6A*, and *FCGR3A*]). Data are on the basis of 392 kidney allograft biopsies performed in the first year after transplantation. (A) Unsupervised hierarchical clustering. Each variable in an individual patient is colored according to the threshold for each parameter (zero to three, with higher score including more severe injury or transcript expression level). Cluster A was enriched with patients with complement-activating donor-specific anti-HLA antibodies, and cluster B was enriched with patients with noncomplement-activating donor-specific anti-HLA antibodies and patients without donor-specific anti-HLA antibodies. Immediately beside the cluster bars, the tricolor bar indicates the patients with complement-activating donor-specific anti-HLA antibodies (red), those with noncomplement-activating donor-specific anti-HLA antibodies (blue), and those without donor-specific anti-HLA antibodies (black). (B) Principal component analysis: projection of individuals segregated into three distinct histomolecular patterns on the basis of histologic variables and the five genes associated with complement-activating donor-specific anti-HLA antibodies. (C) Principal component analysis: correlation circle showing the contribution of each histologic and molecular parameter for segregating the three patterns.

Terminal Complement Pharmacologic Blockade Abrogates the Complement-Activating Anti-HLA DSA Histomolecular Allograft Rejection Phenotype

In the terminal complement blockade study (n=116), we evaluated the effects of complement pharmacologic blockade by eculizumab (Soliris; Alexion Pharmaceuticals, Cheshire, CT; n=52) compared with noncomplement-directed standard of care (SOC; plasma exchange and intravenous Ig; n=64) for rejection prophylaxis in kidney transplant recipients with anti-HLA DSAs at the time of transplantation. We compared between patients with pretransplant complement-activating anti-HLA DSAs and those with noncomplement-activating anti-HLA DSAs (Supplemental Figure 3) (*1*) the histomolecular allograft phenotype on day 14 biopsies according to rejection prophylaxis and (*2*) the clinical response to rejection prophylaxis defined by the 3-month incidence of biopsy-proven antibody-mediated rejection. The baseline characteristics of the patients were similar between the two treatment groups (Supplemental Tables 10 and 11).

Effect of Eculizumab on Allograft Histomolecular Phenotype

In patients with complement-activating anti-HLA DSAs (n=69), compared with patients receiving SOC, eculizumab treatment was associated with abrogation of the complement-activating antibody-mediated histomolecular allograft rejection phenotype at day 14, with decreased glomerulitis (0.9 [0.9] versus 1.7 [0.9]; P=0.001), peritubular capillaritis (0.7 [0.9] versus 1.6 [0.8]; P<0.001), interstitial inflammation (0.1 [0.3] versus 0.9 [1.0]; P<0.001), and tubulitis (0.1 [0.3] versus 0.9 [1.0]; P<0.001) as well as a significant decrease in CXCL11 (−4.0-fold change; P<0.001), CCL4 (−2.9-fold change; P<0.001), MS4A6A (−2.5-fold change; P<0.001), MS4A7 (−2.4-fold change; P<0.001), and FCGR3A (−2.9-fold change; P<0.001) (Table 2). In contrast, compared with patients receiving SOC, eculizumab treatment was not associated with histomolecular changes in patients with noncomplement-activating anti-HLA DSAs (n=47) (Table 2).

The histomolecular changes associated with eculizumab treatment were consistent within the two terminal complement blockade study subsets (Supplemental Table 12).

Clinical Response to Rejection Prophylaxis

Patients receiving eculizumab treatment (n=52) showed a decreased 3-month incidence of rejection (17%; 95% CI, 8 to 30) compared with that of patients receiving SOC (n=64; 33%; 95% CI, 22 to 46; P=0.06). Stratified analysis further indicated that the benefit of eculizumab treatment compared with SOC was observed in patients with complement-activating anti-HLA DSAs (19%; 95% CI, 8 to 35 versus 56%; 95% CI, 38 to 74, respectively; P=0.001) but not in those with noncomplement-activating anti-HLA DSAs (13%; 95% CI, 2 to 40 versus 9%; 95% CI, 2 to 25, respectively; P=0.65) (Figure 4). The histologic characteristics of patients with ABMR are provided in Supplemental Table 13.

DISCUSSION

This study defined the specific histomolecular phenotype of complement-activating anti-HLA antibody-mediated rejection

Table 2. Clinical and histologic 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 complement pharmacologic blockade study

