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Donor–Recipient Matching Based on Predicted Indirectly Recognizable HLA Epitopes Independently Predicts the Incidence of *De Novo* Donor-Specific HLA Antibodies Following Renal Transplantation

N. Lachmann[1],*, M. Niemann[2], P. Reinke[3],†,
K. Budde[3], D. Schmidt[3], F. Halleck[3], A. Pruß[4],
C. Schönemann[1], E. Spierings[5] and O. Staeck[3]

[1]*Center for Tumor Medicine, H&I Laboratory, Charité University Medicine Berlin, Berlin, Germany*
[2]*PIRCHE AG, Berlin, Germany*
[3]*Department of Nephrology, Charité University Medicine Berlin, Berlin, Germany*
[4]*Universitary Tissue Bank, Charité University Medicine Berlin, Berlin, Germany*
[5]*UMC Utrecht, Laboratory of Translational Immunology, Utrecht, The Netherlands*
*Corresponding author: Nils Lachmann,
nils.lachmann@charite.de
†*Coordinator EU FP7 BIODrIM*

De novo **donor-specific HLA antibodies (dnDSA) are recognized as a risk factor for premature allograft failure. Determinants of DSA specificity are generated via the indirect allorecognition pathway. Here, we present supportive data for the relevance of predicted indirectly recognizable HLA epitopes (PIRCHE) to predict dnDSA following kidney transplantation. A total of 2787 consecutive kidney transplants performed between 1995 and 2015 without preformed DSA have been analyzed.** *De novo* **DSA were detected by single antigen bead assay. HLA epitope mismatches were determined by the HLAMatchmaker and PIRCHE approach and correlated in uni- and multivariate analyses with 10-year allograft survival and incidence of dnDSA. The PIRCHE-II score moderately predicted allograft survival. However, the predictive value of elevated PIRCHE-II scores >9 for the incidence of dnDSA was statistically significant (p < 0.001). In a multivariate Cox regression analysis adjusted for antigen mismatch and HLAMatchmaker epitopes, the PIRCHE-II score could be identified as an independent risk factor for dnDSA. The PIRCHE-II score independently from the antigen mismatch and HLAMatchmaker epitopes could be revealed as being a strong predictor for dnDSA. PIRCHE may help to identify acceptable mismatches with decreased risk of dnDSA and thus improve long-term renal allograft survival.**

Introduction

It is well accepted among transplant clinicians and immunologists that donor-specific HLA antibodies (HLAab) detected posttransplant are an independent risk factor for late deterioration of renal allograft function (1). However, the complexity of the underlying mechanisms is still not fully understood. Relevant antibody effector functions damaging the allograft may involve (i) activation of the complement cascade, (ii) activation of intracellular signal transduction pathways leading to tissue remodeling, and (iii) recruitment of immune effector cells (2,3). One important cornerstone of antibody-mediated rejection diagnosis is the presence of HLAab directed against the allograft (i.e. donor-specific HLAab [DSA]). As a response to the encountered mismatched donor HLA, corresponding antibodies may be produced by the recipient at any time point posttransplant. Several risk factors for the *de novo* development of HLAab have been identified so far: HLA class II mismatch, young recipient age, inadequate immunosuppression, and nonadherence (4). To date, histocompatibility of donor organs has been evaluated by classical alphanumeric matching of HLA class I and II antigens (5). However, it is well known that HLAab are directed against functional epitopes comprising a limited number of mismatched polymorphic amino acid (AA) residues (6). The prediction of HLA epitopes is performed by the HLAMatchmaker algorithm developed by Rene Duquesnoy (7). The algorithm is based on

theoretically designated patches of antibody-accessible polymorphic AA residues (eplets) present on HLA of the donor but not the recipient. Importantly, HLAMatchmaker may predict the humoral alloimmune response following pregnancies and transplantation but is not intended to predict T cell alloresponse (8).

DSA production occurs exclusively via the indirect allorecognition pathway in which foreign HLA is processed by the recipient's professional antigen-presenting cells and presented via HLA class II (primarily HLA-DRB1) to CD4+ T cells (2,9). Consequently, activated CD4+ T cells provide further help to other effector immune cells in the generation of alloreactive CD8 + T cells and antibody-producing B cells.

A novel tool to predict an alloimmune response is the PIRCHE (predicted indirectly recognizable HLA epitopes) algorithm (10), which predicts donor-HLA-derived peptides presented by the recipient's HLA-DRB1 molecules (PIRCHE-II). It is well known that AA variations in each HLA binding groove lead to unique peptide binding repertoires. It has been shown that PIRCHE-II predicts child-specific HLAab resulting from pregnancy (11). Otten et al (10) demonstrated in a rather small cohort of 21 renal transplant recipients that the PIRCHE algorithm may even predict DSA after allograft failure and nephrectomy. HLA mismatches that triggered *de novo* donor-specific HLA antibodies (dnDSA) revealed a higher PIRCHE-II score than the nonimmunogenic mismatches. However, both publications exclusively focused on HLA class I–derived PIRCHE-II, but in fact the majority of dnDSA are directed against HLA class II (12).

We hypothesized that the PIRCHE algorithm may be a better predictor for histocompatibility of kidney transplants, dnDSA formation, and allograft survival than classical antigen matching.

Materials and Methods

Patient population
We systematically reviewed our electronic patient record database "TBase" (13) for all consecutive renal transplants January 1995–December 2015. We included recipients ≥18 years of age of a kidney or combined kidney/pancreas transplant with complete HLA typing (at least HLA-A, B, DRB1) as well as comprehensive pre- and posttransplant HLAab follow-up. Most importantly, we included only patients with DSA surveillance by Luminex(R)-based assays (One Lambda, Canoga Park, CA) during follow-up and excluded transplants with primary nonfunction due to surgical complications. Finally, we enrolled a total of 2787 patients (Table 1).

According to standard center practice, the vast majority of patients analyzed in this study initially received a standard triple immunosuppressive protocol (calcineurin inhibitor, mycophenolate, and steroids) with induction therapy by anti-IL-2 receptor antibody. A few patients enrolled in clinical studies were treated with different protocols. Changes in immunosuppression and tapering of steroids were performed according to clinical needs or research protocols. Due to the heterogeneity of

Table 1: Patient characteristics (n = 2787)

Follow-up, years (SD)	7.2 (4.8)
Recipient age, years (SD)	49.9 (13.9)
Donor age, years (SD)	50.2 (15.8)
Female gender	1095 (39%)
Time on dialysis, years (IQR)	4.8 (2.1–7.0)
Prior kidney transplantation	322 (12%)
Living donor	623 (22%)
ABO incompatible	88 (3%)
Split-HLA-mismatches (A,B,DR) (IQR)	3 (2–4)
Split HLA-mismatches (A,B,C,DR,DQ) (IQR)	5 (3–7)
Combined kidney–pancreas transplantation	159 (6%)
Cold ischemia time, hours (SD)	9.8 (5.7)
Lowest serum creatinine posttransplant, mg/dL (IQR)	1.1 (0.9–1.4)

Data are mean (standard deviation, SD), median (interquartile range, IQR), or n (%).

immunosuppressive therapies over time and partially incomplete corresponding data, immunosuppression and adherence were not incorporated as variables in these analyses.

