SUPPLEMENTARY MATERIAL

Data collection procedures

The transplant allocation system was identical between the 2 centers, corresponding to the rules of the French National Agency for Organ Procurement (Agence de la Biomédecine). All of the data from Necker Hospital regarding the donors and recipients were extracted from the DIVAT clinical prospective cohort (official Web site: www.divat.fr). Data from the Saint Louis Hospitals were obtained from the French national registry agency database CRISTAL (official Web site: https://www.sipg.sante.fr/portail/). The DIVAT and CRISTAL database networks were approved by the National French Commission for bioinformatics data and patient liberty (DIVAT: CNIL, registration number: 1016618, validated June 8, 2004; and CRISTAL: CNIL, registration number: 363505, validated April 3, 1996). Codes were used to ensure strict donor and recipient anonymity and blinded assays. Informed consent was obtained from the participants at the time of transplantation. The data were inputted into the databases in real time, as well as at each transplant anniversary, and they were submitted for annual audits.

Per protocol screening biopsy

All of the graft biopsies were scored and graded from 0 - 3 by trained pathologists (JPDVH, DN, JV, MR), according to the updated Banff criteria, (6, 34) based on the following histological factors: glomerular inflammation (glomerulitis), tubulitis, interstitial inflammation, endarteritis, peritubular capillary inflammation (capillaritis), transplant glomerulopathy, interstitial fibrosis, tubular atrophy, and arteriosclerosis. The microcirculation inflammation score was defined as the sum of glomerular and peritubular capillary inflammation, and the tubular and interstitial score was the sum of interstitial inflammation and tubulitis.

Post-transplantation induction protocols and maintenance immunosuppressive therapy

All of the patients received induction therapy, consisting of rabbit antithymocyte globulin (1.5 mg/kg/day for 10 days) or basiliximab (20 mg at day 0 and day 4), immediately after transplantation. Subsequent maintenance immunosuppressive therapy consisted of prednisone; mycophenolate mofetil (1000 mg twice daily); tacrolimus, administered to maintain a target blood level of 8 - 10 ng/mL for the first 3 months and 6 - 8 ng/mL after 3 months; or cyclosporine, administered to maintain a target blood level of 800 - 1200 ng/mL for the first 3 months and 600 - 800 ng/mL after 3 months. Patients with preexisting donor-specific anti-HLA antibodies, as detected by the techniques in current use at the time of transplantation, received intravenous immune globulin (2 g/kg of body weight on day 0, day 20, and day 40) with or without rituximab given twice (375 mg/m^2 of body-surface area on day 0 and day 7) as prophylaxis against acute rejection, according to center practice (26, 35, 36).

Treatment of allograft-rejection episodes

Of the 142 patients with subclinical antibody-mediated rejection, 56 received antibody-targeting therapies consisting of 4 courses of high-dose intravenous immune globulin (2 g/kg of body weight over 96 hours), plasma exchange (5 rounds), and anti-CD-20 Rituximab® at a dose of 375 mg/m2 of body surface, based on the biopsy results. The remaining 86 patients with subclinical antibody-mediated rejection did not receive antibody-targeting-based therapy following biopsy (25 received methylprednisolone pulses alone, and 61 did not receive any treatment based on the biopsy findings). Among the 56 (39.4%) patients with subclinical ABMR who received antibody-directed therapy, 8 lost their grafts (14.29%). Among the remaining 86 (60.5%) patients with subclinical ABMR who did not receive antibody-directed therapy, 21 (24.42%) lost their grafts (p=0.14, compared with subclinical ABMR patients who received antibody-directed therapy).