Characteristics	Patients with C1q+ anti-HLA DSAs, n=69			Patients with C1q− Anti-HLA DSAs, n=47		
	SOC, n=32	Eculizumab, n=37	P Value	SOC, n=32	Eculizumab, n=15	P Value
Clinical parameters, mean (SD)						
eGFR, ml/min per 1.73 m^2	44.8 (15.7)	47.2 (18.1)	0.65	46.2 (15.6)	48.1 (13.8)	0.63
Proteinuria, g/g	0.6 (0.6)	0.3 (0.3)	0.02	0.3 (0.2)	0.3 (0.2)	0.61
Histology (Banff scores), median (IQR)						
g score	2 (1–2)	1 (0–1)	0.001	1 (0–2)	1 (0–2)	0.82
ptc score	2 (1–2)	0 (0–1)	<0.001	1 (0–1)	0 (0–1)	0.85
v score	0 (0–0)	0 (0–0)	0.30	0 (0–0)	0 (0–0)	0.42
i score	1 (0–1)	0 (0–0)	<0.001	0 (0–0)	0 (0–1)	0.88
t score	1 (0–2)	0 (0–0)	<0.001	0 (0–1)	0 (0–1)	0.73
cg score	0 (0–0)	0 (0–0)	0.12	0 (0–0)	0 (0–0)	0.51
C4d score	2 (1–2)	3 (0–3)	0.23	0 (0–1)	0 (0–2)	0.64
Gene expression level (log2 OD), mean (SD)						
CXCL11	8.9 (1.8)	4.9 (2.3)	<0.001	4.3 (1.5)	4.1 (1.0)	0.99
CCL4	9.7 (1.8)	6.8 (2.2)	<0.001	6.5 (1.6)	6.1 (1.5)	0.52
MS4A6A	9.3 (2.1)	6.8 (2.6)	<0.001	7.0 (2.4)	6.7 (2.5)	0.78
MS4A7	8.1 (2.1)	5.7 (2.6)	<0.001	5.2 (2.6)	5.4 (2.5)	0.79
FCGR3A	9.2 (1.8)	6.3 (2.2)	<0.001	6.0 (1.8)	5.7 (1.8)	0.66

g, glomerulitis; IQR, interquartile range; ptc, peritubular capillaritis; v, endarteritis; i, interstitial inflammation; t, tubulitis; cg, chronic allograft glomerulopathy; C4d, C4d complement fraction deposition in peritubular capillaries.

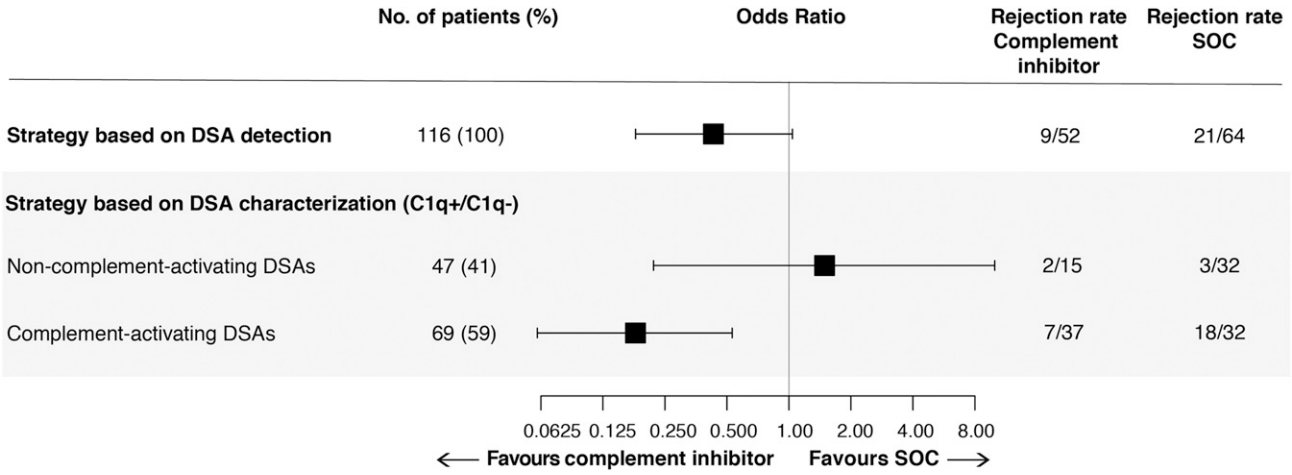

Figure 4. Complement inhibition associated with a decreased 3-month biopsy-proven ABMR incidence in patients with complement-activating anti-HLA DSAs but not in those with noncomplement-activating anti-HLA DSAs. Response to rejection prophylaxis with the complement inhibitor versus SOC in patients with donor-specific anti-HLA antibodies according to the current strategy on the basis of donor-specific anti-HLA antibody detection and a strategy on the basis of the characterization of donor-specific anti-HLA antibody complement-activating capacity. Response to treatment is on the basis of the incidence of biopsy-proven antibody-mediated rejection within the first 3 months after transplantation in 116 kidney recipients who were transplanted with donor-specific anti-HLA antibodies and received rejection prophylaxis with the complement inhibitor eculizumab (n=52) or SOC therapy (plasma exchange and high-dose intravenous Ig; n=64). Rejection rate represents the number of patients with biopsy-proven ABMR within the first 3 months after transplantation among all patients receiving complement inhibitor or SOC.

