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Non-HLA Agonistic Anti-Angiotensin II Type 1 Receptor Antibodies Induce a Distinctive Phenotype of Antibody-mediated Rejection in Kidney Transplant Recipients

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ABSTRACT

Anti-angiotensin II type 1 receptor (AT1R) antibodies have been associated with allograft rejection. We hypothesized that circulating AT1R antibodies might identify kidney transplant recipients at increased risk of allograft rejection and loss who are not identified by the HLA system. We prospectively enrolled 1845 kidney transplant recipients from two centers. Donor-specific HLA antibodies (DSAs) and AT1R antibodies were measured at the time of the first acute rejection episode or at 1 year post-transplant. Allograft biopsy was performed to evaluate the rejection phenotype and to assess for endothelial activation. Overall, 371 (20.1%) participants had AT1R antibodies, 334 (18.1%) had DSAs, and 133 (7.2%) had both. AT1R antibodies were associated with an increased risk of allograft loss (adjusted HR 1.49, 95% CI 1.07-2.06 for AT1R antibodies alone and 2.26, 95% CI 1.52-3.36 for AT1R antibodies and DSAs). Participants with AT1R antibodies had a higher incidence of antibody-mediated rejection (AMR) compared with participants without AT1R antibodies (25.0% vs. 12.9%). Among 77 participants with histological features of AMR but without DSAs, 51 (66.2%) had AT1R antibodies. Compared to participants with prototypical DSA-mediated rejection, those with AT1R antibody-associated rejection had higher prevalence of hypertension, more vascular rejection with arterial inflammation, higher levels of endothelial-associated transcripts, and lack of complement deposition in allograft capillaries. Thus, AT1R antibodies may identify kidney transplant recipients at high risk of allograft rejection and loss, independent of the HLA system. Recognition of complement-independent AT1R antibody-mediated vascular rejection could lead to the development of new treatment strategies to improve allograft survival.

INTRODUCTION

Incompatibility between genetically disparate donor and recipient pairs has been recognized as the fundamental contributing factor to allograft rejection, which is a main impediment to successful transplantation of various organs, including kidney,¹ heart,² lung³ and liver.⁴ Allograft rejection mainly occurs through the triggering of donor-specific immune responses mediated by T and B cells.⁵ The Human Leucocyte Antigen (HLA) tissue system has been identified as the primary target of donor-specific alloimmune responses, especially by involving recipient production of antibodies directed towards non-self-donor HLA class I and HLA class II proteins that are expressed on the allograft endothelium and show high polymorphism.⁶ The assessment of the HLA system, which consists of measuring the degree of HLA class I and HLA class II tissue antigen matching between the recipient and the donor and then detecting circulating donor-specific anti-HLA antibodies (DSAs) in the recipient's serum, represents the contemporary gold standard for defining patients' access to transplantation, post-transplant immunological monitoring and allograft rejection diagnosis.^{6,7} However, it is commonly acknowledged that this HLA-centric approach does not provide a sufficient level of accuracy for the evaluation of immunological complications in transplant recipients.⁷ Accumulating evidence has highlighted that allograft rejection with pathological features of antibody-mediated rejection can occur in patients without detectable anti-HLA DSAs using highly sensitive contemporary assays,⁸ even in recipients of transplants from HLA-identical siblings.⁹

The allograft endothelium represents the first barrier between the transplanted organ and the immune system of the recipient; this endothelium expresses the most potentially relevant antigens in transplant immunobiology.⁵ Among antibodies to non-HLA endothelial antigens, agonistic antibodies directed towards angiotensin II type 1 receptor (AT1R), a G-protein coupled receptor that is expressed at the endothelial cell surface, have been the most

commonly reported non-HLA antibodies that are associated with allograft rejection. The presence of anti-AT1R antibodies in transplant recipients was initially reported in 16 patients with steroid-refractory vascular rejection¹⁰ and was further associated in small studies with the occurrence of allograft dysfunction, rejection or loss in adult and pediatric kidney,¹¹⁻²³ heart,²⁴ lung²⁵ and liver²⁶ transplant recipients. However, these findings have not been reproduced by other investigators, who reported a lack of association between anti-AT1R antibodies and allograft outcomes or associations limited to patients with concomitant anti-HLA DSAs,²⁷⁻³⁰ particularly in the largest study conducted to date.³¹ Therefore, the impact of anti-AT1R antibodies on transplant outcomes at the population level, with respect to the presence of concomitant anti-HLA antibodies and to what extent they can induce allograft rejection independently and/or synergistically with anti-HLA antibodies, remains largely unclear. Given the low level of evidence linking anti-AT1R antibodies to allograft rejection and loss at the population level, screening for these antibodies is neither considered standard of care for risk stratification and monitoring of allograft rejection by international guidelines^{7,32,33} nor for the diagnosis of allograft rejection by international classifications.³⁴⁻³⁷

We sought to determine, in a large, prospective cohort of well-phenotyped kidney transplant recipients, whether post-transplant circulating anti-AT1R antibodies might play a role in the failure of kidney allografts and to what extent their detection might identify patients at risk of allograft rejection and loss who are unrecognized by current diagnostic standards based on HLA assessment.

RESULTS

Patient characteristics

Among 1845 kidney recipients transplanted between January 1, 2008 and December 31, 2012, we identified 504 (27.3%) patients with post-transplant anti-AT1R antibodies and 1341 (72.7%) patients without post-transplant anti-AT1R antibodies. **Table 1** shows the demographic and clinical characteristics of the patients at the time of allograft evaluation according to the presence of post-transplant anti-AT1R antibodies. Compared with patients without post-transplant anti-AT1R antibodies, patients with post-transplant anti-AT1R antibodies were significantly younger (49.3 (12.6) vs. 46.9 (14.5) years old; $p=0.001$), more often women ($n=497/1341$ (37.1%) vs. $n=247/504$ (49.0%); $p<0.001$), had a higher prevalence of previous kidney transplantation ($n=216/1341$ (16.1%) vs. $n=118/504$ (23.4%); $p<0.001$), and showed a higher level of sensitization against HLA antigens at the time of transplantation, with a mean calculated panel of reactive HLA antibodies of 38.3 (34.2) vs. 45.2 (36.3) % ($p<0.001$).

