Human Immunology 80 (2019) 79–84

Contents lists available at ScienceDirect

Human Immunology

journal homepage: www.elsevier.com/locate/humimm

Review

Continue to focus clinical decision-making on the antigen recognition domain for the present

Carolyn Katovich Hurley[a,*], Jennifer Ng[b]

[a] Department of Oncology, Georgetown University, United States
[b] Department Pediatrics, Georgetown University, United States

ARTICLE INFO

Keywords:
Antigen recognition domain
HLA typing
Histocompatibility matching

ABSTRACT

Next generation DNA sequencing has facilitated the routine characterization of complete HLA gene sequences. These data complement structural and functional studies of HLA elements encoded outside of the exons specifying the antigen recognition domain. This commentary is focused on evaluating whether the interpretation of HLA clinical typing results should expand the region of the HLA gene considered in the assignment from the exon (s) encoding the antigen recognition domain to the full gene sequence. Our recommendation is that, at present, there is insufficient data to support considering variation in the regions outside of the antigen recognition domain in clinical decision-making.

1. Introduction

The introduction of next generation sequencing (NGS) methods has made it easier to routinely assess the complete nucleotide sequence of an HLA allele [1,2]. A comprehensive picture of the nature of HLA genomic diversity is beginning to emerge from these efforts focused on the regions of the HLA gene outside of the antigen recognition domain (ARD)-encoding exons. Studies have shown the impact of the non-ARD regions on: (1) our understanding of the evolution of the HLA system [3]; (2) HLA expression and levels on the cell surface [4–7]; (3) the polypeptide sequence of HLA [8]; (4) trafficking, peptide loading, and intracellular signaling [9,10]; and (5) intercellular interactions [11,12]. HLA gene polymorphism outside of ARD-encoding exons can also impact other immune responses. HLA-B intron encoded microRNA appears to regulate the expression of IgA transcripts [13]. Dimorphism in HLA-B leader peptides modulates HLA-E binding and surface expression, impacting natural killer (NK) cell receptor CD94/NKG2A binding and NK education [14]. Thus, variation outside the ARD can impact many different aspects of HLA and can affect immune responses.

Considering this information, this commentary is focused on a reassessment of the resolution required for HLA clinical testing. Clinical testing is defined as HLA typing for: (1) donor selection for cell, tissue and organ transplantation and transfusion support, (2) assessment of risk of disease and adverse drug reactions, and (3) determination of vaccine eligibility. Donor selection includes both an evaluation of HLA matching between potential donor and recipient and HLA-specific antibody analysis testing. Attempts to distinguish among the over 17,000 class I and class II alleles is a challenge for the clinical laboratory in terms of effort, time, and cost. **Our recommendation is that clinical decision-making should focus only on the sequence of the antigen recognition domain (ARD) with the exception of common non-expressed alleles that are distinguished by variation outside of the ARD.** If further information supporting clinical impact on the non-ARD regions is obtained, the HLA typing resolution can be expanded in the future.

2. Definition of ARD resolution

The majority of the ARD is encoded by class I exons 2 and 3 (e.g., encoding HLA-A amino acids 1–182) and class II exon 2 (e.g., encoding DRB1 amino acids 5–94). Alleles that share the nucleotide sequence of these exons fall into a G group [15]. For example, based on the HLA nomenclature website from September 2017 [16], there are 39 alleles that share the DNA sequence designated C*04:01:01G (Table 1, Supplementary Fig. 1). Named after the reference allele C*04:01:01:01, 20 of these alleles display non-coding (e.g., C*04:01:01:02) and/or synonymous (e.g., 04:01:54) variation when compared to C*04:01:01:01, one allele encodes a non-expressed allele (C*04:09N) and 17 encode nonsynonymous substitutions designated by the second nomenclature field (e.g., C*04:28). A G designation can be readily assigned by several different DNA-based HLA typing strategies [1,2,17–19].

Alleles that share the polypeptide sequence of the ARD region fall into a P group (e.g., C*04:01P) [15]. No DNA-based typing method can

* Corresponding author.
E-mail address: hurleyc@georgetown.edu (C.K. Hurley).

https://doi.org/10.1016/j.humimm.2018.04.010
Received 4 January 2018; Received in revised form 10 April 2018; Accepted 15 April 2018
Available online 21 April 2018

Table 1
Alleles included in HLA-C*04:01:01G.

Primary Allele N = 1	Synonymous and Non-coding[a] N = 20	Nonsynonymous[b] N = 17	Expression Variation N = 1
C*04:01:01:01	C*04:01:01:02	C*04:28	C*04:09N
	C*04:01:01:03	C*04:30	
	C*04:01:01:04	C*04:41	
	C*04:01:01:05	C*04:79	
	C*04:01:01:06	C*04:82	
	C*04:01:01:07	C*04:84	
	C*04:01:01:08	C*04:106	
	C*04:01:01:09	C*04:144	
	C*04:01:01:10	C*04:146	
	C*04:01:01:11	C*04:161	
	C*04:01:01:12	C*04:162	
	C*04:01:01:13	C*04:165	
	C*04:01:01:14	C*04:195	
	C*04:01:54	C*04:226	
	C*04:01:57	C*04:267	
	C*04:01:69	C*04:274	
	C*04:01:78	C*04:275	
	C*04:01:79		
	C*04:01:82		
	C*04:01:83		

[a] Synonymous substitutions are silent nucleotide changes in exons that do not alter the amino acid sequence. Non-coding substitutions are alterations outside of the exons (i.e., in introns or 5′ and 3′ untranslated regions). These substitutions may have no impact on expression or may alter regulatory regions (e.g., RNA splicing sites).