Primary and secondary end points for the analysis included death-censored allograft loss with return to dialysis as well as the detection of dnDSA by single antigen beads (SAB).

The study was approved by the institutional review boards of the Charité hospital (EA1/048/14, 118/16).

HLA antibody testing
Transplants were performed based on a negative complement-dependent cytotoxicity (CDC) crossmatch using isolated T and B lymphocytes. Pretransplant HLAab were determined by CDC in combination with solid-phase immunoassays. Between 1995 and 2005, pretransplant solid-phase HLAab screening and specification were achieved by the enzyme-linked immunosorbent assay (ELISA) Lambda Antigen Tray (LAT) (One Lambda, Canoga Park, CA). Beginning in 2006, all patients on the kidney waiting list were tested by the Luminex®-based LABScreen® mixed and SAB assay (One Lambda, Canoga Park, CA). A cross-sectional posttransplant HLAab monitoring scheme by SAB including all kidney transplants with a functioning allograft was started in 2002, continued in 2004, 2006, and annually thereafter (14). In addition, HLAab monitoring was initiated in case of clinical signs of impaired allograft function. All tests were performed according to the manufacturer's instructions. For the SAB assay, a normalized mean fluorescence intensity value exceeding 1000 was defined as positive in the pre- and posttransplant setting. The HLA loci A, B, C, DRB, and DQB were considered for the definition of dnDSA. Due to missing typing, DQA, DPA, and DPB could not be considered for this analysis. Patients transplanted before 2006 were only considered for the analysis if at least one Luminex®-based posttransplant antibody monitoring was negative for DSA. Therefore, all dnDSA have been designated based on Luminex® SAB.

Subgroup analysis
The limitations of such a retrospective analysis of a large-sized cohort with long follow-up urged us to perform a subgroup analysis on 1247 patients with more stringent inclusion criteria to internally verify our results. Thereby, only patients transplanted after 2002 with at least two pretransplant serum samples (median of six tests and interquartile range

[IQR]: 4–11) and complete posttransplant testing (median of six tests and IQR: 3–9) performed by Luminex® were included, although this inevitably resulted in limitations due to a significantly decreased power and shorter follow-up. The same analyses were applied to both the whole cohort and this subgroup.

PIRCHE analysis

The HLA-derived mismatched peptide epitopes that can be presented by the recipient's HLA-DRB1 molecules were calculated using the latest version of the PIRCHE algorithm (Version 2.4.21). Tools to predict the presentation of allopeptides by the recipient's DRB3/4/5, DQB, DQA, DPB, and DPA molecules have not been implemented yet. However, in contrast to earlier implementations (10), the current version predicts presentation of both HLA class I and class II derived peptides (i.e. HLA-A, B, C, DRB, and DQB). Briefly, each mismatched HLA allele of the donor is translated into the AA sequence of the mature molecule covering exons 1 to 7 or 1 to 5 depending on HLA class (15). After removing the signal peptide, each hypothetical candidate peptide fragment is derived from these molecules. In resemblance to the naturally occurring thymic negative selection process, only candidate peptides proven to be absent in the patient's self-peptide background are considered as allopeptides. The netMHCIIpan predictor version 3.0 (16) is used to estimate the binding probability of these allopeptides. All ligands, likely to be bound (IC50 < 1000) in one of the recipient's DRB1 molecules binding grooves, are considered as PIRCHE-II, increasing the PIRCHE-II score by 1. HLA typing of the recipient was achieved by serological (HLA class I) and DNA-based techniques (HLA class I and II). In detail, sequence-specific primer (SSP) (Olerup, Stockholm, Sweden) and reverse sequence-specific oligonucleotide (SSO) (One Lambda, Canoga Park, CA) assays were used according to the manufacturer's instructions. Recipients were typed twice. HLA typing of a deceased donor was provided by the donor center and was confirmed in-house by SSO. Living donors were typed twice by serology and SSO or SSP. HLA typing results retrieved from the databases were restricted to serological split equivalents. For 32% of patients, HLA-C and/or DQB typing could not be retrieved from the databases. Missing typings were extrapolated from HLA-ABCDRDQ-haplotype frequencies based on the National Marrow Donor Program database 2007 for Americans of European descent (17). Similarly, low-resolution typing data of patients and donors was extrapolated using a multiple imputation approach (18). Haplotype frequencies for estimated high-resolution genotypes were taken into account as weight for the PIRCHE-II score of each probable high-resolution patient–donor pair. Genotypes with a normalized probability of <0.01% were omitted. The IMGT database version 3.20 was used.

HLAMatchmaker analysis

HLAMatchmaker eplets for HLA-A, -B, -C, -DRB, and –DQB were assigned based on HLAMatchmaker Version 02 (downloaded October 2015, www.epitopes.net). Similarly to the PIRCHE-II score, an HLAMatchmaker score was calculated as the weighted sum of the count of eplets of each pair of high-resolution patient and donor genotypes. Weighting for each estimated high-resolution genotype was based on haplotype frequencies as described for the PIRCHE-II score.

Statistics

Patient cohort characteristics and parameters were summarized as mean with standard deviation or, in case of nonparametric distribution of continuous variables, as median with IQR. The relationship between HLAMatchmaker score and PIRCHE-II score was investigated by means of Spearman's rank-correlation coefficient (rho). Multiple Cox regression models were created to identify predictors of dnDSA posttransplant. The proportional hazard assumption was tested by Schoenfeld residuals for each covariate over time. Cox proportional regression was performed using the ln-transformed PIRCHE-II score, taking into account the logarithmic correlation between the PIRCHE-II score and the cumulative incidence of dnDSA (Figure 4). Time-to-event outcome data with respect to dnDSA development and death-censored allograft survival were assessed by Kaplan–Meier plots and log-rank test. Analyses were conducted using SPSS 23 (IBM Corp., Armonk, NY). Goodness-of-fit according to Grønnesby and Borgan (19,20) was tested by R version 3.2.3. Two-tailed p-values of <0.05 were considered statistically significant.