Kidney allograft survival

The median follow-up after antibody screening was 7.23 years (interquartile range, 4.13-9.03). **Figure 1A** shows kidney allograft survival according to anti-AT1R antibody status after transplantation. Patients with post-transplant anti-AT1R antibodies had decreased kidney allograft survival compared with patients without post-transplant anti-AT1R antibodies (7-year allograft survival after antibody screening of 76.4%; 95%CI, 72.1-80.1 vs. 84.8%; 95%CI, 82.6-86.7; $p<0.001$). Higher levels of circulating anti-AT1R antibodies were associated with increasing incidence of allograft loss (**Figure S1**). Patients with anti-AT1R antibodies and anti-HLA DSAs had the worst allograft survival after antibody screening (60.2%; 95%CI, 50.4-68.7) compared with patients with only anti-HLA DSAs (75.3%; 95%CI, 69.9-79.8), those with only

anti-AT1R antibodies (81.9%, 95%CI; 77.3-85.7) and those without these antibodies (87.9%; 95%CI, 85.6-89.9, $p<0.001$) (**Figure 1B**).

The univariate associations of clinical, histological and immunological factors with kidney allograft loss are shown in **Table 2**. In multivariable analysis, the following independent determinants of kidney allograft loss were identified: glomerular filtration rate (HR, 0.21; 95%CI, 0.16-0.29; $p<0.001$), proteinuria (HR, 2.25; 95%CI, 1.85-2.73; $p<0.001$), anti-HLA DSA and anti-AT1R antibody status (HR, 1.49; 95%CI, 1.07-2.06 for anti-AT1R antibodies alone; HR, 1.38; 95%CI, 1.05-1.84 for anti-HLA DSAs alone and HR, 2.26; 95%CI, 1.52-3.36 for both antibodies ($p<0.001$)), glomerulitis and peritubular capillaritis (HR, 1.79; 95%CI, 1.32-2.43; $p<0.001$) and interstitial fibrosis and tubular atrophy (HR, 1.29; 95%CI, 1.00-1.67; $p=0.046$) (**Table 3**).

Kidney allograft injury

Kidney allograft injury according to anti-AT1R antibody status

Patients with anti-AT1R antibodies showed a higher incidence of histological features of active antibody-mediated rejection at one year after transplantation as defined by the international classification for allograft rejection³⁴ (microvascular inflammation with sum of glomerulitis and peritubular capillaritis Banff scores >1) compared with patients without anti-AT1R antibodies: 126/504 (25.0%) vs. 173/1341 (12.9%); $p<0.001$. Patients with anti-AT1R antibodies had a greater incidence of vascular rejection lesions, including microcirculation inflammation (incidence of glomerulitis of 147/504 (29.2%) vs. 277/1341 (20.7%); $p<0.001$ and incidence of peritubular capillaritis of 145/504 (28.8%) vs. 232/1341 (17.3%); $p<0.001$) and arterial inflammation (incidence of intimal arteritis of 56/504 (11.1%) vs. 53/1341 (4.0%); $p<0.001$), compared with patients without anti-AT1R antibodies (**Table 4**). Higher levels of circulating anti-AT1R antibodies were associated with increased risk of glomerulitis, peritubular capillaritis and intimal arteritis (**Figure 2**). Stratified analysis according to the type of allograft biopsy showed the consistency of these associations in patients undergoing clinical indication biopsy within the first year after transplantation and in patients undergoing protocol biopsy one year after transplantation (**Table S1**).

Stratified analysis according to anti-HLA DSA status

Stratified analysis according to anti-HLA DSA status revealed the consistency of the associations between anti-AT1R antibodies and allograft injury in patients without anti-HLA DSAs and in those with anti-HLA DSAs (**Table S2**). The incidence of histological features of active antibody-mediated rejection at one year after transplantation was increased in patients with anti-AT1R antibodies compared with patients without anti-AT1R antibodies among patients without anti-HLA DSAs (n=51/371, 13.7% vs. n=26/1007, 2.6%; p<0.001) as well as in patients with anti-HLA DSAs (n=75/133, 56.4% vs. n=147/334, 44.0%; p=0.016). In patients without anti-HLA DSAs (n=1378), those with anti-AT1R antibodies (n=371) showed greater incidence of microvascular (incidence of glomerulitis of 68/371 (18.3%) vs. 114/1007 (11.3%); p=0.001 and incidence of peritubular capillaritis of 64/371 (17.3%) vs. 73/1007 (7.3%); p<0.001) and arterial inflammation (incidence of intimal arteritis of 40/371 (10.8%) vs. 34/1007 (3.4%); p<0.001), compared with patients without anti-AT1R antibodies (n=1007). Among patients with anti-HLA DSAs (n=467), those with anti-AT1R antibodies (n=133) showed greater incidence of microvascular (incidence of glomerulitis of 79/133 (59.4%) vs. 163/334 (48.8%); p=0.039 and incidence of peritubular capillaritis of 81/133 (60.9%) vs. 159/334 (47.6%); p=0.009) and arterial inflammation (incidence of intimal arteritis of 16/133 (12.0%) vs. 19/334 (5.7%); p=0.019), compared with patients without anti-AT1R antibodies (n=334).

Anti-angiotensin II type 1 receptor antibody-associated rejection phenotype

Among 299 (16.2%) patients with histological features of active antibody-mediated rejection (microvascular inflammation with sum of glomerulitis and peritubular capillaritis Banff scores >1) (**Table 5**), the detection of anti-AT1R antibodies identified 51/77 (66.2%) patients as having active antibody-mediated rejection in patients without anti-HLA DSAs. Among them, 36 (70.6%) patients had pre-existing anti-AT1R antibodies detected in the sera collected at the time of transplantation and 15 (29.4%) patients were found to have de novo anti-AT1R antibodies. Patients with pre-existing anti-AT1R antibodies (n=36) showed increased levels of

anti-AT1R antibodies between the time of transplantation and the time of ABMR diagnosis ($p<0.001$, **Figure S2**).

Compared with patients with prototypical anti-HLA DSA-mediated rejection (HLA-DSA+, AT1R-Ab-, $n=147$), patients with anti-AT1R antibody-associated rejection (HLA-DSA-, AT1R-Ab+, $n=51$) exhibited greater prevalence of hypertension ($n=46/147$ (31.3%) vs. $n=32/51$ (62.7%); $p<0.001$), similar glomerular filtration rate (32.5 (20.7) vs. 34.2 (18.9) mL/min/1.73 m²; $p=0.61$) and proteinuria level (1.08 (1.60) vs. 0.93 (1.57); $p=0.56$), and similar cold ischemia time (18.3 (9.5) vs. 17.4 (10.4); $p=0.57$). Antibody-mediated rejection was diagnosed at a median time since transplantation of 11.3 months (IQR, 2.8 - 12.0) in patients with anti-AT1R antibody-associated rejection vs. 5.6 months (IQR, 2.8 - 11.3) in patients with anti-HLA DSA-mediated rejection ($p=0.11$). Patients with anti-AT1R antibody-associated rejection showed greater prevalence of arterial inflammation ($n=18/147$ (12.2%) vs. $n=22/51$ (43.1%); $p<0.001$), decreased prevalence of complement deposition in peritubular capillaries ($n=73/147$ (49.7%) vs. $n=7/51$ (13.7%); $p<0.001$) and increased endothelial activation in allografts reflected by higher levels of expression of ENDATs ($p=0.013$) compared to patients with prototypical anti-HLA DSA-mediated rejection. **Figure 3** illustrates the pattern of complement-independent antibody-mediated vascular rejection observed in kidney allografts of patients with circulating anti-AT1R antibodies.