[b] Nonsynonymous substitutions alter the polypeptide sequence. The alleles listed all encode amino acid sequence differences outside of the ARD-encoding exons 2 and 3.

achieve an assignment that includes all of the alleles forming a P group and only those alleles. However, some laboratories list a P assignment in their final report as a means of indicating that the ambiguity remaining after typing does not alter the sequence of the HLA proteins present. Because this designation does not coincide with the actual typing result, some applications will not accept a P assignment. For example, the National Marrow Donor Program (NMDP) utilizes its registry data to predict HLA allele and haplotype frequencies used for their match algorithm HapLogic© [17]; thus, assignments that do not reflect the precise set of alleles identified by the testing contribute inaccurate data to the algorithm and are not accepted. Further explanation of the P assignment can be found in Supplementary Material.

A published definition of “high resolution” defines it as discriminating among alleles that encode different polypeptide sequences in the ARD and excluding all non-expressed alleles [18]. Our local definition of “high resolution” differs in that it includes only alleles that share their nucleotide sequence in the ARD and it specifies only common null alleles to be considered in the typing strategy. While consideration of all relevant null alleles was included in the original definition of high resolution typing [18], the very low frequency of many of the known null alleles suggests that typing to specifically eliminate these nulls might not be cost effective, may be less robust, and is likely to cause delays in finalizing clinical decisions. For example, A*01:04N was listed as a common allele in the 2012 listing of common and well-documented alleles [19]; this allele is included in the A*01:01:01G group. Since that time, the NMDP has required testing for this and similar null alleles during the extended typing phase of donor selection. Allele level typing of large numbers of volunteer donors in the United States has now determined the frequency of A*01:04N to be very low, appearing only twice compared to A*01:01:01:01 which was assigned over 68,000 times (J. Dehn, NMDP, personal communication). In contrast, C*04:09N [20,21], DRB4*01:03:01:02N [22], and DRB5*01:08N [23] are found frequently suggesting that these common null alleles be included in clinical testing of the relevant loci. Other null alleles may also be identified as common as frequency data accumulates.

3. HLA typing methods for ARD resolution

This commentary is focused on the region of the gene that should be considered in making an HLA assignment during clinical testing. There are several DNA-based HLA typing methods that can assign HLA at ARD resolution. Sequencing either by Sanger chain termination chemistry [24–26] or by next generation strategies [1,2,27] provides the complete nucleotide sequence of the targeted region. Phasing (i.e., assignment of polymorphic residues to either paternal or maternal chromosome) in order to resolve alternative genotypes with the same consensus sequence can often, but not always, be obtained by next generation sequencing (NGS) [27,28]. Preparation of sequencing templates containing one of the two alleles in a heterozygote by allele (or group)-specific amplification has been used for phasing in both Sanger [24] and NGS [20] protocols. Other phasing strategies couple Sanger sequencing with sequence specific oligonucleotide hybridization [26], sequence specific priming [29], or sequence-specific sequencing primers [25]. If high resolution is not required for the clinical assay, SSOP and SSP assays alone may suffice. All of these DNA-based typing methods will yield ARD-focused HLA assignments.

4. Functional importance of ARD variation

The evidence supporting clinical decision-making based on the ARD itself is not controversial. The majority of the polymorphic residues within an HLA protein reside in the ARD [30] making it the target of natural selection [3]. The ARD binds peptides [31] and is the ligand for T cell receptors [32] and natural killer cell immunoglobulin-like receptors (KIR) [33].

Typing to assess the risk of an autoimmune disease or an off-target drug response or for vaccine eligibility is focused on the ARD exons as these control peptide binding and T cell recognition. While the mechanisms resulting in an autoimmune disease or drug hypersensitivity linked to HLA are not known for all diseases or drugs, the presumed mechanisms include a significant role for the peptide binding site of the HLA molecule (i.e., the ARD) [34,35].

While X-ray crystallographic data on the location of binding of donor-specific antibodies directed to HLA is limited to antibodies binding to the peptide-HLA complex [36,37], analysis of antibody specificity based on shared amino acid sequences and on site-directed mutagenesis has suggested that the majority of antibodies bind to epitopes localized in the ARD [37–40]. The presence of antibodies directed to HLA may require typing resolution higher than serologic antigens to evaluate, for example, the presence of specific alleles of serologic antigens [41,42] but the focus remains on the ARD.