and showed the potential of complement inhibition for the prophylaxis of ABMR in kidney transplant recipients with complement-activating anti-HLA DSAs. First, we identified in a prospective cohort study a distinct histomolecular subtype of rejection associated with complement-activating anti-HLA antibodies within the landscape of kidney allograft rejection. This was characterized by endothelial activation with microcirculation inflammation by monocytes/macrophages and NK cells, complement deposition in capillaries, and selective changes in allograft gene expression, including overexpression of CXCL11, CCL4, MS4A7, MS4A6A, and FCGR3A. Second, in a complement pharmacologic blockade study, we showed that the anti-C5 mAb eculizumab specifically abrogated this allograft rejection phenotype in patients with complement-activating anti-HLA DSAs. Compared with the current SOC, including plasma exchange and intravenous Ig, terminal complement inhibition was associated with a significant decrease of the 3-month incidence of ABMR in patients with complement-activating anti-HLA DSAs but was not associated in those with noncomplement-activating anti-HLA DSAs.

Anti-HLA DSAs have a strong and frequently considered universally deleterious effect on solid organ allografts.[26] Thus, understanding the pathophysiology of anti-HLA DSA-mediated injury is critical for improving the longevity of existing allografts and developing new drugs to address relevant pathways.[7] Significant progress has been made over the last few years in our ability to diagnose patients with antibody-mediated rejection[7] and predict patients at risk for antibody-mediated rejection and allograft loss.[17] One of these major advances is represented by the recent recognition of complement-activating anti-HLA antibodies as strong determinants of allograft loss in kidney and other solid organ transplants.[8,9,12,14–16,27–29] Despite demonstrations of the strength and reproducibility of the association between complement-activating anti-HLA DSAs and solid organ transplant outcome, the biologic role of these antibodies in allograft rejection is unknown.

Our study provides converging evidence showing that circulating complement-activating anti-HLA DSAs are associated with a specific histomolecular phenotype of allograft rejection. The complement-activating anti-HLA DSA histomolecular allograft rejection phenotype relies on the combination of acute histologic features of allograft rejection and gene expression levels of CXCL11, CCL4, MS4A7, MS4A6A, and FCGR3A. Our study supports the specificity of this integrated phenotype of allograft injury rather than the individual value of each histologic or molecular feature, because this is currently the case for the Banff classification, which combines nonspecific elementary histologic lesions for defining diagnostic categories. First, we showed that patients with complement-activating anti-HLA DSAs had a distinct histomolecular allograft rejection pattern compared with those with noncomplement-activating anti-HLA DSAs and those without anti-HLA DSAs, which was confirmed by unsupervised clustering. Second, we showed the biologic relevance of the selective molecular changes associated with complement-activating anti-HLA DSAs using human rejection effector cell cultures. Third, we provided experimental evidence in humans with pharmacologic complement inhibition showing a specific abrogation of the complement-activating antibody-mediated histomolecular allograft rejection phenotype in patients with complement-activating anti-HLA DSAs. Fourth, we confirmed the consistency of the effect of pharmacologic

complement inhibition on the complement-activating anti-HLA DSA histomolecular allograft rejection phenotype in the two subsets of the therapeutic study.

By using a non-*a priori* approach, we identified a set of five genes (*CXCL11*, *CCL4*, *MS4A7*, *MS4A6A*, and *FCGR3A*) that was strongly associated with complement-activating circulating anti-HLA DSAs and outperformed the histologic features of acute allograft rejection. The expression levels of these genes improved the information provided by allograft histology to distinguish subtypes of antibody-mediated rejection according to the capacity of anti-HLA DSAs to activate complement. The biologic relevance of this gene set associated with complement activation, reflecting IFNG effects, endothelial activation, and NK cell and monocyte/macrophage burden, was supported by immunohistochemical analysis of biopsies performed in patients with complement-activating anti-HLA DSAs, which showed microcirculation inflammation with extensive monocyte/macrophage and NK cell infiltration in allograft capillaries and complement deposition in capillaries. The biologic relevance of this gene set was also reinforced by gene expression analysis in a primary human cell panel composed of primary human cell types that are likely to be affected and/or involved in the rejection process, which showed overexpression of CXCL11 by IFNG-stimulated endothelial cells, CCL4 and FCGR3A by CD16-stimulated NK cells, and MS4A6A and MS4A7 by monocytes.