DISCUSSION

In a prospective cohort of 1845 kidney transplant recipients, we observed that the presence of circulating non-HLA agonistic anti-AT1R antibodies was associated with increased incidence of antibody-mediated allograft rejection at one year after transplantation and decreased long-term allograft survival. These increased risks of allograft rejection and allograft loss associated with anti-AT1R antibodies were present in patients without anti-HLA DSAs and in those with anti-HLA DSAs, with both a main effect and a synergistic effect of these antibodies on allograft injury. Anti-AT1R antibody-associated rejection was characterized by hypertension, active vascular lesions (microcirculation inflammation and arterial inflammation), increased expression of ENDATs, which revealed antibody interaction with the vascular endothelium,³⁴ and low levels of complement deposition in allograft capillaries. The detection of anti-AT1R antibodies identified 51 (66.2%) patients as having antibody-mediated rejection among 77 patients with histological features of antibody-mediated rejection, in whom a definitive diagnosis could not be reached due to the lack of serological evidence of anti-HLA DSAs.

The broadest evidence suggesting a role of non-HLA immunity in transplantation was provided by a large registry study that observed a significant association between the presence of lymphocytotoxic antibodies detected before transplantation and allograft survival in kidney recipients from HLA-identical sibling donors, although the nature of these antibodies and their pathogenic expression in allografts were not evaluated.³⁸ Description of antibody-mediated AT1R activation in vascular cells and proof of concept in animal model emphasized the vasculature as the critical interface between the recipient immune system and the transplanted organ even in the absence of classical complement activation features.¹⁰ Several studies have evaluated the potential role of agonistic antibodies that target AT1R and have reported

conflicting results. These studies were limited by small sample size, the inclusion of selected populations, the lack of systematic allograft biopsy performance for assessing correlations between immunological status and injury phenotype, and/or the lack of evaluation of concomitant anti-HLA DSA status using a sensitive technique.^{10-22,24,26-31} Therefore, no definitive conclusion has been reached regarding the clinical value of anti-AT1R antibody screening as part of the immunological assessment of transplant recipients, which, to date, remains entirely based on the HLA system.^{7,33}

Our data support that post-transplant non-HLA agonistic anti-AT1R antibodies may contribute to a specific type of kidney allograft rejection characterized by vascular injury with microvascular and arterial inflammation, endothelial activation and lack of complement activation. These findings are consistent with previous reports of kidney transplant recipients with anti-AT1R antibody-associated allograft injury who often demonstrated involvement of allograft arteries and lack of complement deposition.¹⁰ Moreover, we observed a biological gradient between the level of circulating anti-AT1R antibodies and allograft antibody-mediated injury and allograft loss, with exposure to higher levels of anti-AT1R antibodies being associated with greater prevalence of concomitant microcirculation and arterial injuries and greater incidence of allograft loss, reinforcing the potential causal role of these antibodies as mediators of allograft rejection. Biological plausibility is supported by the current knowledge regarding the direct endothelial pathogenicity of agonistic anti-AT1R antibodies attributed to AT1R activation process in different disease contexts ranging from allograft rejection with vascular involvement to severe systemic autoimmune vasculopathies.³⁹⁻⁴¹

One of the major limitations faced by current diagnostic standards in organ transplantation is represented by the diagnostic uncertainty in patients showing histological features of antibody-mediated rejection without evidence for donor-specific anti-HLA antibodies, with discordances between clinicians' diagnosis and reference standard in nearly 50% of cases.⁴² This diagnostic uncertainty leads to unresolved therapeutic questions because current approaches in patients with antibody-mediated rejection are directed towards removal of circulating anti-HLA DSAs,

blockade of their effects and reduction of their production.⁴³ The identification of anti-AT1R antibodies as a potential mediator of allograft rejection and loss, independent of the presence of anti-HLA DSAs, has important implications for the clinical management of transplant recipients by enabling the development of specific therapeutic strategies. Future studies should evaluate the efficacy of current antibody-targeting therapies, such as plasma exchange and intravenous immune globulins, which are the standard of care treatment for anti-HLA antibody-mediated allograft rejection.⁴³ Moreover, our study paves the way for future therapeutical approaches; a selective blockade of AT1Rs using sartans could alleviate allograft vascular injury and prevent long-term allograft loss mediated by agonistic anti-AT1R antibodies. Reports have actually indicated good outcomes using AT1R blockers for treating kidney transplant recipients with anti-AT1R antibody-associated rejection in terms of clinical and histological course⁴⁴ and allograft survival¹⁰, as well as for preventing kidney allograft rejection in recipients with high levels of circulating anti-AT1R antibodies.⁴⁵ However, no clinical trial has been conducted to date to demonstrate the benefit of such approaches. In addition, AT1R blockers may exert a clinically relevant immunomodulatory effect exemplified by a reduction in IFN- γ generation by T cells as demonstrated in vitro and in vivo.⁴⁶ Finally, the low level of complement activation observed in patients with anti-AT1R antibody-mediated rejection suggests that strategies based on complement inhibition, such as terminal C5 or proximal C1 component blockade that have been used in patients with anti-HLA DSAs⁴⁷ may not be relevant in patients with anti-AT1R antibody-mediated rejection. Potential therapeutic advances in anti-AT1R antibody-mediated vascular rejection may offer new opportunities for the management of systemic autoimmune vasculopathies related to AT1R activation.

Our study has some limitations. The assessment of anti-AT1R antibody status and allograft injury was limited to the first year after transplantation to limit the confounding background of previous allograft injuries. One has to consider potential inter-laboratory variability of anti-AT1R antibody measurement, which remains to be investigated, although most studies have reported a comparable cut-point for anti-AT1R antibody positivity. Additionally, there might be residual confounding in the association between anti-AT1R antibodies and allograft survival

and injury represented by other non-HLA antibodies. Finally, although our data indicate a clinical value of anti-AT1R antibody assessment in patients with pathological features of antibody-mediated rejection (with or without detectable anti-HLA DSAs), specific studies dedicated to the evaluation of cost-effectiveness, including the consideration of resource-limited settings and the cost related to allograft biopsies triggered by anti-AT1R antibody positivity, are needed to substantiate the utility of a systematic monitoring of anti-AT1R antibodies as part of the standard of care management of kidney transplant recipients. Nevertheless, given the strong phenotypic association with C4d negative ABMR one should consider anti-AT1R antibodies as an important diagnostic tool.