X-ray crystallography shows that alloreactive T cell receptors [43] bind to the ARD. Evaluation of the role of HLA matching at the level of the ARD in transplant outcome has been closely evaluated in hematopoietic stem cell donor-recipient pairs [44–47]. Survival is improved by ARD matching of HLA-A, -B, -C, and -DRB1. In solid organ transplant, HLA mismatching at the level of serologic split antigens increases the risk of graft failure and reduces patient survival [42,48,49]. Thus, the modulation of critical immune interactions by ARD variability and the retrospective outcome analysis based on ARD matching clearly justifies the continued focus on this region of the protein. Whether variation encoded in other regions of the HLA genes will impact outcome and should be also be considered in clinical decision-making in addition to the ARD is discussed in the following paragraphs of this commentary.

5. Null and nonsynonymous variation encoded outside the ARD-encoding exons is infrequent

One consideration for a clinical HLA typing resolution strategy is

how likely it is that a donor and recipient matched for ARDs will be mismatched for a non-expressed (i.e., null) allele or a non-ARD variation in amino acid sequence. It is not yet known how often different alleles within a G group are found associated with different haplotypes so that matching for multiple loci might routinely match for most non-ARD variation.

A mismatch for a non-expressed allele is clearly relevant to donor selection [50]. In June 2017, there were 12,375 HLA-A, -B, -C alleles; 455 (3%) of these alleles have been described as not expressed (http://hla.alleles.org). Only 31 (0.2% of all known class I alleles) of the class I null alleles carry their critical variation outside of the ARD-encoding exons and only six of these have been observed in 2 or more unrelated individuals in the IPD-IMGT/HLA database version 3.29.0 (2017-07) [16]. In our study of random individuals from Argentina (n = 1472) [20] and the United States (n = 1196) [26], the only non-ARD class I null allele observed was C*04:09N which appeared 6 times. Other studies of large U.S. populations (n = 10,000) have also noted the low frequency of outside-ARD null alleles [21]. It is, therefore, likely that most null alleles are rare.

Within G groups, most alleles encoding different amino acid sequences only outside the ARD are also uncommon. In a study of a registry population from Argentina [20], of the 2944 HLA-B alleles identified, only 25 (0.8%) are non-ARD amino acid sequence variants. For example, members of B*15:01:01G, B*15:01:01:01 appears 71 times and B*15:146 appears once. There are exceptions; B*07:06:01 appears 14 times and B*07:05:01:01, the primary allele in the B*07:05:01G group, six times. These two alleles differ by a single amino acid (ile/val) in the transmembrane domain. The limited frequency of variation outside of the ARD is similar for HLA-A (0.3%) and HLA-C (1.9%).

Our laboratory has also provided full gene sequences for several hundred uncommon class I alleles with incomplete sequences in the IPD-IMGT/HLA database (unpublished data). Our observation is that most of these alleles differ from the most closely matched reference allele by a single nucleotide substitution that defined them originally as novel alleles. The remainder of the full gene sequence, exons and introns, are identical to the reference. For example, we have provided full gene sequences of A*03:01:11, A*03:01:16, A*03:06, A*03:47, and A*03:71 to the database. All of these alleles differ from A*03:01:01:01 by a single nucleotide when comparing 3321 nucleotides beginning -118 nucleotides before exon 1 through the 3′UTR. These data suggest that the gene regions outside of the ARD will be highly conserved. Our observations are consistent with the data from others [30].

The frequency of non-ARD amino acid mismatches between hematopoietic stem donor and recipient in 10/10 matches is also low. Of the 3600 allele comparisons made between 360 donor and recipient pairs, only 15 (HLA-A, 2; -B, 3; -C, 4; -DRB1, 2; -DQA1, 4) were encountered in the 360 pairs in transplants facilitated by the NMDP [51]. Similar survival times of hematopoietic stem cell transplantations from matched unrelated donors compared to HLA identical sibling donors suggest that either the frequency of non-ARD mismatches in the unrelated setting are low and/or these mismatches do not impact outcome [52]. From our study of 360 transplantations, it was estimated that 6000–9000 donor-recipient pairs must be typed for full gene sequences in order to achieve enough power to analyze the impact on variation outside ARD-encoding regions in outcome. The Center for International Blood and Marrow Transplant Research has a sample repository of over 39,000 transplant pairs and is expected to complete this full gene sequencing project within the next two years (S. Spellman, personal communication).

The examples provided in this section have focused primarily on the frequency of class I alleles as defined by full gene sequencing. The reason for this is that assessing class II diversity based on full length gene sequences remains a challenge as described below. We have no reason to expect that the general characteristics of class II diversity will be different from that of class I.