Our study highlights the roles of complement activation as well as NK cells and monocytes/macrophages as major pathways triggered by complement-activating anti-HLA DSAs in kidney allografts. To date, there is only circumstantial evidence for enhanced "innate" monocyte infiltration into transplanted organs between genetically nonidentical hosts and donors, and the evidence of NK cells has been underestimated for various technical reasons. It has not been shown that "innate" cells initiate rejection. A recent study[30] showed that transplanted hearts (CB6F1-OVA into B6 recipients) might have contained NK cells that may have reacted against the host and initiated danger signals that led to T cell rejection. The deletion of the adaptor molecule MyD88, which is required for signaling by most Toll-like receptors, prevents the rejection of single minor antigen–mismatched grafts[31]; however, later studies failed to show a significant decrease in allograft rejection if the donor and recipient differed by major or multiple minor histocompatibility antigens.[32] Our data in kidney recipients and cell cultures converged to highlight the major role of NK cells and monocytes/macrophages in the occurrence of a specific antibody-mediated injury in kidney allografts triggered by complement-activating anti-HLA DSAs. This reflects engagement of NK cell CD16 Fc receptors (FCGR3A) with HLA antibodies bound to the microcirculation, suggesting a mechanism related to antibody-dependent cell-mediated cytotoxicity. Evidence for the CD16-related signaling pathway (reviewed in the work by Nimmerjahn and Ravetch[33]) included increased expression of FCGR3A, FCGR3B, and PTPRC, which were highly associated with complement-activating anti-HLA antibodies. Monocytes/macrophages also share features with NK cells, including CD16 expression.[34] Engagement of HLA-bound antibody with CD16 on NK cells triggers IFNG protein production,[35] which induces CXCL10, CXCL11, and CXCL13 in the endothelium, as revealed by the presence of IFNG-inducible transcripts in the allograft biopsies of patients with complement-activating anti-HLA DSAs. Blocking the complement activation pathway ameliorates antibody-dependent cell-mediated cytotoxicity damage to the donor microcirculation and induces a dramatic decrease in NK, IFNG production, and macrophage transcripts (CXCL11, CCL4, MS4A7, MS4A6A, and FCGR3A), supporting a role for NK cell triggering and cytotoxicity.

Our study provides an important step toward pathogenesis-based therapies in kidney transplant recipients by showing that the response to targeted complement inhibition may be dependent on the complement-activating capacity of circulating anti-HLA DSAs. Compared with the current approach to treatment of patients with anti-HLA DSAs, which only considers the presence of circulating anti-HLA DSAs, we showed that a stratified approach on the basis of the complement-activating capacity of anti-HLA DSAs might significantly improve the response rate to complement inhibition. The validity of this approach has also recently been suggested in a clinical trial,[36] showing that the effect of eculizumab on allograft function depends on the complement-activating capacity of anti-HLA DSAs in kidney recipients with chronic antibody-mediated rejection. The lack of knowledge regarding the complement-activating capacity of anti-HLA DSAs in the previous studies investigating complement inhibition for the prevention or treatment of antibody-mediated rejection may have biased their interpretation and might explain their conflicting results.[18–25]

One significant limitation of the therapeutic part of our study is that it was on the basis of *post hoc* analyses of clinical trials that were not primarily designed to assess the molecular response to complement inhibition compared with SOC. These trials (NCT01567085 and NCT01399593) only included kidney transplant recipients with preformed anti-HLA DSAs receiving eculizumab for rejection prophylaxis. However, including patients enrolled in the only two available clinical trials investigating the effect of complement inhibition in kidney transplant recipients with anti-HLA DSAs assured rigorous patient selection, homogeneous treatment protocol, and prospective collection of data. These patients received eculizumab according to the same therapeutic schema and were evaluated in a homogeneous manner across these two studies. Our findings should be confirmed by future prospective randomized trials specifically designed to assess the response to complement inhibition according to the complement-activating status of anti-HLA DSAs. Although we showed that the complement-activating anti-HLA DSA histomolecular rejection phenotype was not affected by the preformed/*de novo* status of anti-HLA DSAs, future studies should also specifically address the effect of eculizumab according to anti-HLA DSA complement–activating status in patients with *de novo* anti-HLA DSAs as well as in a therapeutic setting in patients with ABMR.

In conclusion, using a combination of high-dimensionality molecular assessments and extensively phenotyped kidney recipient populations together with cellular models, we defined the specific histomolecular phenotype of kidney allograft rejection associated with circulating complement-activating anti-HLA DSAs. We also showed that complement-activating anti-HLA DSAs may help to define the population in which complement-targeting intervention would provide the greatest benefit. Moreover, the stratification of clinical interventions targeting complement in patients with transplants represents a significant advance for designing efficient clinical trials by reducing sample sizes and costs. Further studies are needed for defining whether complement-activating anti-HLA DSA has the potential to inform therapeutic decision making for timely intervention before irreversible allograft damage occurs and streamline the use of expensive complement inhibitors in kidney transplantation.

