Towards HLA epitope matching in clinical transplantation

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CHAPTER

8

GENERAL DISCUSSION

Since the beginning of solid organ transplantation HLA matching has been shown to be beneficial for graft survival,[1] which is still the case even in the modern era of immunosuppressive drugs. However, due to high polymorphism of the HLA system and scarcity of organs, most recipients receive a (partially) HLA mismatched graft. The allogeneic HLA molecules of the donor can be recognised as foreign by the immune system of the recipient, which may result in the development of antibodies directed against donor HLA, known as donor-specific antibodies (DSA). These *de novo* DSA can develop early or late after transplantation,[2,3] and the presence of these antibodies often leads to graft injury and eventually rejection.[4] In addition, the presence of DSA severely impacts the chance of finding a suitable donor for repeat transplantation.[5] This is also the case for patients on the transplant waiting list that developed HLA antibodies upon pregnancy or blood transfusion.

Towards definition of immunogenic amino acid configurations (epitopes)

Interestingly, not every HLA antigen mismatch leads to a humoral alloimmune response. As reviewed in **Chapter 2** and **Chapter 3**, while each HLA antigen consists of unique set of epitopes, or configurations of polymorphic amino acids, epitopes can be shared by several HLA antigens. Therefore, the number of foreign antibody epitopes present on mismatched donor HLA antigen varies and depends on the HLA phenotype of the recipient. In addition, the type and physicochemical properties of the amino acid substitution, as well as the presence of accompanying T helper cell epitopes contribute to the immunogenicity of a mismatched antibody epitope. These aspects have been implemented in several algorithms aiming at the prediction of the immunogenicity of a mismatched HLA antigen, such as eplets,[6,7] amino acid mismatches, electrostatic mismatch scores,[8,9] and Predicted Indirectly ReCognizable HLA Epitopes presented by recipient HLA class II (PIRCHE-II).[10,11] Indeed, mismatch scores based on these approaches have shown to predict the chance of *de novo* DSA formation on the population level, while none of these algorithms is superior over the others.[12,13] However, we argue that on the level of the individual patient it is not a numbers game, as a single amino acid or configuration present on a mismatched HLA allele can already be sufficient to induce an antibody response. This was observed previously for HLA class I configuration (triplet) mismatches,[14] and confirmed in our cohort study on amino acid mismatches for HLA class II (**Chapter 7**).

Therefore, as highlighted in **Chapter 2** and **Chapter 3**, it is essential to define immunogenic configurations of amino acids (Figure 1), eplets, or single amino acids so that these can be avoided during donor selection in order to prevent *de novo* DSA formation. With HLA-EMMA software, we developed a tool that enables analyses on amino acid compatibility between

donor and recipient (**Chapter 6**). This tool can perform analyses for large number of donor-recipient pairs at once due to batch option, and for HLA class I and HLA class II simultaneously, which is either very laborious or impossible with the other available algorithms. Additionally, HLA-EMMA considers all available HLA alleles from the IMGT, so there are no restrictions in analysing donor-recipient pairs with rare HLA alleles. Therefore, HLA-EMMA is a very useful and user-friendly tool, which can be used in cohort studies to define the relevant/immunogenic amino acids and positions. This software was further validated in a cohort study of non-immunised male recipients of a first renal allograft (**Chapter 7**).

While the aim of defining the most immunogenic HLA class II amino acids and/or positions was not accomplished in this cohort study due to small numbers, we did observe that a high number of amino acid mismatches is not always a guarantee for the induction of an antibody response. The latter suggests that not all mismatches are immunogenic, as previous mentioned (**Chapter 2** and **Chapter 3**), but also that not all defined polymorphic solvent accessible positions included in HLA-EMMA are equally important for the induction of an antibody response. Narrowing down the solvent accessible positions to only those that are proven to be able to induce an antibody response can be achieved by using human HLA-specific monoclonal antibodies (mAbs), as reactivity analysis of these mAbs allows for identification of amino acids and/or positions that are involved in antibody binding (**Chapter 5**), and thus amino acid and/or positions that can be immunogenic. Including only confirmed immunogenic or relevant polymorphic amino acid and/or positions will improve the prevention of DSA formation without unnecessarily preventing allocation based on non-immunogenic epitope mismatches.[15] Additionally, amino acids or amino acid configurations associated with *de novo* DSA formation identified in clinical cohort studies can be verified by human HLA mAbs, using methods described in **Chapter 5**.