Detection of antibodies against donor-specific HLA molecules

We retrospectively determined the presence of circulating donor-specific anti-HLA antibodies at the time of biopsy in all patients with biopsy-proven acute rejection using single-antigen flow bead assays (One Lambda, Inc., Canoga Park, CA, USA) on a Luminex platform. All beads showing a normalized mean intensity of fluorescence greater than 500 were considered positive, as previously described (26). For each patient, we recorded the number, specificity, and mean intensity of fluorescence of all donor-specific anti-HLA antibodies also detected. The maximum mean intensity of fluorescence of donor-specific anti-HLA antibodies was defined as the highest-ranked donor-specific bead. HLA typing of transplant recipients was performed by molecular biology (Innolipa HLA typing kit; Innogenetics, Belgium). For all of the kidney transplant donors, HLA-A/B/DR/DQ tissue typing was performed using the microlymphocytotoxicity technique with One Lambda Inc. tissue-typing trays, and the typing was controlled by molecular biology technique.

Independent validation cohort

The external validation cohort consisted of 321 kidney recipients transplanted at Saint Louis Hospital, Paris, France, between January 2006 and January 2010. This time period corresponds to the time since Saint Louis implemented a policy of protocol biopsy at 1-year post-transplant. Therefore, for the survival analysis in the validation group, we used a maximum follow-up of 7 years post-transplant. Similar immunological rules for kidney transplantation were applied, with all of the transplantations being ABO-compatible and negative current IgG T-cell and B-cell complement-dependent cytotoxicity cross-matching required for all of the patients. Data were retrieved on December 19, 2012, and their use was based on an agreement between centers participating in the national database system (Agence de la Biomédecine).

SUPPLEMENTARY FIGURES AND TABLES

Supplementary Figure 1: Graft survival by rejection profile in the external validation cohort (n=321)

Supplementary Figure 2: Risk of graft loss according to kidney allograft function and subclinical ABMR phenotype in the external validation cohort

Supplementary Table 1: Comparison of baseline characteristics between kidney allograft recipients included and excluded from the study

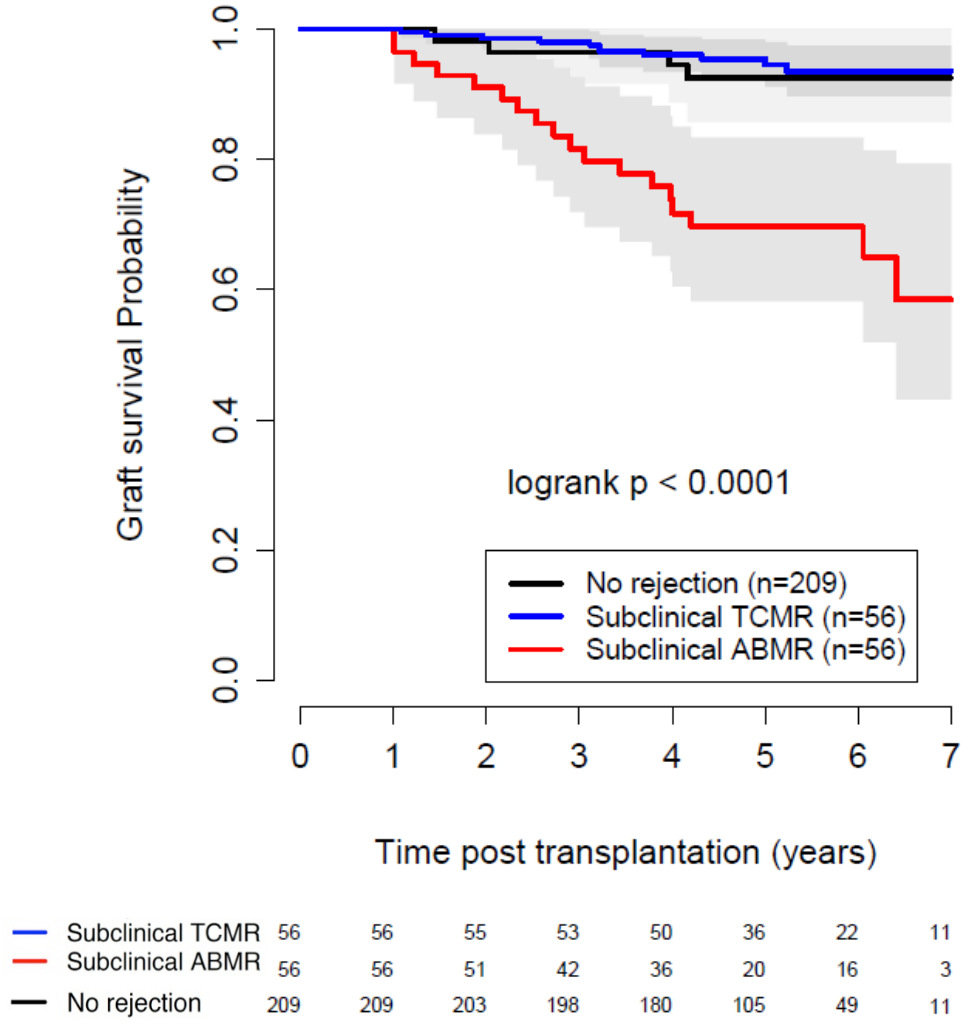
Supplementary Table 2: Histopathological lesions found in for-cause biopsies taken after 1 year post-transplant