6. Limited data exists on the functional impact of variation outside the ARD-encoding exons

The burden of proof that a specific genetic variation must be considered in clinical decision-making should rely on functional and, preferably, on outcomes research. Information on the functional impact of amino acid variation outside of the ARDs in stimulating allorecognition is very limited and is based mainly on *in vitro* studies. B*44:02 and B*44:27 represent one of the most extreme cases of variation within a G group, differing by four amino acids in alpha 3, transmembrane, and cytoplasmic domains. This relatively large variation does not impact peptide binding profiles [53] and alloreactive T cell recognition [54]. Likewise, a study of alloreactive T cell recognition of DPB1*03:01 and DPB1*104:01 which differ by one amino acid in the transmembrane region shows no functional differences [55]. Cytotoxic T lymphocyte precursor assays of several class I non-ARD mismatches and mixed lymphocyte reactions of multiple DRB1*14:01/DRB1*14:54 mismatch combinations differing by a single amino acid in the beta2 domain also demonstrated little to no reactivity [56]. Retrospective outcome studies of the effect of mismatches outside ARD regions in transplantation will require large numbers of donor-recipient pairs to obtain a sufficient number of mismatches to measure the impact of mismatches outside ARD-encoding regions [51].

Much interest has focused on sites in the regions 5′ and 3′ of the HLA gene that might impact HLA expression [57–59]. For example, a microRNA binding site in the 3′ region of HLA-C genes modulates expression of HLA-C allotypes at the cell surface [7]. Some autoimmune diseases, for example, systemic lupus erythematosus and Crohn's disease, have been linked to HLA expression levels [34]. Several outcome studies in hematopoietic stem cell transplantation suggest that higher cell surface levels of the mismatched HLA-C or HLA-DP trigger allorecognition [60–62] although a large registry cohort study did not support the HLA-C observation [63]. Subsequent studies of HLA-C have now shown that the peptide sequences of the alpha1 and alpha2 domains (i.e., the ARD) and their impact on the spectrum of bound peptides appear to play an even stronger role in modulating HLA-C expression than the 3′UTR [64]. Whether expression levels will ultimately define permissive mismatches remains to be more fully investigated. Because of linkage disequilibrium, the 5′ and 3′ gene flanking polymorphisms are strongly associated with specific ARD sequences [28,60]. For this reason, targeted typing of the 5′ and 3′ regions will likely not be necessary to predict expression levels.

7. Assigning HLA alleles based on non-ARD sequences will be a challenge

Finally, there are challenges in routinely obtaining full gene sequences, especially with next generation sequencing [65]. First the sequences of many of the alleles are incomplete in these regions. The length of the class II genes makes full gene sequences difficult to obtain. Non-coding regions, especially in the class II genes, can contain repetitive sequences, insertions/deletions, and GC-rich regions that make interpretation of the sequence difficult [66]. Artifacts caused by the presence of pseudogenes or other co-amplified loci may complicate interpretation. New alleles, varying predominantly in the introns, will be often observed. External proficiency samples to monitor full gene typing assignments are not yet available from vendors. In time, comprehensive typing of the full HLA genes will become more robust but, at present, maintaining a focus on the ARDs of the HLA molecule for clinical decision-making is recommended. Single phased genotype assignments can be obtained by several different strategies including both Sanger [24–26] and next generation [1,2] sequencing protocols.

Another advantage to a focus on the ARD in contrast to the full gene is the ability to readily summarize frequencies of clinically relevant HLA assignments in large registry populations. Ambiguity in HLA assignments has always been a challenge in merging data for a population

study. With more and more alleles exhibiting noncoding variation, the ability to assign a single allele with full field nomenclature has begun to degrade. For example, there are now 12 different nucleotide sequences for B*51:01:01 alleles in the 3′UTR spanning 1072 base pairs following the termination codon. Depending on the annealing position of the primer used to amplify the HLA-B gene, it is possible for a laboratory to detect none, some or all of these polymorphisms. This is also dependent on whether the laboratory can navigate its analysis through a region rich in guanine and cytosine that may include a deletion in one of the two alleles just 3′ of the last exon. Situations like this will lead to ambiguous results for a full field nomenclature assignment. Typing at a G level with phasing to yield a single genotype will provide less ambiguity and allow registries to assess the relevant HLA diversity of their volunteer donor pool.

8. Searching and matching based on full gene assignments may delay or prevent transplantation

The assignment of HLA based on full gene sequences may be misleading in terms of donor search and match status. If matching is decided based on the ARD, then searches using typings with a higher resolution assignment may go astray. For example, a patient typed as A*02:690 will likely find no matches in the current registry; however, converting that typing to its G group, A*02:01:01G, will find many matches. Assignments at allele resolution will appear to be mismatched to the search coordinator or physician, delaying the final donor selection. For example, a patient typed based on the full gene sequence as A*23:17 and a donor typed as A*23:01:01:01 will appear mismatched even though both alleles are members of the A*23:01:01G group. Since there is little information suggesting that the non-ARD mismatches are detrimental to patient survival, other secondary donor selection factors such as donor age [67] and rapid availability [47,68] may be more critical in donor selection. If full gene sequences are used to type donor and/or recipient, these assignments should be converted to ARD resolution for search and match.

9. Research to define the impact of non-ARD variation

Research projects using full gene sequencing of HLA loci will add to our knowledge of HLA diversity and will likely provide additional genetic markers to define haplotypes and disease associations. This information is best collected through large population/registry studies rather than individual observations.