Results

Descriptive analysis and correlation of PIRCHE-II score, HLAMatchmaker, and classical antigen mismatches

The study cohort comprised mainly first single-kidney transplants from a deceased donor with a median waiting time of 4.8 (range 2.1–7.0) years on dialysis and a mean follow-up of 7.2 (4.8) years (Table 1). Transplants of donors with a mean age of 50.2 (15.8) years were allocated to recipients with a median cumulative ABCDRDQ mismatch of 5 (3–7). The distributions of PIRCHE-II, HLAMatchmaker scores, and classical antigen mismatches are illustrated in Figure 1. HLAMatchmaker scores ranged from 0 to 85.5 for the overall cohort with a mean of 27.2 (15.8). Ninety percent of patients revealed a score within a range of 0.6 to 52.1 (Figure 1A). For each HLA mismatch there was a wide variation in corresponding HLAMatchmaker scores (Figure 1B). The HLA mismatches translated into a mean PIRCHE-II score of 70.0 (49.9). The PIRCHE-II score ranged for the overall cohort from 0 to 323.9, but 90% of patients revealed a score within a range of 1.2 to 162.7 (Figure 1C). As illustrated in Figure 1D, there is a huge individual range of PIRCHE-II scores for each HLA mismatch. Up to three HLA mismatches may translate into a PIRCHE-II score of zero. Importantly, there is a moderate direct correlation between PIRCHE-II score and HLAMatchmaker score with a Spearman rank-order correlation coefficient (rho) of 0.75 (p < 0.001) (Figure 1E). Notably, high HLAMatchmaker scores do not necessarily translate into high PIRCHE-II scores and vice versa.

PIRCHE-II and HLAMatchmaker scores predict allograft survival and dnDSA

Death-censored kidney allograft survival within the cohort was 79.0% (95% confidence interval [CI]: 77.0–81.0) at 10 years posttransplant. Graft loss (i.e. return to dialysis) was recorded for 439 (16%) patients. A total of 449 (16%) patients developed dnDSA during follow-up, which translates into an overall projected incidence of dnDSA at 10 years posttransplant of 20.6% (95% CI: 18.6–22.6).

Importantly, the probability of dnDSA was directly correlated with the HLAMatchmaker and PIRCHE-II score. At 10 years of follow-up, patients with a HLAMatchmaker score <5 (n = 323), ≥5 to <18 (n = 476), ≥18 to <36 (n = 1136), and ≥36 (n = 852) revealed a predicted incidence of dnDSA (95% CI) of 3.0% (0.6–5.4), 13.4% (9.5–17.3), 22.0% (18.7–25.3), and 30.5% (26.2–34.8).
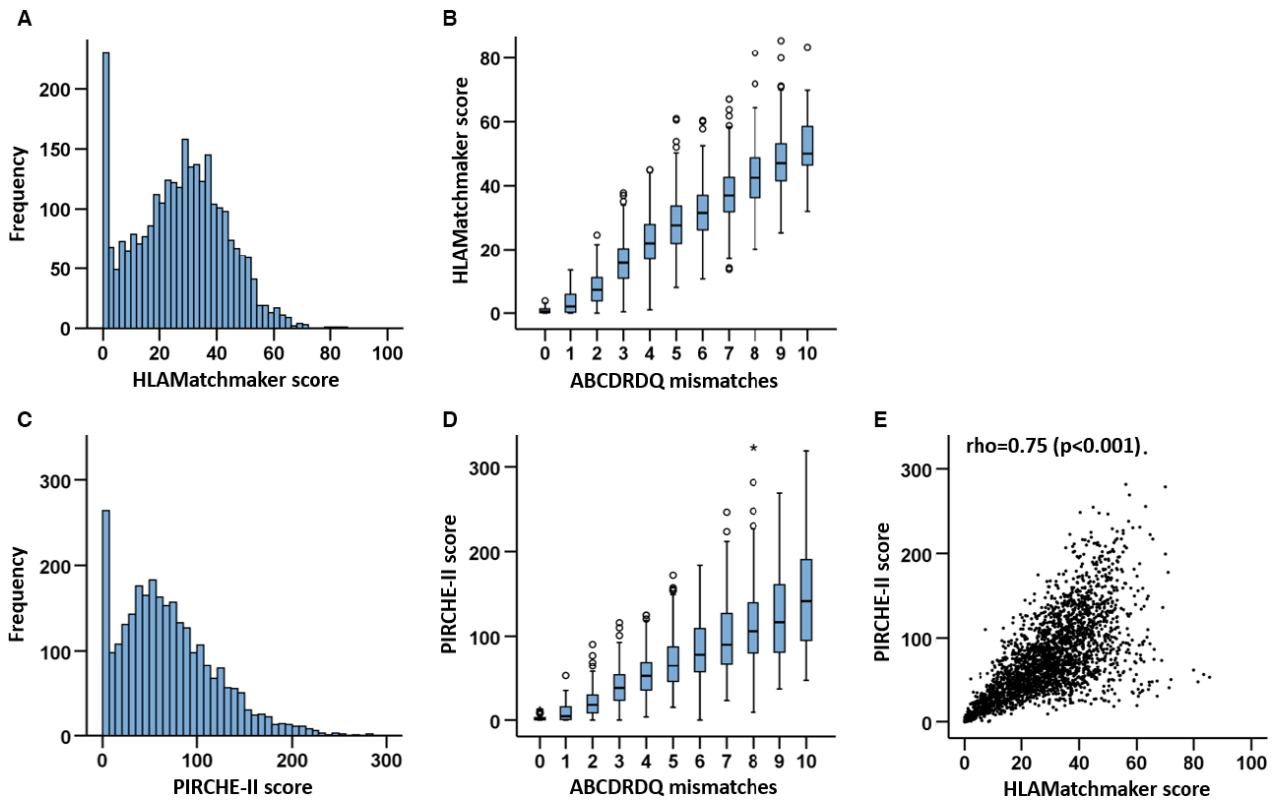
Figure 1: Descriptive statistics of the PIRCHE-II scores in our cohort of 2787 kidney transplants: (A) frequency distribution of HLAMatchmaker scores, (B) association of the HLAMatchmaker scores with the count of ABCDRDQ mismatches, (C) frequency distribution of PIRCHE-II scores, (D) association of the PIRCHE-II scores with the count of ABCDRDQ mismatches, and (E) association of the PIRCHE-II scores with the HLAMatchmaker scores (Spearman rank-order correlation coefficient rho of 0.75, p < 0.001). The boxplots of panels (B) and (D) depict the median and first to third quartile (box), the highest and lowest value within 1.5x IQR (whiskers), 1.5-3× IQR mild outliers (circles) and >3× IQR extreme outliers (asterisk). IQR, interquartile range; PIRCHE, predicted indirectly recognizable HLA epitopes. [Color figure can be viewed at wileyonlinelibrary.com]

Accordingly, patients with a PIRCHE-II score <9 (n = 285), ≥9 to <35 (n = 446), ≥35 to <90 (n = 1222), and ≥90 (n = 834) had a predicted incidence of dnDSA of 3.6% (1.1–6.1), 13.1% (9.0–17.2), 21.5% (18.4–24.6), and 30.5% (26.0–35.0), respectively (Figure 2A and C). As expected, a classical antigen-matching effect with respect to the incidence of dnDSA and allograft survival was also observed (Figure S1).