In conclusion, our study showed the clinical relevance of the screening for circulating functional non-HLA anti-AT1R antibodies in addition to the current approach for immunological assessment of kidney transplant recipients by identifying patients at high risk of allograft rejection and allograft loss independent of the HLA system. We completed the current understanding of autoimmune endothelial injury related to anti-AT1R autoantibodies, including systemic vascular disorders such as pre-eclampsia and scleroderma, by identifying a new pattern of allograft vascular rejection associated with anti-AT1R antibodies in kidney transplant recipients. Recognition of complement-independent anti-AT1R antibody-mediated vascular rejection could lead to the development of new treatment strategies targeting circulating antibodies and AT1Rs to improve allograft survival, in particular in forms of allograft rejection without evidence for HLA antibody involvement, for which no therapeutic strategy has been defined. The clinical relevance of anti-AT1R antibodies might be extended beyond renal transplantation to heart transplantation and other solid organs.

METHODS

Participants and study design

We prospectively enrolled all consecutive patients who underwent ABO blood group compatible kidney transplantation in Necker Hospital and Saint-Louis Hospital (Paris, France) between January 1, 2008, and December 31, 2012 (n=1957) (ClinicalTrials.gov, NCT03466775). These kidney recipients underwent allograft evaluation including assessment of allograft function, circulating anti-HLA DSAs and anti-AT1R antibodies, and histological parameters measured on allograft biopsy within the first year after transplantation. Allograft evaluation was performed at the time of the first episode of biopsy-proven allograft rejection occurring in the first year after transplantation or by protocol at one year after transplantation in patients without any rejection episodes diagnosed in the first year after transplantation. We excluded patients receiving multi-organ transplantation (n=19), patients without allograft biopsy performed within the first year after transplantation (n=56), patients with inadequate allograft biopsy according to the Banff classification for allograft rejection⁴⁸ (n=21), patients without available serum for the assessment of anti-AT1R antibodies (n=10) or anti-HLA DSAs (n=6). Patients were followed annually up until December 31, 2017.

The transplantation allocation system followed the rules of the French national agency for organ procurement (Agence de la Biomédecine). All transplants were performed with negative standard National Institutes of Health and anti-human globulin T and B cell cytotoxicity crossmatches on serum obtained at the time of transplantation. All patients provided written informed consent.

Clinical data

The clinical data on donors and recipients were extracted from the DIVAT database:

(www.divat.fr). Coding was used to ensure strict donor and recipient anonymity. The data were entered into the database in real time or at each transplant anniversary. This database is approved by the National French Commission for Bioinformatics Data and Patient Liberty (Commission Nationale de l'Informatique et des Libertés registration no. 1016618; validated June 8, 2004). The data were retrieved from the database on December 31, 2017. Renal function was assessed by the estimated glomerular filtration rate with the abbreviated Modification of Diet in Renal Disease formula.⁴⁹ Hypertension was defined by a systolic blood pressure ≥ 140 mmHg and/or a diastolic blood pressure ≥ 90 mmHg.

Detection of anti-angiotensin II type 1 antibodies and donor-specific anti-HLA antibodies

All patients were tested for the presence of circulating anti-AT1R antibodies using a quantitative ELISA (Celltrend, Luckenwalde, Germany). A value of 10 U/mL was considered the cut-off point for anti-AT1R antibody positivity after examining prior studies of its association with allograft rejection and loss;^{12,14-17,20-22,24,28,31} this cut-off point also represented the third quartile value in the distribution of anti-AT1R antibody values in the entire study population (**Figure S3**).

The presence of circulating anti-HLA-A, -B, -Cw, -DR, -DQ, and -DP DSAs was assessed using single-antigen flow bead assays (One Lambda, Inc., Canoga Park, CA, USA) that were used on a Luminex platform (Regional Histocompatibility Laboratory, Paris, France). All beads showing a normalized mean fluorescence intensity (MFI) >1000 were considered positive. HLA typing of all the kidney transplant donors and recipients was performed by molecular biology (Innolipa HLA Typing Kit, Innogenetics, Gent, Belgium).

Histological and immunochemical phenotyping of kidney allograft biopsies

All allograft biopsy specimens (304 allograft biopsies performed for clinical indication, with a median time between transplantation and allograft biopsy of 3.8 months (interquartile range, 2.3-7.3), and 1541 protocol biopsies performed at one year after transplantation) were scored and graded from 0 to 3 according to the updated Banff criteria³⁴ by a trained pathologist. The

pathologist examined the following histological factors: glomerulitis, peritubular capillaritis, mononuclear cell interstitial inflammation, tubulitis, intimal arteritis, chronic allograft glomerulopathy, interstitial fibrosis and tubular atrophy, arteriolar hyaline thickening, and vascular fibrous intimal thickening. The pathologist was blinded to the clinical data and to the original pathological report. Complement split-product C4d staining was performed by immunochemical analysis on paraffin sections using polyclonal rabbit anti-human C4d antibodies (Biomedica Gruppe, Vienna, Austria).

Allograft diagnoses were defined according to the Banff classification. Active ABMR was defined by i) histologic evidence of acute tissue injury, including 1 or more of the following: glomerulitis score >0 and/or peritubular capillaritis score >0 (in the absence of recurrent or de novo glomerulonephritis and with glomerulitis score ≥ 1 in case of acute T cell-mediated rejection, borderline infiltrate, or infection), intimal arteritis score >0 , acute thrombotic microangiopathy in the absence of any other cause, acute tubular injury in the absence of any other apparent cause; ii) evidence of current/recent antibody interaction with vascular endothelium, including 1 or more of the following: C4d score >0 , glomerulitis + peritubular capillaritis scores ≥ 2 ; and iii) serologic evidence of anti-HLA DSAs and/or anti-AT1R antibodies.

RNA extraction and gene expression analysis in kidney allograft biopsies

Kidney allograft biopsies showing pathological features of active antibody-mediated rejection, as defined by the Banff classification for allograft rejection (microcirculation inflammation with sum of glomerulitis and peritubular capillaritis Banff scores >1)³⁴ were processed for microarray analysis as previously described.⁵⁰ One biopsy sample was immediately placed in a dry tube and stored at -80°C . RNA extraction, labelling and hybridization to HG-U219 GeneChip arrays (Affymetrix, Santa Clara, CA, USA) were performed according to the manufacturer's protocols (www.affymetrix.com). The microarrays were scanned using the Gene Array Scanner (Affymetrix) and processed with GeneChip Operating Software Version 1.4.0 (Affymetrix), and robust multiarray averaging was used to normalize the microarrays. The microarray data files were processed using robust multiarray analysis in Bioconductor.