CONCISE METHODS

Prospective Cohort Study to Define Kidney Allograft Rejection Phenotype in Patients with Complement-Activating Anti-HLA Antibodies

Kidney allograft rejection phenotyping was performed in a prospective study that included all consecutive patients who received kidney allografts at two transplantation centers in Paris (Necker Hospital and Saint-Louis Hospital) between January 1, 2011 and January 1, 2014 (n=931). The patients were prospectively screened for the presence of post-transplant circulating anti-HLA DSAs and their complement-activating capacity at the time of any clinical event in the first year post-transplantation and systematically at 1, 3, 6, and 12 months after transplantation. Patients underwent allograft biopsy at the time of post-transplant anti-HLA DSA detection in patients with *de novo* anti-HLA DSAs and at the time of an increase in MFI level according to clinician's judgement in patients with preformed anti-HLA DSAs. Allograft injury was assessed by histopathology, immunochemistry. and allograft gene expression analyses and compared between kidney transplant recipients with post-transplant circulating anti-HLA DSAs (complement activating and noncomplement activating) and those without anti-HLA DSAs (Reference Set in Supplemental Material, Supplemental Table 14).

Detection and Characterization of Circulating Donor-Specific Anti-HLA Antibodies

The presence of circulating donor-specific anti–HLA-A, -B, -Cw, -DR, -DQ, and -DP antibodies was analyzed using Luminex Single Antigen bead assays (One Lambda, Inc., Canoga Park, CA). All beads showing a normalized MFI ≥1000 were considered positive. All of the serum samples were treated with EDTA; a 0.1 M solution of disodium EDTA at pH 7.4 was diluted 1:10 in the serum and incubated for 10 minutes before testing. Patients with post-transplant anti-HLA DSAs were assessed for the presence of C1q-binding anti-HLA DSAs using single-antigen bead assays according to the manufacturer's protocol (C1qScreenTM; One Lambda, Inc.) as previously described.[8] The IgG subclass assay was performed as previously reported[37] using a modified standard single-antigen assay.

Histologic and Immunochemical Phenotyping of Kidney Allograft Biopsies

All patient allograft biopsy specimens were scored and graded from zero to three according to the updated international Banff criteria[4,38–40] by two trained pathologists who were blinded to the clinical data. We analyzed the deposition of complement split product C4d (polyclonal rabbit anti-human C4d antibody; Biomedica Gruppe, Vienna, Austria) and the presence of infiltrating monocytes/macrophages (anti-CD68 antibodies, clone EBM11; DakoCytomation, Glostrup, Denmark) in paraffin-embedded renal allograft tissue in all biopsies. NK cells were stained in frozen kidney sections using NKp46/NCR1 immunohistochemistry (NKp46/NCR1 antibody, clone 195314; R&D Systems Europe, Lille, France) in all biopsies. We used the international Banff score for monocytes/macrophages quantification and the number of cells per ten consecutive high-power fields (including glomeruli and peritubular capillaries) for NK cells quantification.

RNA Extraction and Gene Expression Analyses in Kidney Allograft Biopsies

All biopsies were processed for microarray analysis as previously described.[41] One biopsy bite was immediately placed in a dry tube and stored at −80°C. RNA extraction, labeling, and hybridization to HG-U219 GeneChip arrays (Affymetrix, Santa Clara, CA) were performed according to the manufacturer's protocols (www.affymetrix.com). The microarrays were scanned, and .cel files were generated using GeneChip Operating Software 1.4.0 (Affymetrix).

We measured and compared the intragraft gene expression in patients with complement-activating anti-HLA DSAs, patients with noncomplement-activating anti-HLA DSAs, and patients without anti-HLA DSAs (Reference Set). We used a non-*a priori* gene selection procedure to identify a specific gene set for complement-activating anti-HLA DSAs. First, the microarrays were normalized using robust multiarray averaging and global interquartile range filtering of probe sets, which was performed with a cutoff of 0.10 on the log base 2 scale and resulted in 9954 probe sets remaining for further analyses. Second, we identified the top 50 differentially expressed annotated genes between the patients with complement-activating anti-HLA DSAs and patients with noncomplement-activating anti-HLA DSAs using the false discovery rate–adjusted P values according to the Benjamini and Hochberg procedure at level 0.10. Third, to define the set of individual genes with a higher contribution to complement-activating anti-HLA DSA status than histology assessment, we determined the relative importance of the annotated nonredundant genes from the top 50 genes according to their discriminative performance for complement-activating anti-HLA DSA status by constructing random forests. Genes and histologic parameters were ranked on the basis of their relative variable importance, which was calculated by randomizing of the variable values and measuring the resulting decline in model accuracy. Fourth, for the genes included in the final discriminative set for the complement-activating anti-HLA DSA status, we assessed the correlation between the individual transcript expression measured by microarray and the corresponding RT-PCR expression value in a subset of 150 kidney allograft biopsies as previously described.[42] Fifth, transcripts were also analyzed using an IPA (Ingenuity Systems; www.ingenuity.com), with a focus on

the canonical pathways. IPA Path Designer in combination with the grow function was used to identify the pathways that were over-represented with complement-activating anti-HLA DSA–associated transcripts.