Similarly, however, to a lesser extent, HLAMatchmaker and PIRCHE-II scores predicted death-censored allograft survival at 10 years posttransplant with stratified graft survival probabilities of 83.6% (78.5–88.7), 81.1% (76.4–85.8), 79.7% (76.6–82.8), and 74.9% (71.0–78.8) for patients categorized according to HLAMatchmaker score and 83.1% (77.8–88.4), 83.0% (78.3–87.7), 78.9% (76.0–81.8), and 75.0% (70.9–79.1) for patients categorized according to PIRCHE-II score, respectively (Figure 2B and 2D).
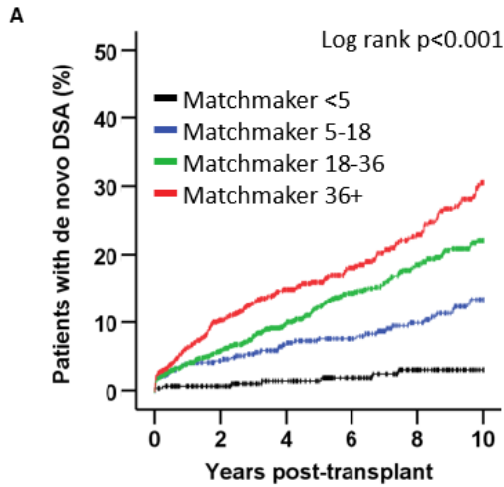
These results could be qualitatively verified in subgroup analyses on 1247 patients with complete and extensive pre- and posttransplant Luminex® monitoring fulfilling more stringent inclusion criteria as described in the Materials and Methods section. Corresponding data are visualized as supplemental data (Figure S2). Interestingly, the effect of PIRCHE matching on the incidence of dnDSA applies not only to deceased but also to living donor organ transplants as revealed by a stratified analysis (Figure S3).

To test the accuracy of our model of PIRCHE-II scores predicting the incidence of dnDSA, we performed a goodness-of-fit test according to Grønnesby and Borgan (19,20). As depicted in Figure 3, PIRCHE-II score stratified into quintiles of each 557 patients predicted well the corresponding observed incidences of dnDSA in our cohort.
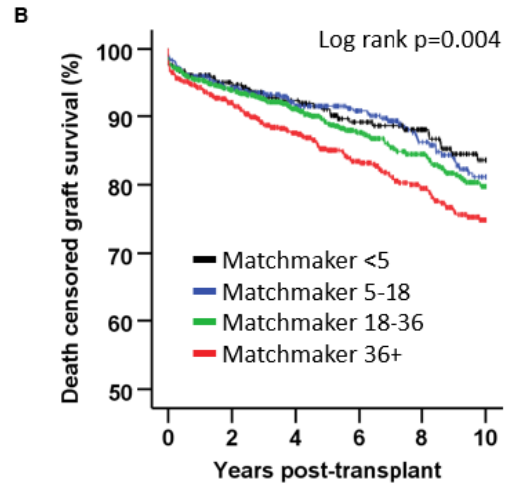
PIRCHE-II score is an independent predictor for de novo DSA

Evaluating the discriminative performance of PIRCHE regarding the development of dnDSA with various IC50 threshold values (i.e., 125, 250, 500, and 1000), a time-to-event Receiver Operating Characteristic analysis (21)
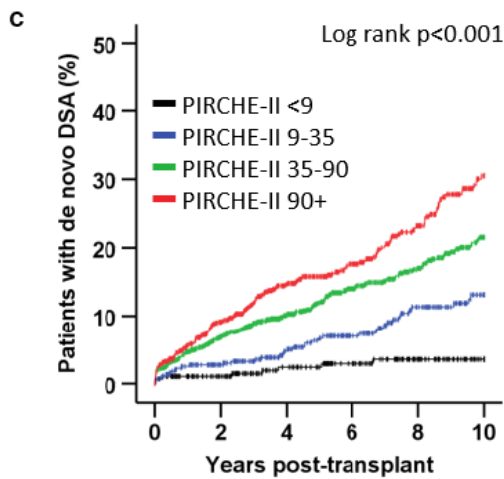
Figure 2: Kaplan–Meier plots illustrating the cumulative incidence of dnDSA, and 10-year death-censored kidney allograft survival both stratified according to arbitrary categories. (A) and (B): HLAMatchmaker score (<5, ≥5 to <18, ≥18 to <36, and ≥36). (C) and (D): PIRCHE-II score (i.e. <9, ≥9 to <35, ≥35 to <90, and ≥90). dnDSA, de novo donor-specific HLA antibodies; PIRCHE, predicted indirectly recognizable HLA epitopes.

revealed a superior area under the curve of 0.641 and R^2 of 0.0326 (22) for IC50 < 1000 (Table S1). We therefore continued analyses using IC50 < 1000 as cutoff.

Figure 4 illustrates the logarithmic correlation of PIRCHE-II score and the predicted incidence of dnDSA at 10 years

posttransplant. This led us to transform the PIRCHE-II score into the natural logarithm of the PIRCHE-II score (i.e. ln[PIRCHE-II]) for subsequent Cox regression analyses.

In addressing the individual contribution of PIRCHE-II for each HLA locus on the prediction of dnDSA at the
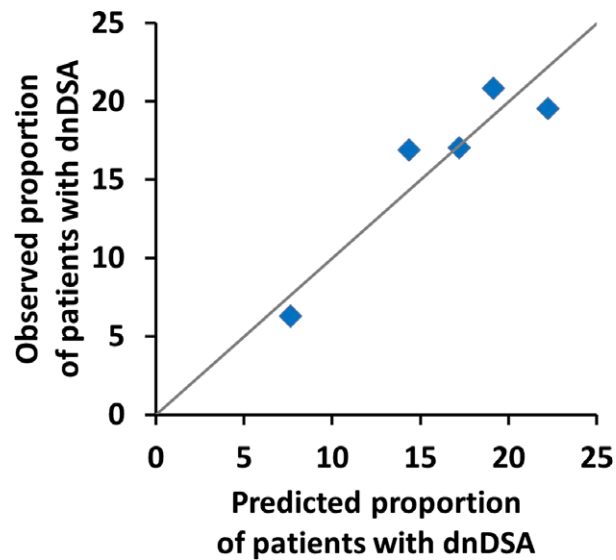
Figure 3: Goodness-of-fit analysis of expected vs. observed events of dnDSA based on quintiles of the PIRCHE-II score (19). There was no statistical difference between the number of predicted vs. observed events. dnDSA, *de novo* donor-specific HLA antibodies; PIRCHE, predicted indirectly recognizable HLA epitopes. [Color figure can be viewed at wileyonlinelibrary.com]