We measured and compared intra-graft expression levels of ENDATs according to anti-AT1R antibodies and anti-HLA DSA status. ENDATs represent a pathogenesis-based transcript set that reflects endothelial activation.⁵¹ The details of the probe set can be found in **Table S3**.

Outcomes

The primary outcomes were kidney allograft loss and the incidence of biopsy-proven allograft rejection at one year post-transplantation. Kidney allograft loss was defined as the patient's return to chronic dialysis or preemptive subsequent kidney transplant. Allograft rejection episodes were diagnosed according to the Banff classification for allograft rejection.³⁴ Secondary outcomes included allograft injury phenotype based on histological allograft elementary lesions defined by the Banff classification and ENDAT expression measured in kidney allograft biopsies showing histological features of active antibody-mediated rejection (sum of glomerulitis and peritubular capillaritis Banff scores >1).

Statistical analysis

Continuous variables were described using means with standard deviations (SDs) unless otherwise stated. We compared means and proportions using Student's t-test and chi-square test, respectively (or Mann-Whitney U test and Fisher's exact test, respectively, if appropriate). Survival was analyzed from the time of post-transplant allograft biopsy to a maximum of seven years after allograft evaluation, with kidney allograft loss as the event of interest. In case of death with a functioning allograft, allograft survival was censored at time of death. Rates of kidney allograft survival were plotted on Kaplan-Meier curves and compared according to anti-AT1R antibodies and anti-HLA DSA status with the use of the log-rank test. Cox proportional hazards models were used to quantify hazard ratios (HR) and 95% confidence intervals (CI) for time to kidney allograft loss. The association of clinical, histological, and immunological parameters evaluated at the time of allograft evaluation with allograft loss were assessed in separate univariate and multivariable Cox regression analyses. The best transformation for continuous variables was determined using

fractional polynomial method. A p-value threshold of 0.20 for entering variables into the multivariable model was used. The factors identified in these analyses were thereafter included in a final multivariable model with stepwise backward elimination. The proportional hazards assumption of the Cox model was verified with the log-graphic method.

We used penalized splines to represent the relationship between anti-AT1R antibody levels and the odds ratio for the presence of glomerulitis, peritubular capillaritis and intimal arteritis and the HR for the occurrence of allograft loss. Biologic plausibility (that is, monotonicity) was considered, along with Akaike's information criterion, in selecting the optimal degrees of freedom.⁵²

All tests were two-sided, and except in the univariate analyses, all p-values <0.05 were regarded as statistically significant. All of the statistical analyses were performed using R version 3.4.3 (R Development Core Team, Vienna, Austria).

Contributors

C Lefaucheur, AL and DD conceived and designed the study. C Lefaucheur, DV, YB, DP, AL and DD drafted the manuscript. C Lefaucheur, DV, YB, AP, OA, JPDVH, JLT, PH, AL, DG, C Legendre and DD analyzed and interpreted the data. AP prepared the serum samples. JPDVH reported pathology results. All authors were involved in revising the article and approved the final version of the manuscript. All the authors had full access to the data and made the decision collectively to submit the manuscript for publication.

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Study approval

This study was approved by Institutional Review Boards of Necker and Saint-Louis Hospitals. The

DIVAT database network has been approved by the National French Commission for bioinformatics data and patient liberty: CNIL, registration number: 1016618, validated 8th June 2004.

Conflict of interests

The authors declare that they had no financial relationships with any organizations that might have an interest in the submitted work in the past three years and no other relationships or activities that could appear to have influenced the submitted work.

Supplementary Material

Supplementary Table 1. Kidney allograft injury at the time of antibody screening according to the presence of circulating anti-angiotensin II type 1 receptor antibodies and stratified on the basis of allograft biopsy indication.

Supplementary Table 2. Kidney allograft injury at the time of antibody screening according to the presence of circulating anti-angiotensin II type 1 receptor antibodies and stratified on the basis of donor-specific anti-HLA antibody status.

Supplementary Table 3. Endothelial-associated transcript (ENDAT) gene list with their corresponding probe set.

Supplementary Figure 1. Risk of kidney allograft loss according to post-transplant circulating anti-angiotensin II type 1 receptor antibody level.

Supplementary Figure 2. Distribution of pre-transplant and post-transplant circulating anti-angiotensin II type 1 receptor antibody levels in patients with pre-existing anti-AT1R antibody-associated rejection without anti-HLA DSAs.

Supplementary Figure 3. Distribution of post-transplant circulating anti-angiotensin II type 1 receptor antibody levels.

Supplementary Methods. Induction and maintenance immunosuppressive therapy protocols.

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Table 1. Demographic and clinical characteristics according to the presence of circulating anti-angiotensin II type 1 receptor antibodies.

Characteristics	All patients (n=1845)	AT1R-Ab negative (n=1341)	AT1R-Ab positive (n=504)	p
Recipient age , mean (SD), years	48.6 (13.2)	49.3 (12.6)	46.9 (14.5)	0.001
Recipient female gender , No. (%)	744 (40.3)	497 (37.1)	247 (49.0)	<0.001
Recipient blood type , No. (%)				0.046
A	852 (46.2)	639 (47.7)	213 (42.3)	
B	162 (8.8)	122 (9.1)	40 (7.9)	
O	754 (40.9)	531 (39.6)	223 (44.2)	
AB	77 (4.1)	49 (3.6)	28 (5.6)	
Chronic kidney disease , No. (%)				0.160
Glomerulopathy	481 (26.1)	335 (25.0)	146 (29.0)	
Vascular nephropathy	141 (7.6)	108 (8.1)	33 (6.5)	
Chronic interstitial nephropathy	224 (12.1)	166 (12.4)	58 (11.5)	
Congenital nephropathy	355 (19.3)	269 (20.1)	86 (17.1)	
Diabetes	167 (9.1)	114 (8.4)	53 (10.5)	
Other	76 (4.1)	61 (4.5)	15 (3.0)	
Not determined	401 (21.7)	288 (21.5)	113 (22.4)	
Previous transplantation , No. (%)	334 (18.1)	216 (16.1)	118 (23.4)	<0.001
Preemptive transplantation , No. (%)	242 (13.1)	176 (13.1)	66 (13.1)	0.987
Time since dialysis , mean (SD), years	4.9 (4.6)	4.9 (4.6)	4.9 (4.6)	0.852
Donor age , mean (SD), years	51.5 (16.3)	52.1 (16.0)	50.0 (17.0)	0.015
Donor male gender , No. (%)	1008 (54.6)	715 (53.3)	293 (58.1)	0.064
Donor type , No. (%)				0.001
Living	336 (18.2)	220 (16.4)	116 (23.0)	
Deceased	1509 (81.8)	1121 (83.6)	388 (77.0)	
Donor serum creatinine , mean (SD), $\mu\text{mol/L}$	86.6 (51.2)	87.4 (54.1)	84.7 (42.4)	0.266
Cold ischemia time , mean (SD), hours	17.7 (9.9)	18.0 (10.0)	16.8 (9.8)	0.017