Supplementary Table 3: Sensitivity analysis of acute rejection based on a multivariate analysis of factors associated with kidney graft loss adjusted for acute rejection

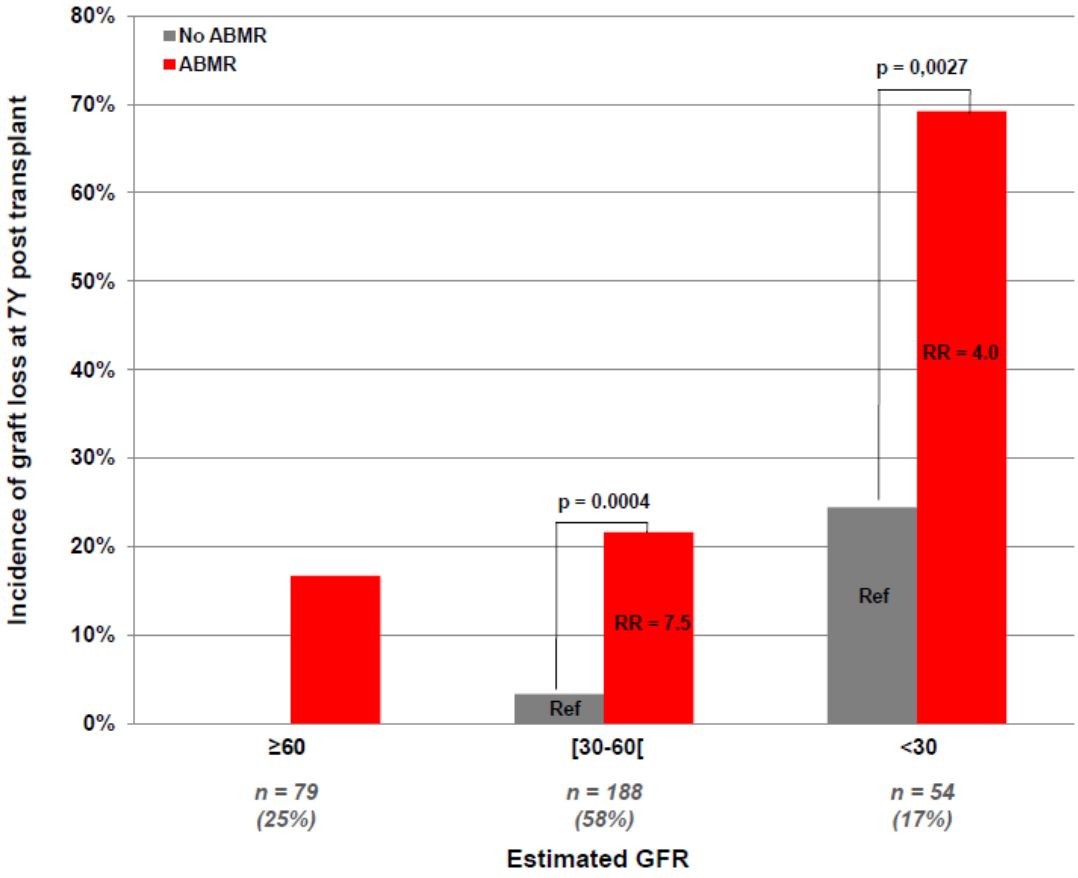
Supplementary Table 4: Baseline characteristics of the external validation cohort