Figure 4: Logarithmic correlation between the cumulative incidence of dnDSA and PIRCHE-II score. dnDSA, *de novo* donor-specific HLA antibodies; PIRCHE, predicted indirectly recognizable HLA epitopes. [Color figure can be viewed at wileyonlinelibrary.com]
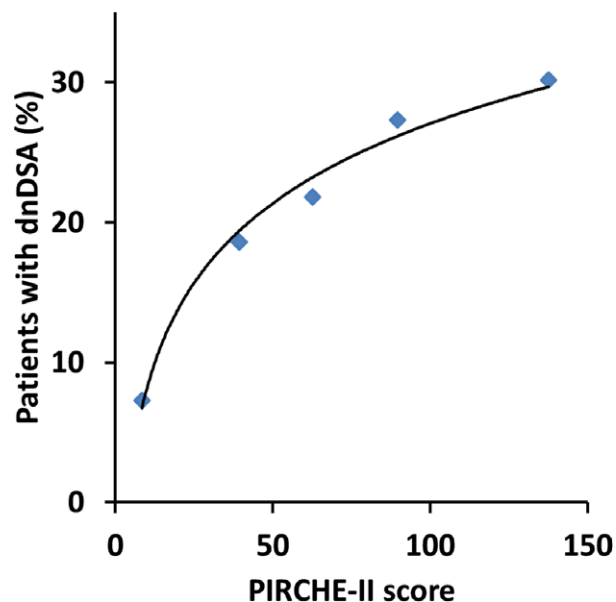
corresponding locus, the univariate analysis revealed hazard ratios significantly exceeding 1.0 for all loci (Table 2). More interestingly, the multivariate analysis adjusted for the number of HLA antigen mismatches at the corresponding locus revealed a significant increase in risk for dnDSA with increasing ln(PIRCHE-II) score. The multivariate hazard ratio reached its maximum for HLA-DQB with 1.82 (95% CI 1.56–2.12,

$p < 0.001$) and the minimum for HLA-C with 1.39 (95% CI 1.03–1.87, $p = 0.031$).

The individual contributions of a low or high PIRCHE-II score (first quartile vs. fourth quartile) on the development of locus-specific dnDSA for patients with one HLA mismatch at the corresponding locus are illustrated in Figure 5. The stratification into low or high PIRCHE

Table 2: Univariate and multivariate Cox regression analysis for ln(PIRCHE-II) at each HLA locus on the prediction of dnDSA at the corresponding locus (n = 2787). The multivariate analysis was performed adjusting for the count of antigen mismatch at the corresponding locus to reveal the PIRCHE score as independent predictor of dnDSA at the HLA loci A, B, C, DRB, and DQB

	Predicted incidence of dnDSA 10 years posttransplant	ln(PIRCHE-II) for each HLA locus univariate analysis		ln (PIRCHE-II) for each HLA locus multivariate analysis[1]	
		HR (95% CI)	p	HR (95% CI)	p
HLA locus A	7.2%	1.91 (1.63–2.22)	<0.001	1.72 (1.40–2.12)	<0.001
HLA locus B	7.1%	1.90 (1.59–2.26)	<0.001	1.68 (1.32–2.13)	<0.001
HLA locus C	3.7%	1.61 (1.29–2.01)	<0.001	1.39 (1.03–1.87)	0.031
HLA locus DRB	7.6%	1.92 (1.63–2.27)	<0.001	1.72 (1.38–2.15)	<0.001
HLA locus DQB	12.9%	1.99 (1.75–2.26)	<0.001	1.82 (1.56–2.12)	<0.001

[1]Adjusting for the count of HLA antigen mismatches at the corresponding locus. CI, confidence interval; dnDSA, *de novo* donor-specific HLA antibodies; HR, hazard ratio; PIRCHE, predicted indirectly recognizable HLA epitopes.
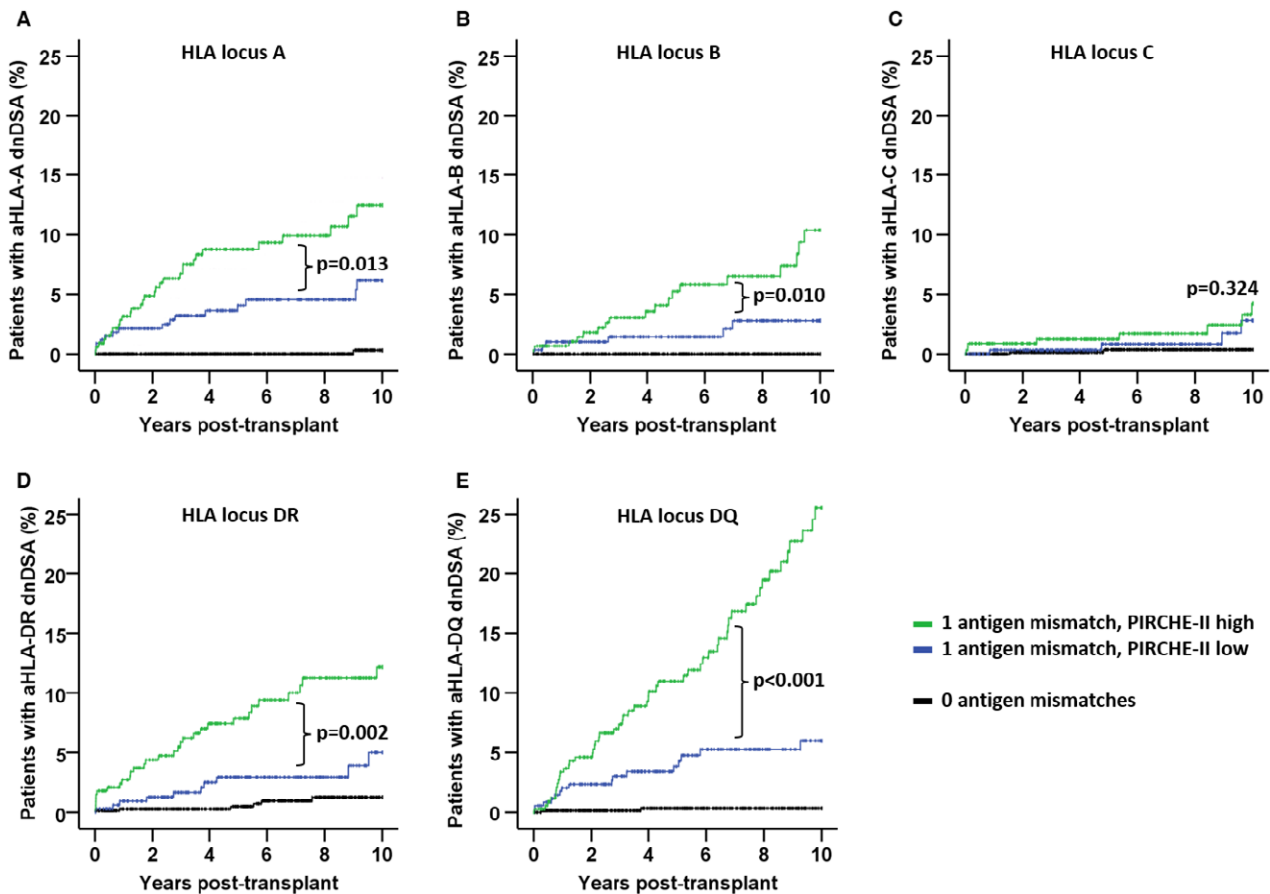