10. Summary

How relevant is non-ARD variation? Does it have a significant clinical impact? Should non-ARD variation be considered for donor-recipient matching? If it is, how should it be prioritized in comparison to other donor selection criteria? We base our recommendation to continue to focus clinical decision-making on the ARD at present on: (1) the low frequency of variation outside the ARD, especially for non-expressed alleles, suggesting that mismatching will be uncommon; (2) the role of the ARD and linkage of all allelic polymorphisms to the ARD in determining expression variation; (3) data from limited cellular studies of allorecognition; (4) the complexity of typing regions outside the ARD; and (5) the potential confusion with matching and search.

It may seem short-sighted to suggest a continued focus on the ARD exons but, at this point in time, we do not understand the clinical impact of variation in the non-ARD regions. Researchers studying these regions and their impact on the immune response will continue to focus their efforts on full gene sequences and their information will fill in the gaps in knowledge needed to refine the resolution of clinical HLA assignments. With the exception of common non-expressed alleles, incorporating non-ARD regions into clinical decision-making is premature.

Acknowledgements

This study is supported by Office of Naval Research grants N00014-16-1-3133 and N00014-15-1-0052 to Georgetown University. The Office of Naval Research did not participate in the design or conduct of the study. The views expressed in this article do not reflect the official policy or position of the Department of the Navy.

Conflicts of interest

Georgetown University has filed a patent application on which Hurley and Ng are inventors of a HLA typing and Sanger-based sequencing technology.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.humimm.2018.04.010.

References

[1] K. Hosomichi, T. Shiina, A. Tajima, I. Inoue, The impact of next-generation sequencing technologies on HLA research, J. Hum. Genet. 60 (2015) 665–673.
[2] V. Bravo-Egana, D. Monos, The impact of next-generation sequencing in immunogenetics: current status and future directions, Curr. Opin. Organ. Transplant 22 (2017) 400–406.
[3] D. Meyer, C.A. Vr, B.D. Bitarello, C.B. Dy, K. Nunes, A genomic perspective on HLA evolution, Immunogenetics (2017).
[4] V.R. Sutton, R.W. Knowles, An aberrant DRB4 null gene transcript is found that could encode a novel HLA-DRb chain, Immunogenetics 31 (1990) 112–117.
[5] A. Balas, S. Santos, M.J. Aviles, F. Garcia-Sanchez, R. Lillo, A. Alvarez, L.M. Villar-Guimerans, J.L. Vicario, Elongation of the cytoplasmic domain, due to a point deletion at exon 7, results in an HLA-C null allele, Cw*0409 N, Tissue Antigens 59 (2002) 95–100.
[6] P. Perrier, A. Dormoy, C. Andre-Botte, N. Froelich, HLA-A*02010102L: a laborious assignment, Tissue Antigens 68 (2006) 442–445.
[7] C. O'hUigin, S. Kulkarni, Y. Xu, Z. Deng, J. Kidd, K. Kidd, X. Gao, M. Carrington, The molecular origin and consequences of escape from miRNA regulation by HLA-C alleles, Am. J. Hum. Genet. 89 (2011) 424–431.
[8] T.V. Hviid, S. Hylenius, C. Rorbye, L.G. Nielsen, HLA-G allelic variants are associated with differences in the HLA-G mRNA isoform profile and HLA-G mRNA levels, Immunogenetics 55 (2003) 63–79.
[9] G. Basha, G. Lizee, A.T. Reinicke, R.P. Seipp, K.D. Omilusik, W.A. Jefferies, MHC class I endosomal and lysosomal trafficking coincides with exogenous antigen loading in dendritic cells, PLoS One 3 (2008) e3247.
[10] D. Makhadiyeva, L. Lam, M. Moatari, J. Vallance, Y. Zheng, E.C. Campbell, S.J. Powis, MHC class I dimer formation by alteration of the cellular redox environment and induction of apoptosis, Immunology 135 (2012) 133–139.
[11] E. Martinez-Naves, L.D. Barber, J.A. Madrigal, C.M. Vullo, C. Clayberger, S.C. Lyu, R.C. Williams, C. Gorodezky, T. Markow, M.L. Petzl-Erler, P. Parham, Interactions of HLA-B*4801 with peptide and CD8, Tissue Antigens 50 (1997) 258–264.
[12] L.E. Hudson, R.L. Allen, Leukocyte Ig-like receptors – A model for MHC class I disease associations, Front. Immunol. 7 (2016) 281.
[13] N. Chitnis, P.M. Clark, M. Kamoun, C. Stolle, J.F. Brad, D.S. Monos, An expanded role for HLA Genes: HLA-B encodes a microRNA that regulates IgA and other immune response transcripts, Front. Immunol. 8 (2017) 583.
[14] A. Horowitz, Z. Djaoud, N. Nemat-Gorgani, J. Blokhuis, H.G. Hilton, V. Beziat, K.J. Malmberg, P.J. Norman, L.A. Guethlein, P. Parham, Class I HLA haplotypes form two schools that educate NK cells in different ways, Sci. Immunol. 1 (2016).
[15] S.G. Marsh, E.D. Albert, W.F. Bodmer, R.E. Bontrop, B. Dupont, H.A. Erlich, M. Fernandez-Vina, D.E. Geraghty, R. Holdsworth, C.K. Hurley, M. Lau, K.W. Lee, B. Mach, M. Maiers, W.R. Mayr, C.R. Muller, P. Parham, E.W. Petersdorf, T. Sasazuki, J.L. Strominger, A. Svejgaard, P.I. Terasaki, J.M. Tiercy, J. Trowsdale, Nomenclature for factors of the HLA system, 2010, Tissue Antigens 75 (2010) 291–455.
[16] J. Robinson, J.A. Halliwell, J.D. Hayhurst, P. Flicek, P. Parham, S.G. Marsh, The IPD and IMGT/HLA database: allele variant databases, Nucleic Acids Res. 43 (2015) D423–D431.
[17] J. Dehn, M. Setterholm, K. Buck, J. Kempenich, B. Beduhn, L. Gragert, A. Madbouly, S. Fingerson, M. Maiers, HapLogic: a predictive human leukocyte antigen-matching algorithm to enhance rapid identification of the optimal unrelated hematopoietic stem cell sources for transplantation, Biol. Blood Marrow Transplant 22 (2016) 2038–2046.
[18] E. Nunes, H. Heslop, M. Fernandez-Vina, C. Taves, D.R. Wagenknecht, A.B. Eisenbrey, G. Fischer, K. Poulton, K. Wacker, C.K. Hurley, H. Noreen, N. Sacchi, Definitions of histocompatibility typing terms: harmonization of histocompatibility typing terms working group, Hum. Immunol. 72 (2011) 1214–1216.
[19] S.J. Mack, P. Cano, J.A. Hollenbach, J. He, C.K. Hurley, D. Middleton, M.E. Moraes, S.E. Pereira, J.H. Kempenich, E.F. Reed, M. Setterholm, A.G. Smith, M.G. Tilanus,