HLA A mismatch, No. (%)				0.713
0	451 (24.5)	328 (24.5)	123 (24.4)	
1	949 (51.4)	696 (51.9)	253 (50.2)	
2	445 (24.1)	317 (23.6)	128 (25.4)	
HLA B mismatch, No. (%)				0.030
0	344 (18.6)	231 (17.2)	113 (22.4)	
1	894 (48.5)	667 (49.7)	227 (45.1)	
2	607 (32.9)	443 (33.1)	164 (32.5)	
HLA DR mismatch, No. (%)				0.768
0	548 (29.7)	393 (29.3)	155 (30.7)	
1	967 (52.4)	704 (52.5)	263 (52.2)	
2	330 (17.9)	244 (18.2)	86 (17.1)	
HLA DQ mismatch, No. (%)				0.959
0	482 (26.1)	350 (26.1)	132 (26.2)	
1	1011 (54.8)	733 (54.7)	278 (55.2)	
2	352 (19.1)	258 (19.2)	94 (18.6)	
Calculated panel reactive antibody, No. (%)				<0.001
0%	471 (25.5)	393 (29.3)	78 (15.5)	
1-24%	289 (15.7)	215 (16.0)	74 (14.7)	
25-49%	250 (13.5)	169 (12.6)	81 (16.1)	
50-84%	356 (19.3)	241 (18.0)	115 (22.8)	
85-100%	479 (26.0)	323 (24.1)	156 (30.9)	
Induction therapy, No. (%)				0.443
Thymoglobuline	1287 (69.8)	925 (69.0)	362 (71.8)	
Anti-CD25 therapy	554 (30.0)	413 (30.8)	141 (28.0)	
None	4 (0.22)	3 (0.22)	1 (0.20)	
Maintenance immunosuppression, No. (%)				
IMDPHi	1776 (96.3)	1296 (96.6)	480 (95.2)	0.156
Tacrolimus	1267 (68.7)	905 (67.5)	362 (71.8)	0.073
Cyclosporine	559 (30.3)	423 (31.5)	136 (27.0)	0.058
mTOR inhibitor	23 (1.2)	16 (1.2)	7 (1.4)	0.814
Steroids	1612 (87.4)	1161 (86.6)	451 (89.5)	0.094
Glomerular filtration rate, mean (SD), mL/min/1.73 m²	54.8 (20.9)	55.9 (21.5)	51.9 (19.0)	<0.001
Urine protein/creatinine ratio, mean (SD), g/g	0.34 (0.78)	0.28 (0.60)	0.52 (1.14)	<0.001
Post-transplant HLA DSA, No. (%)	467 (25.3)	334 (24.9)	133 (26.4)	0.514
All HLA DSA				
No. of specificities, mean (SD)	1.9 (1.3)	1.9 (1.2)	2.0 (1.5)	0.684
HLA class, No. (%)				0.817
I	96 (20.5)	71 (21.3)	25 (18.7)	
II	207 (44.2)	146 (43.7)	61 (45.5)	

I+II	165 (35.3)	117 (35.0)	48 (35.8)	
Immunodominant HLA DSA*				
HLA class, No. (%)				0.373
I	144 (30.8)	107 (32.0)	37 (27.8)	
II	323 (69.2)	227 (68.0)	96 (72.2)	
MFI, mean (SD, range)	5177 (4434, 1103- 20746)	5055 (4481, 1103- 20746)	5486 (4314, 1215- 18755)	0.255
HLA DSA detectable before transplantation	342 (18.5)	233 (17.5)	109 (21.4)	0.053
Highest MFI, mean (SD, range)	3974 (4074, 1012- 12323)	3917 (4139, 1012- 12323)	4096 (3936, 1158- 11827)	0.669
Desensitization therapy, No. (%)	136 (7.4)	89 (6.6)	47 (9.3)	0.049

Ab, antibody; AT1R, angiotensin II type 1 receptor; DSA, donor-specific antibody; HLA, human leukocyte antigen; IMPDHi, inosine-5'-monophosphate dehydrogenase inhibitor; MFI, mean fluorescence intensity; mTOR, mammalian target of rapamycin; SD, standard deviation

*The highest mean fluorescence intensity value toward a donor-specific allele was considered to be the immunodominant DSA

Table 2. Determinants of kidney allograft loss assessed at the time of antibody screening: Univariate analysis.

	Number of patients	Number of events	HR	95%CI	p
Clinical					
Recipient age , per 1-year increment	1845	285	0.99	0.98-1.00	0.117
Recipient gender					
Male	1101	178	1		
Female	744	107	0.88	0.69-1.12	0.309
Type of allograft biopsy					
One-year biopsy	1541	189	1		
Clinical indication biopsy in the 1 st year	304	96	2.68	2.09-3.42	<0.001
GFR (log transformed) , per 1-mL/min/1.73 m ² increment	1845	285	0.14	0.11-0.18	<0.001
Proteinuria (square root value) , per 1-g/g increment	1845	285	3.31	2.80-3.92	<0.001
Previous transplantation					
No	1511	210	1		
Yes	334	75	1.75	1.34-2.27	<0.001
Preemptive transplantation					
No	1603	277	1		
Yes	242	8	0.16	0.08-0.33	<0.001
Time since dialysis , per 1-year increment	1603	277	1.03	1.00-1.05	0.031
Donor age , per 1-year increment	1845	285	1.01	1.00-1.02	0.002
Donor gender					
Male	1008	150	1		
Female	837	135	1.09	0.87-1.38	0.458
Donor type					
Living	336	33	1		
Deceased	1509	252	1.81	1.26-2.61	0.001
Cold ischemia time , per 1-hour increment	1845	285	1.02	1.01-1.04	<0.001
No. of HLA A/B/DR/DQ mismatch , per 1-unit increment	1845	285	1.02	0.92-1.12	0.749
Pre-transplant anti-HLA DSA					
No	1503	200	1		
Yes	342	85	2.06	1.60-2.65	<0.001
Immunology					
AT1R and HLA antibody status					<0.001