Upon transplantation antibodies directed against HLA class II and more specifically against HLA-DQ are most prominent. Therefore, understanding of the immunogenicity of especially HLA-DQ is of great interest. One of the features of HLA-DQ is that both the alpha and beta chain are polymorphic. Therefore we analysed the HLA-DQ chains separately with respect to their ability to induce an antibody response (**Chapter 7**), in contrast to previous studies that consider the whole HLA-DQ molecule.[16-19] Of course, once an antibody is formed the whole HLA molecule should be considered as the antibody-footprint could cover both alpha and beta chain of the HLA-DQ molecule. However, we observed that a single amino acid mismatch on either the alpha or beta chain was sufficient to induce an antibody response that was clearly directed against that specific chain. In addition, we often detected an antibody response in

case only the HLA-DQB1 or HLA-DQA1 allele was mismatched. Important to note that this analysis was performed on first transplant recipients that did not have any detectable HLA antibodies prior to transplantation by sensitive single antigen bead assays. This indicates that for predicting immunogenicity not the whole HLA-DQ molecule should be considered, because then recipients can be incorrectly classified as being at low risk for allloimmunisation.[17,19]

One could argue that our tools are of no additive value, as on the group level eplets have shown to be good predictors of sensitization,[18,20-22] graft damage,[23] and subsequently rejection.[24,25] This has resulted in the introduction of cut-offs of numbers of eplets to identify alloimmunisation risk,[17,18,26,27] which has even been applied in allocation strategy for paediatric patients.[28] However, as discussed in **Chapter 2** and **Chapter 3** eplets are theoretically defined and not every eplet mismatch is immunogenic.[29] Furthermore, eplets require experimental verification to establish if an antibody indeed can bind to the eplet. Both absorption and elution studies[30,31] as well as human mAbs[32,33] have been shown to be very useful for antibody-verification of eplets.[34,35] However, we emphasised that there is a need for HLA class II eplet verification, and as shown in **Chapter 5** the newly generated human HLA-DR mAbs contribute to antibody-verification of eplets. More importantly, our antibody reactivity analysis also highlighted that the current list of eplets on HLA Epitope Registry contains inaccuracies and is subject to change without valid reasoning and validation. In addition, when defining polymorphic solvent accessible positions to incorporate in HLA-EMMA (**Chapter 6**) we observed discrepancies between our definition of surface exposed positions and those considered for defining eplets,[6,7,36] This clearly indicates there is a need for standardisation for defining antibody-verified eplets. Both HLA-EMMA and human HLA mAbs will contribute to define the immunogenic polymorphic amino acids and subsequently immunogenic or relevant amino acids configurations in a more standardised and validated manner.

Our cohort study already highlighted that for defining immunogenic polymorphic amino acids a large number of donor-recipient pairs of diverse population is required (**Chapter 7**). The latter is essential, as currently the proposed cut-offs are based on Caucasian population studies, but just like HLA allele frequency the frequency of the most immunogenic epitope can differ between populations (**Chapter 3**). One of such studies will be the upcoming International Immunogenetics and Histocompatibility Workshop, in which not only HLA-EMMA but all factors that regulate antibody induction will be included, such as T cell epitopes (PIRCHE-II) and physicochemical properties (EMS-3D), for a comprehensive analysis.