Figure 5: Cumulative incidence of dnDSA stratified according to low and high PIRCHE-II score (first vs. fourth quartile) in patients with 0 and 1 HLA mismatch at the specific locus individually illustrated for the loci (A) HLA-A, (B) HLA-B, (C) HLA-C, (D) HLA-DR, and (E) HLA-DQ. The median score for HLA-A, B, C, DRB, and DQB was 9.8, 11.1, 11.0, 8.6, and 15.0, respectively. dnDSA, *de novo* donor-specific HLA antibodies; PIRCHE, predicted indirectly recognizable HLA epitopes.

scores compares the first quartile (PIRCHE-II low) with the fourth quartile (PIRCHE-II high) based on the score for the individual HLA locus. The probability of dnDSA for HLA-A, B, DRB, and DQB was significantly higher for patients with a high PIRCHE-II score. Stratification according to the PIRCHE-II score allowed identification of patients with low risk for the development of dnDSA despite one MM at the specific locus. However, the

strongest impact of the PIRCHE-II score on differentiation into low- and elevated-risk patients could be revealed for patients with one MM in HLA-DRB or DQB. The maximum difference of 19.6% points in the incidence of dnDSA at 10 years posttransplant was calculated for patients with one HLA-DQB mismatch and a low versus high PIRCHE score (6.0% vs. 25.6%, p < 0.001). The data for all four quartiles of PIRCHE-II scores are illustrated in Figure S4. Interestingly, the PIRCHE-II score failed to significantly predict the incidence of locus-specific dnDSA independently from the count of mismatches in case of two mismatches at the loci A, B, C, DRB, or DQB.

Finally, we aimed at the comparison of the predictive power for dnDSA between ln(PIRCHE-II) score, HLAMatchmaker score, and number of antigen mismatches in Cox regression analyses. Table 3 summarizes the uni- and multivariate models adjusted for known predictors of dnDSA (i.e. donor and recipient age). Although all three variables significantly contributed to univariate Cox regression models, only ln(PIRCHE-II) and HLAMatchmaker score were found to be independent predictors for dnDSA.

These results were verified in the subgroup of 1247 patients with more extensive pre- and posttransplant Luminex® monitoring. The significantly higher probability of dnDSA for HLA-A, B, DRB, and DQB in patients with a high PIRCHE-II score was confirmed in this subgroup (Figure S5). Furthermore, the multivariate analysis adjusted for the number of HLA antigen mismatches at the corresponding locus (corresponding to Table 2) qualitatively confirmed the significant increase in risk for dnDSA with increasing ln(PIRCHE-II) scores revealing hazard ratios >1 for HLA-A (1.54, p = 0.013), B (1.31, p = 0.188), C (1.50, p = 0.163), DRB (1.54, p = 0.034), and DQB (1.53, p < 0.001). Corresponding to Table 3, the adjusted multivariate model in the subgroup of 1247 patients resulted in hazard ratios of 1.33 (HLAMatchmaker score per 10 increment, p = 0.002), 1.18 (ln(PIRCHE-II) score, p = 0.212), and 0.98 (ABCDRDQ mismatches per MM, p = 0.781). Due to the limited sample size and shorter follow-up in this cohort, the statistical significance level was partly reduced in these analyses.

Discussion

In contrast to the previous publications (10,11,23), we evaluated an adjusted PIRCHE algorithm to allow the prediction of not only donor HLA class I but also class II-derived epitopes. HLA class II-derived epitopes are of special interest as posttransplant dnDSA are predominantly directed against HLA class II (14). In this study, we could confirm the predominance of HLAab and dnDSA directed against class II. The data further emphasize the importance of HLA class II matching to prevent the formation of dnDSA (Figure 5A-E) (24). Importantly, the PIRCHE-II score had major impact on dnDSA formation for DRB and DQB. Patients with one antigen mismatch but a low PIRCHE-II score showed significantly decreased 10-year incidences of dnDSA as compared to patients with a high PIRCHE-II score. The predictive power of the PIRCHE approach for dnDSA appeared to be outweighed in case of two antigen mismatches per locus due to the relatively increased epitope-load. In the multivariate Cox regression analysis adjusted for the count of HLA mismatch at each locus, increasing PIRCHE-II scores could be associated with a significantly increased risk for dnDSA at all loci (Table 2). The impact was strongest for DRB and DQB followed by A and B.

Most of the current literature on HLA epitope matching to mitigate alloimmunization in the setting of kidney transplantation exclusively focus on HLA-DRB and DQB antibodies. Exemplarily, Wiebe et al demonstrated that HLAMatchmaker matching for HLA-DRB and DQB among 286 donor–recipient pairs was predictive for locus-specific DSA. Thereby, an eplet mismatch count of <10 for HLA-DRB and 17 for DQB was associated with minimal development of DSA (25). In another study by the same authors, it was concluded that higher eplet mismatch counts for HLA-DRB and DQB and poor adherence among 195 renal transplant recipients acted synergistically to increase the risk of rejection or graft loss. Unfortunately, DSA detection was not performed in this study, so that the differential contribution of nonadherence and epitope matching could not be determined (26). Others found a correlation between eplet counts and transplant glomerulopathy but again

Table 3: Uni- and multivariate Cox regression models of HLAMatchmaker score, ln(PIRCHE-II) score, and count of ABCDRDQ mismatches to predict the incidence of dnDSA (n = 2787)