Expression of Transcripts in Human Cultured Cells

We isolated PBMCs from the whole blood of healthy volunteers by density gradient centrifugation using Ficoll and then purified the following cell populations for expression analysis on HG_U133_Plus_2.0 GeneChip arrays as previously described[34,43]: effector T cells (CD4+ and CD8+), B cells and monocytes, NK cells, macrophages, endothelial cells (human umbilical vein endothelial cells), and epithelial cells.

Effector T Cells

CD4+ and CD8+ T cells from healthy donors were generated through allostimulation starting with PBMCs cultured at a ratio of 3:1 with mitomycin C (Sigma-Aldrich, St. Louis, MO)–treated chronic myelogenous leukemic B cells (RPMI8866; ATCC, Manassas, VA). Recombinant human IL-2 (Affymetrix eBioscience, San Diego, CA) was added to the cultures at 50 U/ml and cultured for 5 days per round. After four rounds of stimulation, live cells were collected by Ficoll density gradient centrifugation followed by CD4+ and CD8+ cell purification using EasySep negative selection kits (StemCell Technologies, Vancouver, BC, Canada) according to the manufacturer's instructions. Cell purity varied between 92% and 98% (assessed by flow cytometry). The effector phenotype was shown by intracellular staining: 95%±3% of CD8+ T cells stained positive for Granzyme B after the final stimulation and 96%±2% of CD4+ and 90%±3% of CD8+ T cells stained positive for IFNG on restimulation.

B Cells and Monocytes

B cells were purified from PBMCs using EasySep negative selection kits (StemCell Technologies). Purified cell populations remained unstimulated until the time of RNA extraction. B cells were >97% CD19+. Monocytes were isolated directly from the PBMCs using the EasySep Human CD14+ Selection Kit (StemCell Technologies).

NK Cells

NK cells were purified from PBMCs using EasySep negative selection kits (StemCell Technologies). Cells were selected from donors with similarly high ratios of CD56dim to CD56bright NK cells, which are suggestive of a cytolytic phenotype. The majority (average, 96%) of NK cells showed a cytotoxic phenotype (CD56dim), as expected, in whole blood.

NK cells were stimulated by being coated with anti-CD16a LEAF antibodies (BioLegend, San Diego, CA) followed by crosslinking with plate-bound goat anti-mouse IgG F(ab′)2. Cells received 200 U/ml recombinant human IL-2 (Affymetrix eBioscience).

Macrophages

Monocytes were resuspended in complete RPMI, allowed to adhere on 100-mm plates (BD Falcon), and left for 24 hours or treated with recombinant human IFNG (500 U/ml; Affymetrix eBioscience) for 24 hours (Macrophages + IFNG).

Endothelial and Epithelial Cells

Human umbilical vein endothelial cells (StemCell Technologies) and human renal proximal tubule cells (Lonza Inc., Allendale, NJ) were maintained in tissue culture according to the supplier's recommendations and left untreated or treated with recombinant human IFNG (500 U/ml) for 24 hours.

Terminal Complement Pharmacologic Blockade Study

We assessed in a multicenter study the effects of complement pharmacologic blockade by eculizumab (Soliris, a humanized mAb that is a terminal complement inhibitor) on the complement-activating antibody-mediated allograft rejection histomolecular phenotype in kidney transplant recipients with anti-HLA DSAs at the time of transplantation receiving rejection prophylaxis with eculizumab or noncomplement-directed SOC (n=116). We also evaluated the clinical response to rejection prophylaxis defined by the 3-month incidence of biopsy-proven antibody-mediated rejection. 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. In the first trial, kidney recipients from the single-arm NCT01567085 study underwent kidney transplantation from deceased donors and received rejection prophylaxis with eculizumab (n=32), and they were compared with patients from the same centers meeting the same inclusion criteria but receiving noncomplement-directed SOC (n=44) (Supplemental Material). In the second trial, kidney recipients from the randomized, controlled NCT01399593 study underwent kidney transplantation from living donors and received rejection prophylaxis with either eculizumab (n=20) or SOC (n=20). In both studies, patients treated with eculizumab received the drug in the first 9 weeks post-transplantation (1200 mg 1 hour before transplantation, 900 mg/wk for 4 weeks, and 1200 mg every other week for weeks 5, 7, and 9); patients treated with SOC received plasma exchange and intravenous Ig according to the transplant center's SOC 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 and underwent kidney allograft biopsy at day 14 after transplantation, and they were assessed for clinical and histologic characteristics and allograft gene expression (Supplemental Material).

Statistical Analyses

Continuous variables are described using means with SDs or SEMs. We compared means and proportions using the t test and the chi-squared test, respectively (or the Mann–Whitney U test and the Fisher exact test, respectively, if appropriate). Death-censored allograft survival was assessed using the Kaplan–Meier estimator and compared with the log rank test. Random forest was performed using the randomForest package in R. Principal component analysis was performed using the dudi.pca function of the ade4 package in R. Calibration of logistic regression models was assessed by examination of calibration plots and tested with the Hosmer–Lemeshow test. Statistical significance was set at $P<0.05$. All tests were two sided. Unless otherwise indicated, all of the statistical analyses were performed using R, version 3.3.2 (R Development Core Team, Vienna, Austria).