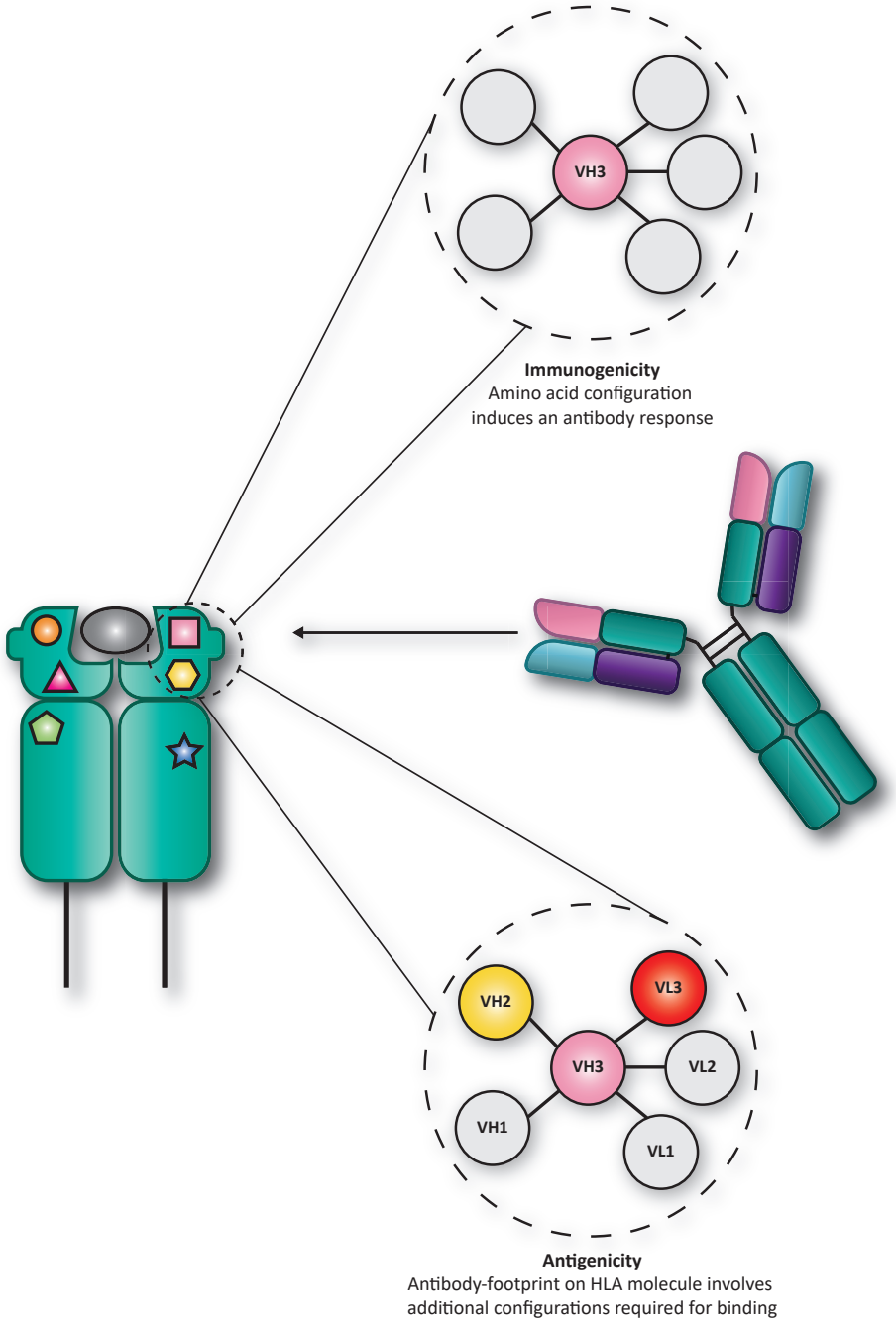

Figure 1: Immunogenicity and antigenicity. An immunogenic amino acid configuration (epitope) on mismatched donor HLA induces alloantibody response and determines the specificity of antibody as it interacts with CDR-H3 of the antibody. However, the antibody-footprint on HLA molecules involves additional configurations required for binding, which is the antigenicity of HLA antibody.

Antigenicity of formed HLA antibodies

Besides immunogenicity, definition of relevant amino acid configurations is also essential for understanding the antigenicity of HLA antibodies (Figure 1), which is imperative for sensitised patients to determine acceptable and unacceptable HLA antigen mismatches to predict a negative crossmatch (**Chapter 2**). HLAMatchmaker was developed to identify uniquely shared eplets by reactive HLA antigens in single antigen bead assay and used for reactivity analysis of mAbs[32,33] and sera[37-40] for antibody-verification of eplets. In recent years, eplets have been incorporated in analysis software of both single antigen bead assay used by HLA laboratories, and also a software tool to perform epitope analysis and virtual crossmatching based on eplets was developed.[41] However, as depicted in **Chapter 2**, reactivity of HLA antibodies is determined by crucial amino acid configurations in addition to the eplet or functional epitope. While some antibody reactivity patterns can indeed be explained by a single eplets/amino acid configuration, which is the functional epitope as it determines the specificity of antibody, this is not always the case as shown for both human HLA class I (**Chapter 3**) and HLA class II (**Chapter 5**) mAbs. In addition, not every reactive HLA antigen detected with single antigen bead assay is relevant, as mAbs can bind to HLA expressed on beads, but not to natively HLA expressed on cells (**Chapter 5**). Many HLA laboratories assign every HLA allele with a mean fluorescence intensity (MFI) value in single antigen bead assay as unacceptable, but our data shows that not every reactive HLA allele is relevant.