M. Torres, M.D. Varney, C.E. Voorter, G.F. Fischer, K. Fleischhauer, D. Goodridge, W. Klitz, A.M. Little, M. Maiers, S.G. Marsh, C.R. Muller, H. Noreen, E.H. Rozemuller, A. Sanchez-Mazas, D. Senitzer, E. Trachtenberg, M. Fernandez-Vina, Common and well-documented HLA alleles: 2012 update to the CWD catalogue, Tissue Antigens 81 (2013) 194–203.
[20] C.K. Hurley, L. Hou, A. Lazaro, J. Gerfen, E. Enriquez, P. Galarza, M.B. Cardoza, M. Halagan, M. Maiers, D. Behm, J. Ng, Next generation sequencing characterizes the extent of HLA diversity in an Argentinian registry population, HLA (2018).
[21] Y. Yin, J.H. Lan, D. Nguyen, N. Valenzuela, P. Takemura, Y.T. Bolon, B. Springer, K. Saito, Y. Zheng, T. Hague, A. Pasztor, G. Horvath, K. Rigo, E.F. Reed, Q. Zhang, Application of high-throughput next-generation sequencing for HLA typing on buccal extracted DNA: results from over 10,000 donor recruitment samples, PLoS One 11 (2016) e0165810.
[22] C.E. Voorter, N.M. Lardy, E.M. van den Berg-Loonen, Presence of the DRB4*0103102N null allele in different DRB1*04-positive individuals, Tissue Antigens 55 (2000) 37–43.
[23] C.E.M. Voorter, H.E.T. Roeffaers, E.D. Du Toit, E.M. Van den Berg-Loonen, The absence of DR51 in a DRB5-positive individual DR2ES is caused by a null allele (DRB5*0108N), Tissue Antigens 50 (1997) 326–333.
[24] K. Kotsch, J. Wehling, S. Kohler, R. Blasczyk, Sequencing of HLA class I genes based on the conserved diversity of the noncoding regions: sequencing-based typing of the HLA-A gene, Tissue Antigens 50 (1997) 178–191.
[25] D. Sayer, R. Whidborne, B. Brestovac, F. Trimboli, C. Witt, F. Christiansen, HLA-DRB1 DNA sequencing based typing: an approach suitable for high throughput typing including unrelated bone marrow registry donors, Tissue Antigens 57 (2001) 46–54.
[26] B. Tu, C. Masaberg, L. Hou, D. Behm, P. Brescia, N. Cha, K. Kariyawasam, J.H. Lee, T. Nong, J. Sells, P. Tausch, R. Yang, J. Ng, C.K. Hurley, Combining one-step Sanger sequencing with phasing probe hybridization for HLA class I typing yields rapid, G-group resolution predicting 99% of unique full length protein sequences, HLA 89 (2017) 90–97.
[27] T.R. Turner, J.D. Hayhurst, D.R. Hayward, W.P. Bultitude, D.J. Barker, J. Robinson, J.A. Madrigal, N.P. Mayor, S.G.E. Marsh, Single molecule real-time DNA sequencing of HLA genes at ultra-high resolution from 126 International HLA and Immunogenetics Workshop cell lines, HLA 91 (2018) 88–101.
[28] B. Schone, S. Bergmann, K. Lang, I. Wagner, A.H. Schmidt, E.W. Petersdorf, V. Lange, Predicting an HLA-DPB1 expression marker based on standard DPB1 genotyping: linkage analysis of over 32,000 samples, Hum. Immunol. (2017).
[29] K. Welsh, M. Bunce, Molecular typing for the MHC with PCR-SSP, Rev. Immunogenet. 1 (1999) 157–176.
[30] J. Robinson, L.A. Guethlein, N. Cereb, S.Y. Yang, P.J. Norman, S.G.E. Marsh, P. Parham, Distinguishing functional polymorphism from random variation in the sequences of > 10,000 HLA-A, -B and -C alleles, PLoS Genet. 13 (2017) e1006862.
[31] P.J. Bjorkman, M.A. Saper, B. Samraoui, W.S. Bennett, J.L. Strominger, D.C. Wiley, The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens, Nature 329 (1987) 512–518.