Supplementary Table 5: Multivariate analysis of factors associated with kidney graft loss in the external validation cohort

Supplementary Figure 1: Graft survival by rejection profile in the external validation cohort (n=321)



Supplementary Figure 2: Risk of graft loss according to kidney allograft function and subclinical ABMR phenotype in the external validation cohort (n=321)



Footnotes:
RR: Relative risk
GFR: Glomerular filtration rate estimated by the MDRD formula
95 % CI of the RR are 7.5 (2.5 - 23.1); 4.0 (1.6 - 9.9)

Supplementary Table 1: Comparison of baseline characteristics between kidney allograft recipients included and excluded from the study

Patient Characteristics	Included (N = 1001)		Excluded (N = 306)		
	n		n		
Recipient age — years **	1001	47.9 ± 13	306	49.2 ± 14	p = 0.1399
Recipient male gender *(n,%)*	994	582 (59%)	305	174 (57%)	p = 0.6429
Re-transplantation *(n,%)*	1001	190 (19%)	306	25 (8%)	p < 0.0001
Time since dialysis — *months* ******	866	5.04 ± 4.4	263	4.8 ± 4.1	p = 0.5405
Donor age (years) **	991	50 ± 16	271	52.6 ± 17	p = 0.0577
Donor male gender *(n,%)*	979	553 (56%)	269	128 (47.6%)	p = 0.0105
Deceased donor *(n,%)*	1001	819 (82%)	306	265 (87%)	p = 0.0560
Cardiovascular cause of donor death *(n,%)*	1001	446 (45%)	306	140 (46%)	p = 0.7427
Cold ischemia time *(hours)*	970	19 ±10	244	20.4 ± 11	p = 0.0168
Delayed graft function (n, %)	995	219 (22%)	248	70 (28%)	p = 0.0436
Graft survival (n, %)[$]	1001	865 (86%)	306	189 (62%)	p < 0.0001
Patient survival (n, %)[$]	1001	928 (93%)	306	255 (83%)	p < 0.0001
Follow-up (years)	1001	4.9 ± 2.4	306	2.9 ± 3.1	p < 0.0001
Causal nephropathy *(n,%)*	1001		306		
Diabetes		85 (8.5%)		40 (13.1%)	
Vascular		68 (6.8%)		25 (8.2%)	
Glomerulopathy		316 (31.6%)		72 (23.5%)	
Congenital		205 (20.4%)		57 (18.6%)	
Other		7 (0.7%)		2 (0.6%)	
Interstitial nephropathy		123 (12.3%)		32 (10.5%)	
Undetermined		197 (19.7%)		78 (25.5%)	p= 0.0155
Immunology					
HLA A+B mismatch **	986	2 ± 1.1	271	2 ± 1.1	p = 0.1599
HLA DR mismatch **	987	0.8 ± 0.7	270	0.8 ± 0.7	p = 0.5312
Recipient blood group type A/B/O/AB	968	438/75/425/30	252	102/25/110/15	p = 0.6345
Number of dialysis treatments post-transplant **	987	1 ± 2.4 (0-29)	247	2.6 ± 10.3 (0-150)	p = 0.0156

The data are from the DIVAT database (Données Informatiques Validées en Transplantation) (24). All of the p values were determined by chi-square tests for the comparison of proportions and by unpaired t tests for the comparison of continuous variables.
** Plus-minus values are means ± SDs.
$ Last follow-up: April 15, 2012

Supplementary Table 2A: Histopathological lesions found in for-cause biopsies taken before 1 year post-transplant[$] (536 biopsies in 396 patients)