In addition, polyclonal serum consists of multiple antibodies, including HLA antibodies recognising different epitopes on same HLA antigen. This is nicely illustrated by our findings on mAbs directed against different epitopes on same HLA antigen that were generated from single memory B cell clones isolated from one individual with one tetramer specificity. The different memory B cell clones obtained showed different V(D)J usage indicating that the clonotypes are unique and not caused by somatic hypermutation (**Chapter 5**). Functional assays with cell expressing the target HLA showed differences in binding strength and differential efficiency in complement mediated cell lysis of the generated mAbs and therefore we surmised that the antibodies had different affinity for target HLA. Overall, this indicates that the abovementioned methods to interpret single antigen bead assay data of neat serum based on shared eplets is not so straightforward, as multiple factors such as immunising event, both immunogenic/functional epitopes and additional crucial configurations, and dilutions[42] should be taken into account to determine the true and relevant HLA alleles.

MFI values are often interpreted as being indicative of the relative concentration of HLA antibodies. However, the different level of reactivity of HLA antigens observed for mAbs

could also reflect the affinity the mAbs for specific HLA alleles (**Chapter 5**). It has been shown that the affinity for immunising antigen is often higher than for other antigens,[43] which might be due to the presence of the optimal set of crucial amino acid configurations. Additionally, amino acid substitution within the structural epitope can affect the affinity as well,[44] on the other hand if amino acid substitution does not affect surface area structure due to similar electrostatic potential, hydrophobicity or size than binding ability most likely remains the same.[45,46] Defining the affinity of HLA antibodies for various reactive HLA alleles will both elucidate on the interaction between antigen and antibody and the corresponding crucial additional contact sites and thus the antibody reactivity patterns observed in single antigen bead assays. Additionally, it will contribute in the understanding of the differential pathogenicity of HLA antibodies. This is essential because while *de novo* DSA are associated with graft rejection, not every recipient with detectable *de novo* DSA has clinical signs of rejection.[3,47] In addition, antibody-mediated rejection is mainly associated with complement activation,[48-50] but also complement independent graft injury has been observed.[51-54] Human HLA class I mAbs have shown to be useful for methodological studies of HLA antibodies to establish the clinical effect of IgG isotype and epitopes recognised.[51,52,55-57] However, as mentioned HLA class II antibodies are the dominant type of antibody to develop upon transplantation and recent studies demonstrated that non-human pan-HLA class II antibodies can induce endothelial cell damage independent of complement system.[53,54,58] The newly generated human HLA class II mAbs can be used to more specifically study the effect of HLA class II antibodies on graft injury, especially the effect of mixture of antibodies recognising different epitopes on same HLA antigen. Additionally, the role of different IgG subclasses can be studied as this method allows for the generation of mAbs of all four IgG subclasses, fully human glycosylated, recognising the same epitope with identical binding affinity (**Chapter 4**).

Besides these methodological studies, crystal structures of antigen-antibody complex are pivotal for studying both the pathogenicity of HLA antibodies as well as defining antigenicity of HLA antibodies. These structures will provide insight on how paratope of the antibody exactly binds to the epitope on the HLA antigen and so which amino acid configurations interact with the different complementary-determining regions of the antibody.[55] This will be especially of interest for HLA-DQ antibodies and how they bind to HLA-DQ molecule. Currently, we are working on isolating HLA-DQ-specific memory B cells similar as described in Chapter 5 but using a sorting strategy with HLA-DQ monomers instead.[59] Preliminary data implies that specificities of memory B cell clones are often directed to one chain, which supports our

HLA-DQ *dn*DSA findings in clinical cohort study (**Chapter 7**), but we suspect to isolate B cell clones that produce antibodies direct against both chains as well.

Clinical application

Once immunogenic epitopes are defined these can be avoided during allocation of kidneys to prevent the formation of *de novo* DSA after transplantation (**Chapter 2** and **Chapter 3**). By basing allocation only on truly immunogenic epitopes, patients will not be denied an organ based on non-immunogenic polymorphisms. Besides allocation, immunogenic epitope mismatch scores can also be used for personalised medicine as these may serve as a biomarker for alloimmunisation risk.[17,19] For individuals at low risk the immunosuppressive drugs can potentially be lowered, which will diminish the risk of side effects.[60] Personalised medicine based on immunogenic epitope mismatch scores will not only apply to kidney transplantation, but also to liver,[61,62] lung,[63] and heart transplant.[64-66] While allocation based on avoiding the most immunogenic epitopes may not always be feasible for these organs due to the necessity of short cold ischemia times and the lower number of available donor organs, immunogenic epitope mismatch scores may be used as indicator of the risk for alloimmunisation and therefore as a parameter upon which treatment can be adjusted.