[32] J. Rossjohn, S. Gras, J.J. Miles, S.J. Turner, D.I. Godfrey, J. McCluskey, T cell antigen receptor recognition of antigen-presenting molecules, Annu. Rev. Immunol. 33 (2015) 169–200.
[33] J.C. Boyington, A.G. Brooks, P.D. Sun, Structure of killer cell immunoglobulin-like receptors and their recognition of the class I MHC molecules, Immunol. Rev. 181 (2001) 66–78.
[34] C.A. Dendrou, J. Petersen, J. Rossjohn, L. Fugger, HLA variation and disease, Nat. Rev. Immunol. (2018).
[35] P.T. Illing, A.W. Purcell, J. McCluskey, The role of HLA genes in pharmacogenomics: unravelling HLA associated adverse drug reactions, Immunogenetics 69 (2017) 617–630.
[36] M. Hulsmeyer, P. Chames, R.C. Hillig, R.L. Stanfield, G. Held, P.G. Coulie, C. Alings, G. Wille, W. Saenger, B. Uchanska-Ziegler, H.R. Hoogenboom, A. Ziegler, A major histocompatibility complex-peptide-restricted antibody and t cell receptor molecules recognize their target by distinct binding modes: crystal structure of human leukocyte antigen (HLA)-A1-MAGE-A1 in complex with FAB-HYB3, J. Biol. Chem. 280 (2005) 2972–2980.
[37] W.E. Biddison, R.W. Anderson, E.P. Cowan, R.V. Turner, J.E. Coligan, K. Hannestad, T. Hansen, W.L. Maloy, Structural studies of an HLA-A03 alloantigenic epitope defined by a human hybridoma antibody, Immunogenetics 30 (1989) 54–57.
[38] R.J. Duquesnoy, Reflections on HLA Epitope-Based Matching for Transplantation, Front. Immunol. 7 (2016) 469.
[39] K.T. Hogan, C. Clayberger, E.J. Bernhard, S.F. Walk, J.P. Ridge, P. Parham, A.M. Krensky, V.H. Engelhard, Identification by site-directed mutagenesis of amino acid residues contributing to serologic and CTL-defined epitope differences between HLA-A2.1 and HLA-A2.3, J. Immunol. 141 (1988) 2519–2525.
[40] N. El-Awar, V. Jucaud, A. Nguyen, HLA epitopes: the targets of monoclonal and alloantibodies defined, J. Immunol. Res. 2017 (2017) 3406230.
[41] B.D. Tait, C. Susal, H.M. Gebel, P.W. Nickerson, A.A. Zachary, F.H. Claas, E.F. Reed, R.A. Bray, P. Campbell, J.R. Chapman, P.T. Coates, R.B. Colvin, E. Cozzi, I.I. Doxiadis, S.V. Fuggle, J. Gill, D. Glotz, N. Lachmann, T. Mohanakumar, N. Suciu-Foca, S. Sumitran-Holgersson, K. Tanabe, C.J. Taylor, D.B. Tyan, A. Webster, A. Zeevi, G. Opelz, Consensus guidelines on the testing and clinical management issues associated with HLA and non-HLA antibodies in transplantation, Transplantation 95 (2013) 19–47.
[42] A.A. Zachary, M.S. Leffell, HLA mismatching strategies for solid organ transplantation – a balancing act, Front. Immunol. 7 (2016) 575.
[43] W.A. Macdonald, Z. Chen, S. Gras, J.K. Archbold, F.E. Tynan, C.S. Clements, M. Bharadwaj, L. Kjer-Nielsen, P.M. Saunders, M.C. Wilce, F. Crawford, B. Stadinsky, D. Jackson, A.G. Brooks, A.W. Purcell, J.W. Kappler, S.R. Burrows, J. Rossjohn, J. McCluskey, T cell allorecognition via molecular mimicry, Immunity 31 (2009) 897–908.
[44] J. Pidala, S.J. Lee, K.W. Ahn, S. Spellman, H.L. Wang, M. Aljurf, M. Askar, J. Dehn, V.M. Fernandez, A. Gratwohl, V. Gupta, R. Hanna, M.M. Horowitz, C.K. Hurley, Y. Inamoto, A.A. Kassim, T. Nishihori, C. Mueller, M. Oudshoorn, E.W. Petersdorf, V. Prasad, J. Robinson, W. Saber, K.R. Schultz, B. Shaw, J. Storek, W.A. Wood, A.E. Woolfrey, C. Anasetti, Nonpermissive HLA-DPB1 mismatch increases mortality after myeloablative unrelated allogeneic hematopoietic cell transplantation, Blood 124 (2014) 2596–2606.