	No Rejection	Subclinical TCMR	Subclinical ABMR
Number of biopsies for cause performed before 1 year (n,%)	323 (60%)	79 (15%)	134 (25%)
Mean time since 1-year screening biopsy (days)	- 275 ± 117	- 242 ± 113	- 281 ± 110
Number of patients with a biopsy for cause performed before 1 year (n,%)	249 (34%)	60 (45%)	87 (61%)
Microcirculation inflammation (g+ptc) Banff score*	0.9 ± 1.5	0.9 ± 1.5	2.6 ± 2.2
Interstitial inflammation (i) Banff score*	0.6 ± 0.9	0.9 ± 1.1	0.8 ± 1.1
Tubulitis (t) Banff score*	0.7 ± 1.1	1.1 ± 1.2	0.9 ± 1.2
Transplant glomerulopathy (cg) Banff score*	0.1 ± 0.4	0.0 ± 0.2	0.2 ± 0.6
Fibrosis-atrophy (IFTA) Banff score*	0.7 ± 1.0	1.1 ± 1.0	0.8 ± 1.0
Arteriosclerosis (cv) Banff score*	1.2 ± 1.0	1.2 ± 1.0	1.1 ± 1.0
TCMR diagnosis (n,%)	43 (13%)	22 (28%)	17 (13%)
ABMR diagnosis (n,%)	49 (15%)	7(8%)	73 (54%)
Other diagnoses (AKI, borderline, BK, CNI toxicity, recurrence)	231 (72%)	50 (63%)	44 (33%)

$ Note that this table is purely descriptive and illustrative given the different time points at which the biopsies for cause were performed before 1 year post-transplant.
*The Banff scores are given as the mean ± SEM

Supplementary Table 2B: Histopathological lesions found in biopsies for cause taken after 1 year post-transplant[€] (317 biopsies in 238 patients)

	No Rejection	Subclinical TCMR	Subclinical ABMR
Biopsy for cause performed after 1-year (n,%)	210 (66%)	51 (16%)	56 (18%)
Mean time since 1-year screening biopsy *(days)*	679.1 ± 580	804 ± 659	467 ± 511
Number of patients with a biopsy for cause performed before 1 year (n,%)	**249 (34%)**	**38 (29%)**	**46 (32%)**
Microcirculation inflammation (g+ptc) Banff score*	1.2 ± 1.7	1.3 ± 1.7	2.6 ± 1.9
Interstitial inflammation (i) Banff score*	0.5 ± 0.9	0.4 ± 0.8	0.4 ± 0.9
Tubulitis (t) Banff score*	0.5 ± 1.0	0.4 ± 0.8	0.3 ± 0.7
Transplant glomerulopathy (cg) Banff score*	0.2 ± 0.8	0.3 ± 0.9	1.0 ± 1.3
Fibrosis-atrophy (IFTA) Banff score*	1.7 ± 1.1	1.6 ± 1.1	1.5 ± 1.2
Arteriosclerosis (cv) Banff score*	1.4 ± 1.0	1.4 ± 1.1	1.6 ± 1.1
TCMR diagnosis	27 (13.0%)	5 (9.8%)	1 (1.8%)
ABMR diagnosis[£]	55 (26.4%)	15 (29.4%)	39 (69.6%)

€ Note that this table is purely descriptive and illustrative given the different time points at which the biopsies for cause were performed after 1 year post-transplant.
*The Banff scores are given as the mean ± SEM
£ Acute ABMR or chronic active ABMR

Supplementary Table 3: Sensitivity analysis of acute rejection based on a multivariate analysis of factors associated with kidney graft loss adjusted for acute rejection[$]

		Number of patients	Number of events	HR	95% CI	P
eGFR* at 1 year	**eGFR ≥ 60**	305	6	1	-	-
	30 ≤ eGFR < 60	577	38	2.824	[1.186- 6.723]	-
	eGFR < 30	79	28	11.541	[4.577- 29.101]	<0.0001
Subclinical ABMR at 1 year[€]	**No**	825	45	1	-	-
	Yes	136	27	2.723	[1.578 - 4.697]	0.0003
Previous acute TCMR	**No**	880	64	1		
	Yes	81	8	0.897	[0.421- 1.909]	0.7773
Previous acute ABMR	**No**	839	56	1		
	Yes	122	16	1.368	[0.725 -2.580]	0.3335
Proteinuria at 1 year (log10 value)		961	72	1.489	[1.248-1.776]	<0.0001