As discussed, the presence of HLA antibodies complicates repeat transplantation, especially for highly sensitised patients. The Eurotransplant Acceptable Mismatch program has shown to be successful in determining acceptable mismatches to which the patient did not form antibodies, and which are used for selection of compatible donors.[67,68] Highly sensitised patients transplanted through this program had a superior graft survival compared to patients transplanted based on merely avoiding unacceptable mismatches.[69,70] In this program epitope analysis to define acceptable/unacceptable mismatches has already been incorporated, but will benefit from an inventory of well-defined immunogenic HLA class I and HLA class II epitopes. However, while it is important to note that for defining acceptable and unacceptable HLA mismatches understanding the exact antibody-antigen interaction and the crucial configurations involved are essential, this is extremely complex and requires additional research. Eventually, this knowledge can be used to define acceptable and unacceptable epitopes to be used for virtual crossmatching, as described in **Chapter 2**. Importantly, this approach allows for defining acceptability of HLA alleles not present in single antigen bead assays. Currently, we are working on implementing defining acceptable and unacceptable HLA mismatches in HLA-EMMA.

While HLA epitope matching is becoming a hot topic in the transplant community and clinicians are eager to start epitope matching, more research is required to introduce HLA epitope matching properly. This thesis forms the basis for these additional studies to be performed.

REFERENCES

1. van Rood JJ, van Leeuwen A, Bruning JW, Porter KA. The importance of leukocyte antigens in renal transplantation. *Folia medica Neerlandica.* 1968;11(1):4-11.
2. 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(10):1505-1513.
3. Wiebe C, Gibson IW, Blydt-Hansen TD, et al. Evolution and clinical pathologic correlations of de novo donor-specific HLA antibody post kidney transplant. *American journal of transplantation.* 2012;12(5):1157-1167.
4. Everly MJ, Rebellato LM, Haisch CE, et al. Incidence and impact of de novo donor-specific alloantibody in primary renal allografts. *Transplantation.* 2013;95(3):410-417.
5. Kosmoliaptsis V, Gjorgjimajkoska O, Sharples LD, et al. Impact of donor mismatches at individual HLA-A, -B, -C, -DR, and -DQ loci on the development of HLA-specific antibodies in patients listed for repeat renal transplantation. *Kidney international.* 2014;86(5):1039-1048.
6. Duquesnoy RJ. A structurally based approach to determine HLA compatibility at the humoral immune level. *Human immunology.* 2006;67(11):847-862.
7. Duquesnoy RJ, Askar M. HLAMatchmaker: a molecularly based algorithm for histocompatibility determination. V. Eplet matching for HLA-DR, HLA-DQ, and HLA-DP. *Human immunology.* 2007;68(1):12-25.
8. Kosmoliaptsis V, Chaudhry AN, Sharples LD, et al. Predicting HLA class I alloantigen immunogenicity from the number and physiochemical properties of amino acid polymorphisms. *Transplantation.* 2009;88(6):791-798.
9. Kosmoliaptsis V, Sharples LD, Chaudhry AN, Halsall DJ, Bradley JA, Taylor CJ. Predicting HLA class II alloantigen immunogenicity from the number and physiochemical properties of amino acid polymorphisms. *Transplantation.* 2011;91(2):183-190.
10. Geneugelijk K, Spierings E. PIRCHE-II: an algorithm to predict indirectly recognizable HLA epitopes in solid organ transplantation. *Immunogenetics.* 2019:10.1007/s00251-00019-01140-x.
11. 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. *Human immunology.* 2013;74(3):290-296.
12. Wiebe C, Kosmoliaptsis V, Pochinco D, Taylor CJ, Nickerson P. A Comparison of HLA Molecular Mismatch Methods to Determine HLA Immunogenicity. *Transplantation.* 2018;102(8):1338-1343.
13. Lachmann N, Niemann M, Reinke P, et al. Donor-Recipient Matching Based on Predicted Indirectly Recognizable HLA Epitopes Independently Predicts the Incidence of De Novo Donor-Specific HLA Antibodies Following Renal Transplantation. *American journal of transplantation.* 2017;17(12):3076-3086.
14. Dankers MK, Witvliet MD, Roelen DL, et al. The number of amino acid triplet differences between patient and donor is predictive for the antibody reactivity against mismatched human leukocyte antigens. *Transplantation.* 2004;77(8):1236-1239.
15. Sapir-Pichhadze R, Zhang X, Ferradji A, et al. Epitopes as characterized by antibody-verified eplet mismatches determine risk of kidney transplant loss. *Kidney international.* 2020;97(4):778-785.
16. Tambur AR, McDowell H, Hod-Dvorai R, Abundis MAC, Pinelli DF. The quest to decipher HLA immunogenicity: Telling friend from foe. *American journal of transplantation.* 2019;19(10):2910-2925.
17. Wiebe C, Kosmoliaptsis V, Pochinco D, et al. HLA-DR/DQ molecular mismatch: A prognostic biomarker for primary alloimmunity. *American journal of transplantation.* 2019;19(6):1708-1719.
18. 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. *American journal of transplantation.* 2013;13(12):3114-3122.
19. Wiebe C, Rush DN, Gibson IW, et al. Evidence for the alloimmune basis and prognostic significance of Borderline T cell-mediated rejection. *American journal of transplantation.* 2020.