Study Approval

This study was approved by the Comité de Protection des Personnes Ile de France II (registration no. DC-2009–955). Each patient from this study provided written informed consent to be included in the French national registry agency (Agence de la Biomédecine) database CRISTAL (official website: https://www.sipg.sante.fr/portail/) and the DIVAT (official website: https://www.divat.fr). The DIVAT and the CRISTAL database networks have been approved by the National French Commission for bioinformatic data and patient liberty (DIVAT: CNIL, registration no. 1016618, validated June 8, 2004 and CRISTAL: CNIL, registration no. 363505, validated April 3, 1996).

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DISCLOSURES

P.F.H. owns shares in Transcriptome Sciences Inc., a company with an interest in molecular diagnostics. The other authors declare that they had no financial relationships with any organizations that might have an interest in the submitted work in the past 3 years and no other relationships or activities that could appear to have influenced the submitted work.

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SUPPLEMENTARY APPENDIX

SUPPLEMENTARY TABLES AND FIGURES

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

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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.

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

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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.

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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.

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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.

Supplementary Figure 2: Expression of complement-activating donor-specific anti-HLA antibody selective transcripts in a panel of primary human cells.

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

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

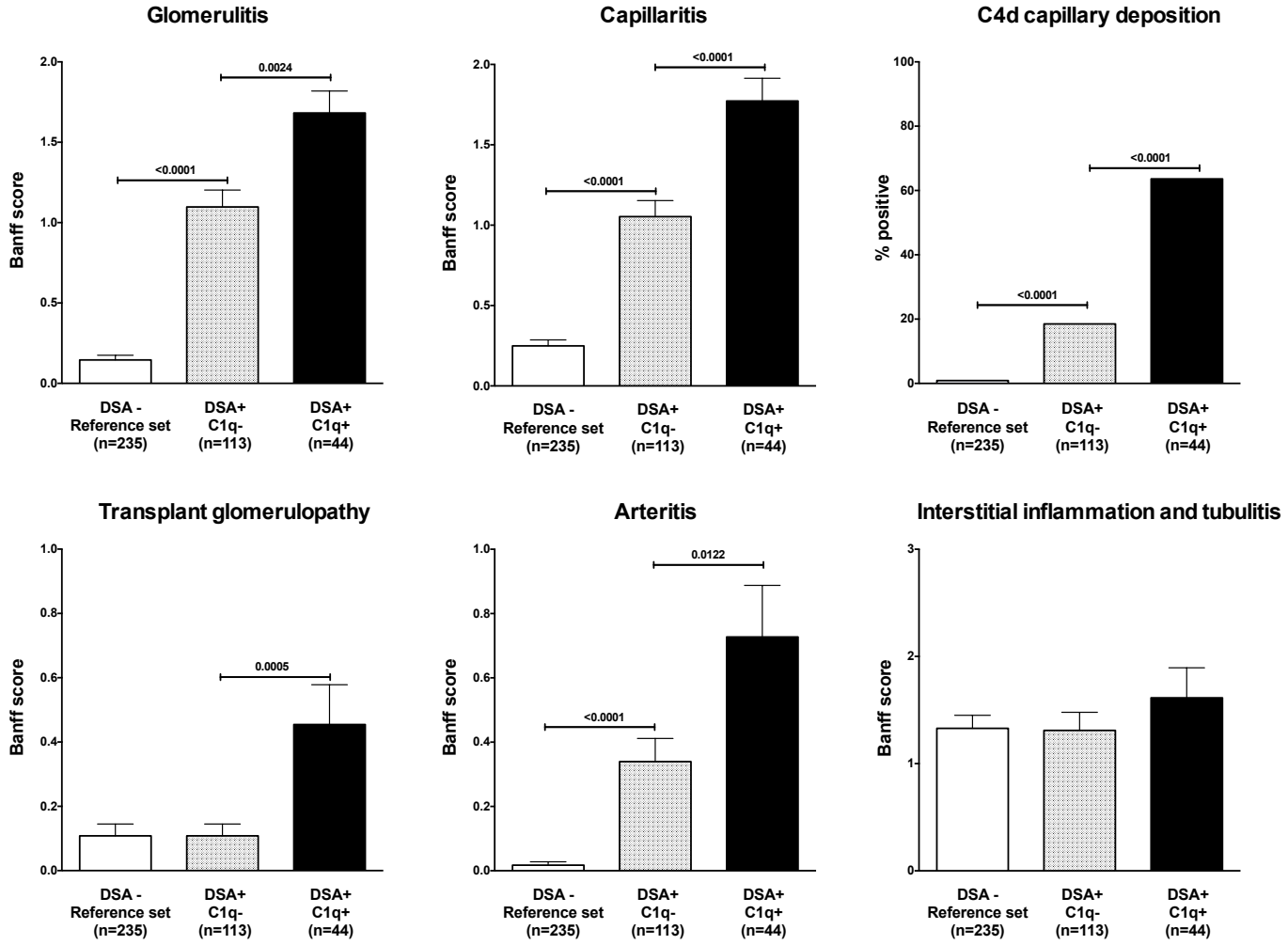
Supplementary Table 14: Baseline characteristics of the Reference Set.