Footnotes:
HR: hazard ratio
CI: confidence interval
* GFR: glomerular filtration rate estimated by the MDRD formula

[$] Final multivariate Cox model obtained by entering risk factors from the univariate model reaching $p \leq 0.10$ as the threshold in a single multivariate proportional hazards model. The final multivariate model is adjusted on the following parameters: i) donor age, ii) donor type, iii) cold ischemia time, iv) graft rank, v) delayed graft function vi) atrophy scarring vii) C4d graft deposition and viii) previous rejection episodes.

[€] Note that subclinical ABMR includes the presence of circulating donor-specific anti-HLA antibodies (DSA) plus microcirculation inflammation lesions (glomerulitis plus peritubular capillaritis). The modeling was performed in 961 patients due to missing data on proteinuria.

Supplementary Table 4: Baseline characteristics of the external validation cohort

** mean ± SD

Patient Characteristics		Study Population (N = 1001)		External Validation Population (N = 321)	P
	N		N		
Recipient age — years **	1001	47.9 ± 13	**321**	**46.2 ± 13.2**	0.042
Recipient gender (male) *(n,%)*	994	582 (59%)	**321**	**187 (58%)**	0.9481
Re-transplantation *(n,%)*	1001	190 (19%)	**321**	**32 (10%)**	0.0001
Time since dialysis — *months* **	866	5.04 ± 4.4	**277**	**5.3 ± 5.1**	0.411
Donor age (years) **	991	50 ± 16	**321**	**47.8 ± 16**	0.032
Donor gender (male) *(n,%)*	979	553 (56%)	**321**	**179 (55.8%)**	0.8459
Deceased donor *(n,%)*	1001	819 (82%)	**321**	**282 (87.9%)**	0.01249
Cardiovascular cause of donor death *(n,%)*	1001	446 (45%)	**-**	**-**	-
Cold ischemia time *(hours)*	970	19 ±10	**321**	**15.1 ± 7.5**	<0.0001
Delayed graft function (n, %)	995	219 (22%)	**-**	**-**	-
Graft survival (n, %)	1001	865 (86%)	**321**	**288 (90%)**	0.1492
Patient survival (n, %)	1001	928 (93%)	**321**	**311 (97%)**	0.003828
Follow-up (years)	1001	4.7 ± 2.1	**321**	**4.4 ± 1.4**	0.06783
Causal nephropathy *(n,%)*	1001		**321**		
Diabetes		85 (8.5%)		**56 (17.4%)**	
Vascular		68 (6.8%)		**40 (12.4%)**	
Glomerulopathy		316 (31.6%)		**67 (20.9%)**	
Congenital		205 (20.4%)		**25 (7.8%)**	
Other		7 (0.7%)		**41 (12.8%)**	
Interstitial nephropathy		123 (12.3%)		**31 (9.7%)**	
Undetermined		197 (19.7%)		**61 (19.0%)**	<0.0001
Immunology					
HLA A/B/DR mismatch	968	2.9 ± 0.9	**321**	**4 ± 1.4**	<0.001
Recipient blood group type A/B/O/AB	968	438/75/425/30	**321**	**136/40/128/17**	0.01627
Number of dialysis treatments post-transplant	987	1.0 ± 2.4	**-**	**-**	-

Supplementary Table 5: Multivariate analysis of factors associated with kidney graft loss in the external validation cohort (N=321)

		Number of patients	Number of events	HR	95% CI	P
eGFR* at 1 year	**eGFR ≥ 60**	79	1	1	-	-
	30 ≤ eGFR < 60	188	13	4.0	(0.5 - 30.9)	-
	eGFR < 30	54	19	27.5	(3.7 - 206.8)	**<0.0001**
Subclinical ABMR	**No**	265	15	1	-	-
	Yes	56	18	5.7	(2.9 - 11.4)	**<0.0001**

Footnotes:
HR: Hazard ratio
CI: Confidence interval
eGFR: Glomerular filtration rate estimated by the MDRD formula