20. Kosmoliaptsis V, Bradley JA, Sharples LD, et al. Predicting the immunogenicity of human leukocyte antigen class I alloantigens using structural epitope analysis determined by HLAMatchmaker. *Transplantation.* 2008;85(12):1817-1825.
21. Sharma A, Taverniti A, Graf N, et al. The association between human leukocyte antigen eplet mismatches, de novo donor-specific antibodies, and the risk of acute rejection in pediatric kidney transplant recipients. *Pediatric nephrology.* 2020;35(6):1061-1068.
22. Singh P, Filippone EJ, Colombe BW, et al. Sensitization trends after renal allograft failure: the role of DQ eplet mismatches in becoming highly sensitized. *Clinical transplantation.* 2016;30(1):71-80.
23. Sapir-Pichhadze R, Tinckam K, Quach K, et al. HLA-DR and -DQ eplet mismatches and transplant glomerulopathy: a nested case-control study. *American journal of transplantation.* 2015;15(1):137-148.
24. Do Nguyen HT, Wong G, Chapman JR, et al. The Association Between Broad Antigen HLA Mismatches, Eplet HLA Mismatches and Acute Rejection After Kidney Transplantation. *Transplantation direct.* 2016;2(12):e120.
25. Tafulo S, Malheiro J, Santos S, et al. Degree of HLA class II eplet mismatch load improves prediction of antibody-mediated rejection in living donor kidney transplantation. *Human immunology.* 2019;80(12):966-975.
26. Snanoudj R, Kamar N, Cassuto E, et al. Epitope load identifies kidney transplant recipients at risk of allosensitization following minimization of immunosuppression. *Kidney international.* 2019;95(6):1471-1485.
27. Daniels L, Naesens M, Bosmans JL, et al. The clinical significance of epitope mismatch load in kidney transplantation: A multicentre study. *Transplant immunology.* 2018;50:55-59.
28. Kausman JY, Walker AM, Cantwell LS, Quinlan C, Sypek MP, Ierino FL. Application of an epitope-based allocation system in pediatric kidney transplantation. *Pediatric transplantation.* 2016;20(7):931-938.
29. Kramer C, Heidt S, Claas FHJ. Towards the identification of the relative immunogenicity of individual HLA antibody epitopes. *Human immunology.* 2019;80(4):218-220.
30. El-Awar N, Lee JH, Tarsitani C, Terasaki PI. HLA class I epitopes: recognition of binding sites by mAbs or eluted alloantibody confirmed with single recombinant antigens. *Human immunology.* 2007;68(3):170-180.
31. El-Awar N, Terasaki PI, Cai J, Deng CT, Ozawa M, Nguyen A. Epitopes of the HLA-A, B, C, DR, DQ and MICA antigens. *Clinical transplants.* 2007:175-194.
32. Duquesnoy RJ, Marrari M, Jelenik L, Zeevi A, Claas FH, Mulder A. Structural aspects of HLA class I epitopes reacting with human monoclonal antibodies in Ig-binding, C1q-binding and lymphocytotoxicity assays. *Human immunology.* 2013;74(10):1271-1279.
33. Duquesnoy RJ, Marrari M, Mulder A, Claas FH, Mostecki J, Balazs I. Structural aspects of human leukocyte antigen class I epitopes detected by human monoclonal antibodies. *Human immunology.* 2012;73(3):267-277.
34. Duquesnoy RJ, Marrari M, Mulder A, Sousa LCDD, da Silva AS, do Monte SJH. First report on the antibody verification of HLA-ABC epitopes recorded in the website-based HLA Epitope Registry. *Tissue antigens.* 2014;83(6):391-400.
35. Duquesnoy RJ, Marrari M, Tambur AR, et al. First report on the antibody verification of HLA-DR, HLA-DQ and HLA-DP epitopes recorded in the HLA Epitope Registry. *Human immunology.* 2014;75(11):1097-1103.
36. Duquesnoy RJ. Update of the HLA class I eplet database in the website based registry of antibody-defined HLA epitopes. *Tissue antigens.* 2014;83(6):382-390.
37. Duquesnoy RJ, Honger G, Hosli I, Marrari M, Schaub S. Antibody-defined epitopes on HLA-DQ alleles reacting with antibodies induced during pregnancy and the design of a DQ eplet map. *Human immunology.* 2016;77(10):824-831.
38. Duquesnoy RJ, Honger G, Hosli I, Marrari M, Schaub S. Identification of epitopes on HLA-DRB alleles reacting with antibodies in sera from women sensitized during pregnancy. *Human immunology.* 2016;77(2):214-222.