	Univariate analysis		Multivariate analysis[1]	
	HR (95% CI)	p	HR (95% CI)	p
HLAMatchmaker score per 10 increment	1.30 (1.23–1.38)	<0.001	1.23 (1.10–1.37)	<0.001
ln(PIRCHE-II) score	1.63 (1.46–1.83)	<0.001	1.44 (1.22–1.69)	<0.001
ABCDRDQ mismatches per MM	1.16 (1.12–1.21)	<0.001	0.96 (0.89–1.04)	0.299

CI, confidence interval; dnDSA, *de novo* donor-specific HLA antibodies; HR, hazard ratio; PIRCHE, predicted indirectly recognizable HLA epitopes.
[1]Adjusting for ln(PIRCHE-II), HLAMatchmaker score, recipient age, donor age, and count of ABCDRDQ mismatches.

without any DSA data (27). As an extension to the HLAMatchmaker and Terasaki's AA mismatch approaches, Kosmoliaptsis et al introduced the electrostatic mismatch concept to explain alloantibody binding based on surface electrostatic potential differences between HLA molecules. In a series of 131 kidney transplants, this approach has been shown to be superior to the HLAMatchmaker and AA mismatch approach in predicting *de novo* alloimmunization against HLA-A, B, DRB, and DQB (28). Similar to our approach, the electrostatic mismatch algorithm aims at the discrimination of immunogenicity of an epitope beyond AA sequence comparison.

Besides dnDSA formation, the main outcome in this analysis was 10-year death-censored allograft survival. There was a significant association between increasing PIRCHE-II scores and impaired allograft survival. The cause of late renal allograft dysfunction is assumed to be multifactorial (29). Thus, it is important that PIRCHE epitope matching at time of transplantation revealed such a strong impact on 10-year graft outcome. Here, PIRCHE matching revealed a very strong impact on the incidence of dnDSA independently of the antigen mismatch, which means that the impact of histocompatibility has even been underestimated over the past. Multivariate analysis clearly demonstrates that epitope matching by PIRCHE better defines histocompatibility than the classical alphanumeric antigen matching.

This is the first single-center analysis of 2787 transplants on the impact of different HLA matching approaches on renal allograft survival and dnDSA formation. Based on our data, PIRCHE and the HLAMatchmaker approach are both independent predictors for dnDSA and may complement each other. Uniquely, patients underwent consecutive posttransplant DSA surveillance by SAB since 2002. However, pretransplant sera of patients transplanted 1995–2005 and posttransplant sera 1995-2002 have been analyzed predominantly by ELISA. Admittedly, ELISA is less sensitive than SAB in the detection of dnDSA. We therefore enrolled patients with HLAab only if at least one posttransplant sample tested by SAB was negative for DSA. All patients developed DSA during our follow-up using the SAB assay and thus have been designated as being true *de novo*. Since this is a retrospective analysis of transplants performed over the last 20 years, we cannot rule out underlying effects due to differing induction and maintenance immunosuppression as well as rejection treatment protocols. However, the large sample size might partially compensate for potential confounders. Nevertheless, given the study's apparent limitations, we initiated an internal verification of the results by applying more stringent inclusion criteria to the total cohort to further minimize confounders and performed the same statistics as we did on the total cohort. As expected, the results could generally be verified by these subgroup analyses, which surely does not spare verification on an independent cohort in a future project. Similar to other epitope models, PIRCHE is dependent on at least two-field resolution HLA typing, which was extrapolated using a multiple imputation approach appropriate for our homogeneous Caucasian cohort. Admittedly, for other studies, the approach may need adaptation considering the ethnic background of the cohort under investigation. In the future, deviation introduced by imputation methods may be avoided, if high-resolution typing becomes widely applied in the kidney transplantation setting. Here, we restricted our analysis to DRB1 but we are aware that further research to elucidate the role of DRB3/4/5, DQA/DQB, and DPA/DPB as allopepetide presenter is necessary.

In conclusion, PIRCHE is a novel HLA epitope matching tool that was shown in this study to predict dnDSA formation following kidney transplantation and thus may help in pretransplant risk stratification to ensure allocation of donor organs to recipients with decreased immunological risk for dnDSA posttransplant. Similar to the acceptable mismatch program for highly immunized patients by Eurotransplant, the PIRCHE algorithm may allow us in the future to define acceptable mismatches associated with a reduced risk for the *de novo* formation of DSA posttransplant for all patients on the kidney waiting list. Importantly, considering these potential wide-ranging implications together with the obvious limitations of this study, cautious interpretation and further validation of the results are warranted.

Acknowledgments

Author Contributions

NL, MN, OS: data compilation, performing statistics, preparation and writing of the manuscript. KB, PR, AP, CS, and ES: supervision, review, and proofreading of the manuscript. DS: data compilation.

Disclosure

The authors of this manuscript have conflicts of interest to disclose as described by the *American Journal of Transplantation*. MN is an employee of PIRCHE AG that runs the PIRCHE web-portal. ES: The UMC Utrecht has filed a patent application on the prediction of an alloimmune response against mismatched HLA. ES is listed as inventor on this patent. The other authors have no conflicts of interest to disclose.

References

1. Loupy A, Hill GS, Jordan SC. The impact of donor-specific anti-HLA antibodies on late kidney allograft failure. Nat Rev Nephrol 2012; 8: 348–357.

2. Hickey MJ, Valenzuela NM, Reed EF. Alloantibody generation and effector function following sensitization to human leukocyte antigen. Front Immunol 2016; 7: 30.

3. Malard-Castagnet S, Dugast E, Degauque N, et al. Sialylation of antibodies in kidney recipients with de novo donor specific antibody, with or without antibody mediated rejection. Hum Immunol 2015; 77: 1076–1083.

4. Wiebe C, Gibson IW, Blydt-Hansen TD, et al. Evolution and clinical pathologic correlations of de novo donor-specific HLA antibody post kidney transplant. Am J Transplant 2012; 12: 1157–1167.

5. Opelz G, Dohler B. Effect of human leukocyte antigen compatibility on kidney graft survival: Comparative analysis of two decades. Transplantation 2007; 84: 137–143.

6. Tambur AR, Claas FH. HLA epitopes as viewed by antibodies: What is it all about? Am J Transplant 2015; 15: 1148–1154.

7. Duquesnoy RJ. Antibody-reactive epitope determination with HLAMatchmaker and its clinical applications. Tissue Antigens 2011; 77: 525–534.

8. Dankers MKA, Heemskerk MBA, Duquesnoy RJ, et al. HLAMatchmaker algorithm is not a suitable tool to predict the alloreactive cytotoxic T-lymphocyte response in vitro. Transplantation 2004; 78: 165–167.