None	1007	106	1		
Anti-AT1R antibody	371	60	1.59	1.16-2.18	
Donor-specific anti-HLA antibody	334	74	2.27	1.69-3.05	
Anti-AT1R and donor-specific anti-HLA antibody	133	45	3.88	2.74-5.50	
Histology					
Glomerulitis + Peritubular capillaritis					
Banff score ≤1	1546	186	1		
Banff score >1	299	99	3.13	2.46-4.00	<0.001
Interstitial inflammation + Tubulitis					
Banff score ≤1	1528	211	1		
Banff score >1	317	74	1.75	1.34-2.28	<0.001
Intimal arteritis					
Banff score 0	1736	254	1		
Banff score >0	109	31	2.16	1.53-3.08	<0.001
Chronic allograft glomerulopathy					
Banff score 0	1755	260	1		
Banff score >0	90	25	2.13	1.41-3.22	<0.001
Interstitial fibrosis and tubular atrophy					
Banff score ≤1	1354	178	1		
Banff score >1	491	107	1.83	1.44-2.33	<0.001
Arteriosclerosis					
Banff score ≤1	1183	166	1		
Banff score >1	662	119	1.40	1.10-1.76	0.006
Arteriolar hyalinosis					
Banff score ≤1	1537	217	1		
Banff score >1	308	68	1.71	1.30-2.25	<0.001
C4d deposition in peritubular capillaries					
Banff score 0	1660	225	1		
Banff score >0	185	60	2.68	2.02-3.57	<0.001

AT1R, angiotensin II type 1; CI, confidence interval; GFR, glomerular filtration rate; HLA, human leukocyte antigen; HR, hazard ratio

Table 3. Determinants of kidney allograft loss assessed at the time of antibody screening: Multivariable model.

	Number of patients	Number of events	HR	95%CI	p
GFR (log transformed) , per 1- mL/min/1.73 m ² increment	1845	285	0.21	0.16-0.29	<0.001
Proteinuria (square root value) , per 1-g/g increment	1845	285	2.25	1.85-2.73	<0.001
AT1R and HLA antibody status					<0.001
None	1007	106	1		
Anti-AT1R antibody	371	60	1.49	1.07-2.06	
Donor-specific anti-HLA antibody	334	74	1.38	1.05-1.84	
Anti-AT1R and donor-specific anti- HLA antibodies	133	45	2.26	1.52-3.36	
Glomerulitis + Peritubular capillaritis					
Banff score ≤1	1546	186	1		
Banff score >1	299	99	1.79	1.32-2.43	<0.001
Interstitial fibrosis and tubular atrophy					
Banff score ≤1	1354	178	1		
Banff score >1	491	107	1.29	1.00-1.67	0.046

AT1R, angiotensin II type 1; CI, confidence interval; GFR, glomerular filtration rate; HLA, human leukocyte antigen; HR, hazard ratio

Table 4. Kidney allograft injury according to the presence of circulating anti-angiotensin II type 1 receptor antibodies.

Data are based on 1845 kidney transplant recipients who were assessed simultaneously for the presence of circulating anti-AT1R antibodies and kidney allograft histology within the first year after transplantation. Histological assessment of kidney allograft injury was performed according to the Banff classification for allograft rejection. Each individual lesion was considered present (Banff score of 1, 2 or 3) or absent (Banff score of 0). Pathological features of active antibody-mediated rejection were defined by microcirculation inflammation with the sum of glomerulitis and peritubular capillaritis Banff scores >1 according to the Banff classification.

	Overall (n=1845)	AT1R-Ab negative (n=1341)	AT1R-Ab positive (n=504)	p
Type of allograft biopsy, No. (%)				0.262
Clinical indication biopsy	304 (16.5)	213 (15.9)	91 (18.1)	
One-year protocol biopsy	1541 (83.5)	1128 (84.1)	413 (81.9)	
Glomerulitis, No. (%)	424 (23.0)	277 (20.7)	147 (29.2)	<0.001
Peritubular capillaritis, No. (%)	377 (20.4)	232 (17.3)	145 (28.8)	<0.001
Interstitial inflammation, No. (%)	393 (21.3)	281 (21.0)	112 (22.2)	0.553
Tubulitis, No. (%)	381 (20.7)	269 (20.1)	112 (22.2)	0.307
Intimal arteritis, No. (%)	109 (5.9)	53 (4.0)	56 (11.1)	<0.001
Chronic allograft glomerulopathy, No. (%)	90 (4.9)	58 (4.3)	32 (6.4)	0.072
Interstitial fibrosis and tubular atrophy, No. (%)	1095 (59.4)	789 (58.8)	306 (60.7)	0.464
Arteriosclerosis, No. (%)	1187 (64.3)	872 (65.0)	315 (62.5)	0.313
Arteriolar hyalinosis, No. (%)	1084 (58.8)	794 (59.2)	290 (57.5)	0.516
C4d deposition, No. (%)	185 (10.0)	125 (9.3)	60 (11.9)	0.100
Pathological features of ABMR with donor-specific anti-HLA antibodies, No. (%)	222 (12.0)	147 (11.0)	75 (14.8)	0.021
Pathological features of ABMR without donor-specific anti-HLA antibodies, No. (%)	77 (4.2)	26 (1.9)	51 (10.1)	<0.001
T-cell mediated rejection, No. (%)	182 (9.9)	128 (9.6)	54 (10.7)	0.453

Ab, antibody; ABMR, active antibody-mediated rejection; AT1R, angiotensin II type 1 receptor; HLA, human leukocyte antigen

Table 5. Kidney allograft injury according to the presence of post-transplant circulating anti-angiotensin II type 1 receptor antibodies and circulating donor-specific anti-HLA antibodies in patients with histological features of active antibody-mediated rejection.

Data are based on 1845 kidney allograft biopsies performed within the first year after transplantation, in which 299 (16.2%) showed histological features of active antibody-mediated rejection (sum of glomerulitis and peritubular capillaritis Banff scores >1). Histological assessment of kidney allograft injury was performed according to the Banff classification for allograft rejection. Each individual lesion was considered present (Banff score of 1, 2 or 3) or absent (Banff score of 0).

	Overall (n=299)	AT1R-Ab- HLA-DSA- (n=26)	AT1R-Ab+ HLA-DSA- (n=51)	AT1R-Ab- HLADSA+ (n=147)	AT1R-Ab+ HLADSA+ (n=75)	p
Type of allograft biopsy, No. (%)						0.001
Clinical indication biopsy	175 (58.5)	7 (26.9)	29 (56.9)	84 (57.1)	55 (73.3)	
One-year protocol biopsy	124 (41.5)	19 (73.1)	22 (43.1)	63 (42.9)	20 (26.7)	
Glomerulitis, No. (%)	285 (95.3)	22 (84.6)	49 (96.1)	141 (95.9)	73 (97.3)	0.094
Peritubular capillaritis, No. (%)	285 (95.3)	20 (76.9)	49 (96.1)	141 (95.9)	75 (100)	<0.001
Interstitial inflammation, No. (%)	117 (39.1)	12 (46.2)	21 (41.2)	57 (38.8)	27 (36.0)	0.805
Tubulitis, No. (%)	102 (34.1)	8 (30.8)	21 (41.2)	49 (33.3)	21 (28.0)	0.484
Intimal arteritis, No. (%)	59 (19.7)	3 (11.5)	22 (43.1)	18 (12.2)	16 (21.3)	<0.001
Chronic allograft glomerulopathy, No. (%)	50 (16.7)	2 (7.7)	6 (11.8)	26 (17.7)	16 (21.3)	0.338
Interstitial fibrosis and tubular atrophy, No. (%)	219 (73.2)	14 (53.9)	41 (80.4)	108 (73.5)	56 (74.7)	0.111
Arteriosclerosis, No. (%)	202 (67.6)	17 (65.4)	31 (60.8)	100 (68.0)	54 (72.0)	0.598
Arteriolar hyalinosis, No. (%)	173 (57.9)	15 (57.7)	32 (62.8)	88 (59.9)	38 (50.7)	0.509
C4d deposition, No. (%)	121 (40.5)	2 (7.7)	7 (13.7)	73 (49.7)	39 (52.0)	<0.001