39. Duquesnoy RJ, Honger G, Hosli I, Marrari M, Schaub S. Detection of newly antibody-defined epitopes on HLA class I alleles reacting with antibodies induced during pregnancy. *International journal of immunogenetics.* 2016;43(4):200-208.
40. Daniels L, Emonds MP, Bosmans JL, Marrari M, Duquesnoy RJ. Epitope analysis of DQ6-reactive antibodies in sera from a DQ6-positive transplant candidate sensitized during pregnancy. *Transplant immunology.* 2016;38:15-18.
41. Anunciacao FA, Sousa LC, da Silva AS, et al. EpViX: A cloud-based tool for epitope reactivity analysis and epitope virtual crossmatching to identify low immunologic risk donors for sensitized recipients. *Transplant immunology.* 2015;33(3):153-158.
42. Tambur AR. Hiding in Plain Sight-A New Look at HLA Epitopes: A Case Report. *American journal of transplantation.* 2016;16(11):3286-3291.
43. Daga S, Moyse H, Briggs D, et al. Direct quantitative measurement of the kinetics of HLA-specific antibody interactions with isolated HLA proteins. *Human immunology.* 2018;79(2):122-128.
44. Visentin J, Leu DL, Mulder A, et al. Measuring anti-HLA antibody active concentration and affinity by surface plasmon resonance: Comparison with the luminex single antigen flow beads and T-cell flow cytometry crossmatch results. *Molecular immunology.* 2019;108:34-44.
45. Kosmoliaptsis V, Dafforn TR, Chaudhry AN, Halsall DJ, Bradley JA, Taylor CJ. High-resolution, three-dimensional modeling of human leukocyte antigen class I structure and surface electrostatic potential reveals the molecular basis for alloantibody binding epitopes. *Human immunology.* 2011;72(11):1049-1059.
46. Mallon DH, Bradley JA, Winn PJ, Taylor CJ, Kosmoliaptsis V. Three-Dimensional Structural Modelling and Calculation of Electrostatic Potentials of HLA Bw4 and Bw6 Epitopes to Explain the Molecular Basis for Alloantibody Binding: Toward Predicting HLA Antigenicity and Immunogenicity. *Transplantation.* 2015;99(2):385-390.
47. Gill JS, Landsberg D, Johnston O, et al. Screening for de novo anti-human leukocyte antigen antibodies in nonsensitized kidney transplant recipients does not predict acute rejection. *Transplantation.* 2010;89(2):178-184.
48. Loupy A, Lefaucheur C, Vernerey D, et al. Complement-binding anti-HLA antibodies and kidney-allograft survival. *The New England journal of medicine.* 2013;369(13):1215-1226.
49. Sutherland SM, Chen G, Sequeira FA, Lou CD, Alexander SR, Tyan DB. Complement-fixing donor-specific antibodies identified by a novel C1q assay are associated with allograft loss. *Pediatric transplantation.* 2012;16(1):12-17.
50. Thurman JM, Panzer SE, Le Quintrec M. The role of complement in antibody mediated transplant rejection. *Molecular immunology.* 2019;112:240-246.
51. Jindra PT, Zhang XH, Mulder A, et al. Anti-HLA antibodies can induce endothelial cell survival or proliferation depending on their concentration. *Transplantation.* 2006;82(1):S33-S35.
52. Valenzuela NM, Mulder A, Reed EF. HLA class I antibodies trigger increased adherence of monocytes to endothelial cells by eliciting an increase in endothelial P-selectin and, depending on subclass, by engaging FcgammaRs. *Journal of immunology.* 2013;190(12):6635-6650.
53. Jin YP, Valenzuela NM, Zhang X, Rozengurt E, Reed EF. HLA Class II-Triggered Signaling Cascades Cause Endothelial Cell Proliferation and Migration: Relevance to Antibody-Mediated Transplant Rejection. *Journal of immunology.* 2018;200(7):2372-2390.
54. Aljabri A, Vijayan V, Stankov M, et al. HLA class II antibodies induce necrotic cell death in human endothelial cells via a lysosomal membrane permeabilization-mediated pathway. *Cell death & disease.* 2019;10(3):235.
55. Gu Y, Wong YH, Liew CW, et al. Defining the structural basis for human alloantibody binding to human leukocyte antigen allele HLA-A*11:01. *Nature communications.* 2019;10(1):893.
56. Honger G, Amico P, Arnold ML, Spriewald BM, Schaub S. Effects of weak/non-complement-binding HLA antibodies on C1q-binding. *HLA.* 2017;90(2):88-94.