9. Conlon TM, Saeb-Parsy K, Cole JL, et al. Germinal center alloantibody responses are mediated exclusively by indirect-pathway CD4 T follicular helper cells. J Immunol 2012; 188: 2643–2652.

10. Otten HG, Calis JJ, Kesmir C, van Zuilen AD, Spierings E. Predicted indirectly recognizable HLA epitopes presented by HLA-DR correlate with the de novo development of donor-specific HLA IgG antibodies after kidney transplantation. Hum Immunol 2013; 74: 290–296.

11. Geneugelijk K, Honger G, van Deutekom HW, et al. Predicted indirectly recognizable HLA epitopes presented by HLA-DRB1 are related to HLA antibody formation during pregnancy. Am J Transplant 2015; 15: 3112–3122.

12. Filippone EJ, Farber JL. Humoral immune response and allograft function in kidney transplantation. Am J Kidney Dis 2015; 66: 337–347.

13. Schröter K, Lindemann-Trzebiatowski G, Fritsche L. TBase2 a web-based electronic patient record. Fundamenta Informaticae 2000; 43: 343–353.

14. Lachmann N, Terasaki PI, Budde K, et al. Anti-human leukocyte antigen and donor-specific antibodies detected by luminex posttransplant serve as biomarkers for chronic rejection of renal allografts. Transplantation 2009; 87: 1505–1513.

15. Geneugelijk K, Niemann M, de Hoop T, Spierings E. Completion of HLA protein sequences by automated homology-based nearest-neighbor extrapolation of HLA database sequences. Hum Immunol 2016; 77: 1030–1036.

16. Karosiene E, Rasmussen M, Blicher T, Lund O, Buus S, Nielsen M. NetMHCIIpan-3.0, a common pan-specific MHC class II prediction method including all three human MHC class II isotypes, HLA-DR, HLA-DP and HLA-DQ. Immunogenetics 2013; 65: 711–724.

17. Maiers M, Gragert L, Klitz W. High-resolution HLA alleles and haplotypes in the United States population. Hum Immunol 2007; 68: 779–788.

18. Geneugelijk K, Wissing J, Koppenaal D, Niemann M, Spierings E. Computational approaches to facilitate epitope-based HLA matching in solid organ transplantation. J Immunol Res 2017; 2017: 9130879.

19. May S, Hosmer DW. A simplified method of calculating an overall goodness-of-fit test for the Cox proportional hazards model. Lifetime Data Anal 1998; 4: 109–120.

20. Gronnesby JK, Borgan O. A method for checking regression models in survival analysis based on the risk score. Lifetime Data Anal 1996; 2: 315–328.

21. Heagerty PJ, Lumley T, Pepe MS. Time-dependent ROC curves for censored survival data and a diagnostic marker. Biometrics 2000; 56: 337–344.

22. Nagelkerke NJD. A note on a general definition of the coefficient of determination. Biometrika 1991; 78: 691–692.

23. Thus KA, Ruizendaal MT, de Hoop TA, et al. Refinement of the definition of permissible HLA-DPB1 mismatches with predicted indirectly recognizable HLA-DPB1 epitopes. Biol Blood Marrow Transplant 2014; 20: 1705–1710.

24. Halleck F, Khadzhynov D, Liefeldt L, et al. Immunologic outcome in elderly kidney transplant recipients: Is it time for HLA-DR matching? Nephrol Dial Transplant 2016; 31: 2143–2149.

25. Wiebe C, Pochinco D, Blydt-Hansen TD, et al. Class II HLA epitope matching-A strategy to minimize de novo donor-specific antibody development and improve outcomes. Am J Transplant 2013; 13: 3114–3122.

26. Wiebe C, Nevins TE, Robiner WN, Thomas W, Matas AJ, Nickerson PW. The synergistic effect of class II HLA epitope-mismatch and nonadherence on acute rejection and graft survival. Am J Transplant 2015; 15: 2197–2202.

27. Sapir-Pichhadze R, Tinckam K, Quach K, et al. HLA-DR and -DQ eplet mismatches and transplant glomerulopathy: A nested case-control study. Am J Transplant 2015; 15: 137–148.

28. Kosmoliaptsis V, Mallon DH, Chen Y, Bolton EM, Bradley JA, Taylor CJ. Alloantibody responses after renal transplant failure can be better predicted by donor-recipient HLA amino acid sequence and physicochemical disparities than conventional HLA matching. Am J Transplant 2016; 16: 2139–2147.

29. Stegall MD, Gaston RS, Cosio FG, Matas A. Through a glass darkly: Seeking clarity in preventing late kidney transplant failure. J Am Soc Nephrol 2015; 26: 20–29.

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Figure S1: Kaplan–Meier plots illustrating the cumulative incidence of dnDSA (A) and 10-year death-censored kidney allograft survival (B) both stratified according to the number of classical antigen mismatches (MM) in the cohort of n = 2787 patients.

Figure S2: Subgroup analysis of 1247 patients with at least two pretransplant serum samples tested using Luminex®-based assays, complete Luminex® testing posttransplant (median number of tests: 6 [IQR 3–9]), transplant date beginning 2002. Kaplan–Meier plots illustrating the cumulative incidence of dnDSA, and death-censored kidney allograft survival at

5 years of follow-up stratified according to arbitrary categories. A) and B): HLAMatchmaker score (<5, $\geq$5 to <18, $\geq$18 to <36, and $\geq$36). C) and D): PIRCHE-II score (i.e. <9, $\geq$9 to <35, $\geq$35 to <90, and $\geq$90).

Figure S3: Kaplan–Meier plots illustrating the cumulative incidence of dnDSA, and 10-year death-censored kidney allograft survival stratified according to categories of the PIRCHE-II score, shown separately for the cohort of patients with living donors (n = 623, panels A and B) and deceased donors (n = 2164, panels C and D).

Figure S4: Cumulative incidence of dnDSA stratified according to quartiles of PIRCHE-II score (first through fourth quartile) in patients with one HLA mismatch at the specific locus individually illustrated for the loci (A) HLA-A, (B) HLA-B, (C) HLA-C, (D) HLA-DR, and (E) HLA-DQ.

Figure S5: Cumulative incidence of dnDSA among the subgroup of 1247 patients fulfilling the most stringent inclusion criteria stratified according to low and high PIRCHE-II score (first vs. fourth quartile) in patients with 0 and 1 HLA mismatch at the specific locus individually illustrated for the loci (A) HLA-A, (B) HLA-B, (C) HLA-C, (D) HLA-DR, and (E) HLA-DQ at 5 years of follow-up.

Table S1: Assessing the discriminative ability (AUC) (25) and calibration (R^2) (22) of PIRCHE predicting dnDSA with different IC50 thresholds.