Ab, antibody; AT1R, angiotensin II type 1 receptor; DSA, donor-specific antibody; HLA, human leukocyte antigen

FIGURE LEGENDS

Figure 1. Kaplan-Meier curves for death-censored kidney allograft survival after antibody screening according to post-transplant circulating anti-angiotensin II type 1 receptor antibody status.

Data are based on 1845 kidney transplant recipients undergoing systematic screening for circulating donor-specific anti-HLA antibodies and anti-angiotensin II type 1 receptor antibodies within the first year after transplantation. Panel A shows Kaplan-Meier curves for death-censored kidney allograft survival on the basis of the presence of post-transplant anti-angiotensin II type 1 receptor antibodies. Panel B shows Kaplan-Meier curves for death-censored kidney allograft survival according to post-transplant anti-angiotensin II type 1 receptor antibody status and post-transplant donor-specific anti-HLA antibody status.

Ab, antibody; AT1R, angiotensin II type 1 receptor; DSA, donor-specific antibody; HLA, human leukocyte antigen

Figure 2. Risk of occurrence of microcirculation and arterial injury according to post-transplant circulating anti-angiotensin II type 1 receptor antibody level.

Data are based on 1845 kidney transplant recipients who were assessed simultaneously for the presence of circulating anti-AT1R antibodies and kidney allograft histology within the first year after transplantation. The blue curve represents the relationship between circulating anti-AT1R antibody level and the risk of occurrence of glomerulitis (Panel A), peritubular capillaritis (Panel B) and intimal arteritis (Panel C) in allografts on the basis of penalized splines. Dashed lines denote 95% confidence intervals. The grey bars represent individual anti-AT1R antibody levels. The spline curves were truncated at anti-AT1R antibody level of 20 U/mL given the low number of patients above this value (n=74/1845, 4.0%). Significant odds ratios are observed from AT1R antibody levels between 10 and 15 U/mL for glomerulitis (A), peritubular capillaritis (B) and intimal arteritis (C), where the lower limit of the 95% confidence intervals are above an odds ratio of 1 (red line).

AT1R, angiotensin II type 1 receptor; OR, odds ratio

Figure 3. Complement-independent antibody-mediated vascular kidney allograft rejection in patients with circulating anti-angiotensin II type 1 receptor antibodies.

Data are based on 1845 kidney transplant recipients who were assessed simultaneously for the presence of circulating anti-angiotensin II type 1 receptor antibodies and kidney allograft histology within the first year after transplantation, in whom 299 (16.2%) showed histological features of active antibody-mediated rejection according to the Banff classification for allograft rejection (microcirculation inflammation with sum of glomerulitis and peritubular capillaritis Banff scores >1). Among patients with histological features of active antibody-mediated rejection, 51 (17.0%) had anti-angiotensin II type 1 receptor antibodies, 147 (49.2%) had donor-specific anti-HLA antibodies, 75 (25.1%) had both antibodies and 26 (8.7%) had no antibody. Anti-angiotensin II type 1 receptor antibody-associated rejection was characterized by vascular inflammation with microvascular and arterial inflammation, endothelial activation reflected by high levels of expression of endothelial-associated transcripts, and lack of complement deposition in allograft capillaries. **(A)** Severe intimal arteritis in a patient with anti-angiotensin II type 1 receptor antibody-associated rejection characterized by subendothelial infiltration of an interlobular artery by inflammatory cells; Masson's trichrome stain, original magnification x200, bar = 200 µm. **(B)** Peritubular capillaritis and lack of complement fraction C4d deposition in peritubular capillaries in the same patient with anti-

angiotensin II type 1 receptor antibody-associated rejection; immunoperoxidase, original magnification x400, bar = 200 μ m. **(C)**. Expression levels of endothelial-associated transcripts (ENDATs) in patients with features of active antibody-mediated rejection according to anti-angiotensin II type 1 receptor antibody and donor-specific anti-HLA antibody status. Expression levels of ENDATs were measured using microarray. ENDATs represent a pathogenesis-based transcript set that reflects endothelial activation and indicates current/recent antibody interaction with the vascular endothelium.

Ab, antibody; AT1R, angiotensin II type 1 receptor; DSA, donor-specific antibody; HLA, human leukocyte antigen

A

Number at risk

AT1R-Ab negative 1341 1264 1210 1118 1033 954 874 734

AT1R-Ab positive 504 469 437 399 364 321 297 257

B

Number at risk

AT1R-Ab negative/HLA-DSA negative 1007 953 915 852 792 740 683 589

AT1R-Ab positive/HLA-DSA negative 371 349 332 303 283 251 236 207

AT1R-Ab negative/HLA-DSA positive 334 311 295 266 241 214 191 145

AT1R-Ab positive/HLA-DSA positive 133 120 105 96 81 70 61 50

Non-HLA Agonistic Anti-Angiotensin II Type 1 Receptor Antibodies Induce a Distinctive Phenotype of Antibody-mediated Rejection in Kidney Transplant Recipients

Prospective cohort

1845 kidney transplant patients

Allograft evaluation within the 1st yr post-tx

- AT1R Abs
- HLA DSAs
- Graft function
- Graft biopsy

AT1R Ab-associated C4d-negative vascular rejection

Allograft survival

Number at risk

AT1R-Ab negative/HLA-DSA negative	1007	953	915	852	792	740	683	589
AT1R-Ab positive/HLA-DSA negative	371	349	332	303	283	251	236	207
AT1R-Ab negative/HLA-DSA positive	334	311	295	266	241	214	191	145
AT1R-Ab positive/HLA-DSA positive	133	120	105	96	81	70	61	50

CONCLUSION:

Circulating AT1R Abs associate with a specific kidney allograft rejection phenotype and graft loss, independent of the HLA system.