57. Kushihata F, Watanabe J, Mulder A, Claas F, Scornik JC. Human leukocyte antigen antibodies and human complement activation: role of IgG subclass, specificity, and cytotoxic potential. *Transplantation.* 2004;78(7):995-1001.
58. Lion J, Taflin C, Cross AR, et al. HLA Class II Antibody Activation of Endothelial Cells Promotes Th17 and Disrupts Regulatory T Lymphocyte Expansion. *American journal of transplantation.* 2016;16(5):1408-1420.
59. Karahan GE, de Vaal YJ, Roelen DL, Buchli R, Claas FH, Heidt S. Quantification of HLA class II-specific memory B cells in HLA-sensitized individuals. *Human immunology.* 2015;76(2-3):129-136.
60. Wiebe C, Rush DN, Nevins TE, et al. Class II Eplet Mismatch Modulates Tacrolimus Trough Levels Required to Prevent Donor-Specific Antibody Development. *Journal of the American Society of Nephrology.* 2017;28(11):3353-3362.
61. Ekong UD, Antala S, Bow L, et al. HLA, Non-HLA Antibodies, and Eplet Mismatches in Pediatric Liver Transplantation: Observations From a Small, Single-Center Cohort. *Experimental and clinical transplantation.* 2019;17(Suppl 1):6-17.
62. Guiral S, Segundo DS, Irure J, et al. Number of Antibody Verified Eplet in Hla-C Locus as an Independent Factor of T Cell Mediated Rejection after Liver Transplantation. *Transplantation.* 2020;104(3):562-567.
63. Walton DC, Hiho SJ, Cantwell LS, et al. HLA Matching at the Eplet Level Protects Against Chronic Lung Allograft Dysfunction. *American journal of transplantation.* 2016;16(9):2695-2703.
64. Nilsson J, Ansari D, Ohlsson M, et al. Human Leukocyte Antigen-Based Risk Stratification in Heart Transplant Recipients-Implications for Targeted Surveillance. *Journal of the American Heart Association.* 2019;8(15):e011124.
65. Sullivan PM, Warner P, Kemna MS, et al. HLA molecular epitope mismatching and long-term graft loss in pediatric heart transplant recipients. *Journal of Heart and Lung Transplantation.* 2015;34(7):950-957.
66. McCaughan JA, Battle RK, Singh SKS, et al. Identification of risk epitope mismatches associated with de novo donor-specific HLA antibody development in cardiothoracic transplantation. *American journal of transplantation.* 2018;18(12):2924-2933.
67. Claas FH, Witvliet MD, Duquesnoy RJ, Persijn GG, Doxiadis, II. The acceptable mismatch program as a fast tool for highly sensitized patients awaiting a cadaveric kidney transplantation: short waiting time and excellent graft outcome. *Transplantation.* 2004;78(2):190-193.
68. Heidt S, Witvliet MD, Haasnoot GW, Claas FH. The 25th anniversary of the Eurotransplant Acceptable Mismatch program for highly sensitized patients. *Transplant immunology.* 2015;33(2):51-57.
69. Heidt S, Haasnoot GW, van Rood JJ, Witvliet MD, Claas FHJ. Kidney allocation based on proven acceptable antigens results in superior graft survival in highly sensitized patients. *Kidney international.* 2018;93(2):491-500.
70. Heidt S, Haasnoot GW, Witvliet MD, et al. Allocation to highly sensitized patients based on acceptable mismatches results in low rejection rates comparable to nonsensitized patients. *American journal of transplantation.* 2019;19(10):2926-2933.