	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.



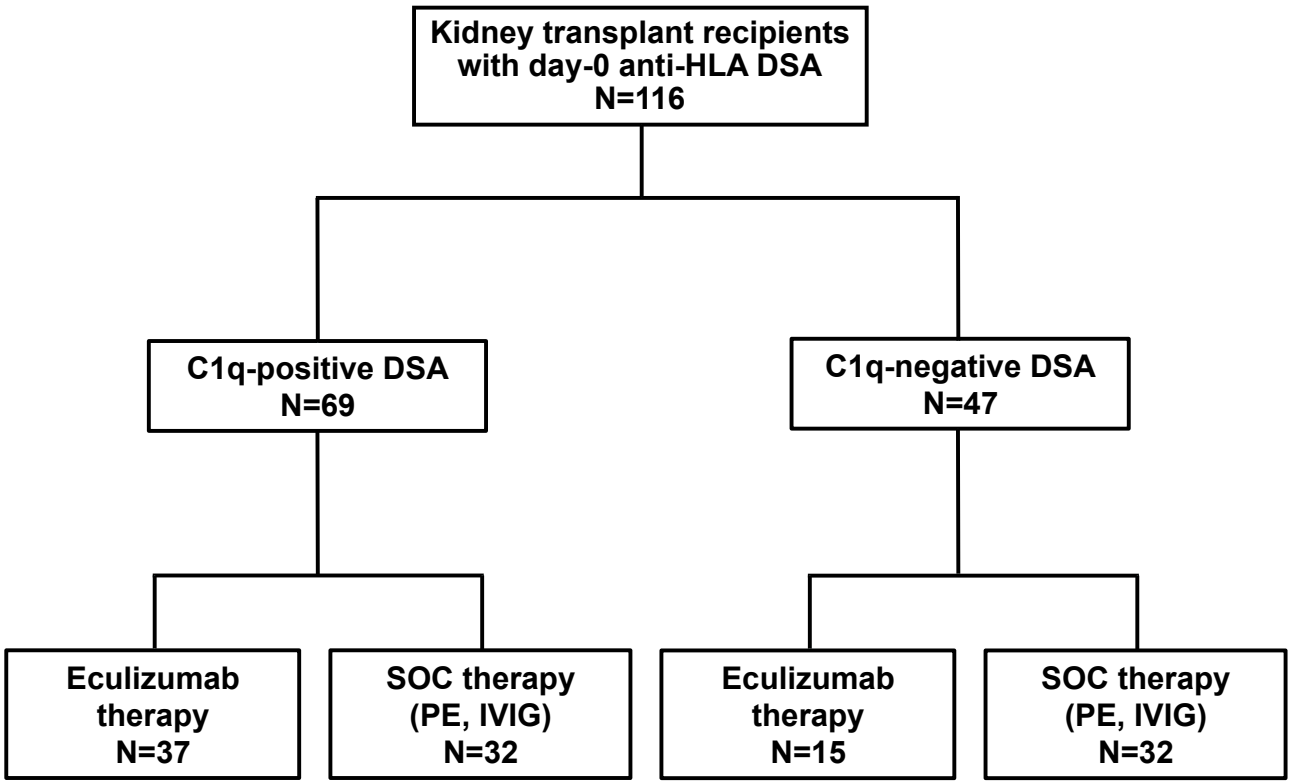
Supplementary Figure 2: Expression of complement-activating donor-specific anti-HLA antibody selective transcripts in a panel of primary human cells, including effector CD8+ and CD4+ T-cells, unstimulated NK cells, CD16-stimulated NK cells, B cells, monocytes, macrophages, IFNG-treated macrophages, and 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.

SIGNIFICANCE STATEMENT

Complement-activating anti-HLA donor-specific antibodies (DSAs) are associated with an increased risk of kidney allograft loss, but their specific effects on kidney allograft injury are unknown. This study uses gene expression analysis as well as histopathology and immunostaining to characterize circulating complement-activating anti-HLA DSA-mediated rejection in kidney allografts and in *in vitro* human cell cultures. The specific phenotype defined, when applied in a stratified analysis, predicted the response of antirejection treatment with eculizumab, the anti-C5 mAb; benefit was restricted to patients with pretransplant complement-activating anti-HLA DSAs. Complement-activating anti-HLA DSAs may help to define the population of kidney recipients for whom complement-targeting intervention will provide the greatest benefit.