[45] D. Furst, C. Muller, V. Vucinic, D. Bunjes, W. Herr, M. Gramatzki, R. Schwerdtfeger, R. Arnold, H. Einsele, G. Wulf, M. Pfreundschuh, B. Glass, H. Schrezenmeier, K. Schwarz, J. Mytilineos, High-resolution HLA matching in hematopoietic stem cell transplantation: a retrospective collaborative analysis, Blood 122 (2013) 3220–3229.
[46] Y. Morishima, K. Kashiwase, K. Matsuo, F. Azuma, S. Morishima, M. Onizuka, T. Yabe, M. Murata, N. Doki, T. Eto, T. Mori, K. Miyamura, H. Sao, T. Ichinohe, H. Saji, S. Kato, Y. Atsuta, K. Kawa, Y. Kodera, T. Sasazuki, Biological significance of HLA locus matching in unrelated donor bone marrow transplantation, Blood 125 (2015) 1189–1197.
[47] S.J. Lee, J. Klein, M. Haagenson, L.A. Baxter-Lowe, D.L. Confer, M. Eapen, M. Fernandez-Vina, N. Flomenberg, M. Horowitz, C.K. Hurley, H. Noreen, M. Oudshoorn, E. Petersdorf, M. Setterholm, S. Spellman, D. Weisdorf, T.M. Williams, C. Anasetti, High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation, Blood 110 (2007) 4576–4583.
[48] R.C. Williams, G. Opelz, E.J. Weil, C.J. McGarvey, H.A. Chakkera, The risk of transplant failure with HLA mismatch in first adult kidney allografts 2: living donors, summary, Guide Transplant Direct 3 (2017) e152.
[49] R.C. Williams, G. Opelz, C.J. McGarvey, E.J. Weil, H.A. Chakkera, The risk of transplant failure with HLA mismatch in first adult kidney allografts from deceased donors, Transplantation 100 (2016) 1094–1102.
[50] C.K. Hurley, A. Woolfrey, T. Wang, M. Haagenson, J. Umejiego, M. Aljurf, M. Askar, M. Battiwalla, J. Dehn, J. Horan, M. Oudshoorn, J. Pidala, W. Saber, V. Turner, S.J. Lee, S.R. Spellman, The impact of HLA unidirectional mismatches on the outcome of myeloablative hematopoietic stem cell transplantation with unrelated donors, Blood 121 (2013) 4800–4806.
[51] L. Hou, C. Vierra-Green, A. Lazaro, C. Brady, M. Haagenson, S. Spellman, C.K. Hurley, Limited HLA sequence variation outside of antigen recognition domain exons of 360 10 of 10 matched unrelated hematopoietic stem cell transplant donor-recipient pairs, HLA 89 (2017) 39–46.
[52] W. Saber, S. Opie, J.D. Rizzo, M.J. Zhang, M.M. Horowitz, J. Schriber, Outcomes after matched unrelated donor versus identical sibling hematopoietic cell transplantation in adults with acute myelogenous leukemia, Blood 119 (2012) 3908–3916.
[53] C. Bade-Doeding, P. Cano, T. Huyton, S. Badrinath, B. Eiz-Vesper, O. Hiller, R. Blasczyk, Mismatches outside exons 2 and 3 do not alter the peptide motif of the allele group B*44:02P, Hum. Immunol. 72 (2011) 1039–1044.
[54] F. Bettens, U. Schanz, J.M. Tiercy, Lack of recognition of HLA class I mismatches outside alpha1/alpha2 domains by CD8+ alloreactive T lymphocytes: the HLA-B44 paradigm, Tissue Antigens 81 (2013) 414–418.
[55] P. Crivello, N. Lauterbach, L. Zito, F. Sizzano, C. Toffalori, J. Marcon, L. Curci, A. Mulder, L. Wieten, E. Zino, C.E. Voorter, M.G. Tilanus, K. Fleischhauer, Effects of transmembrane region variability on cell surface expression and allorecognition of HLA-DP3, Hum. Immunol. 74 (2013) 970–977.
[56] D. Roelen, V.Y. de, C. Vierra-Green, S. Waldvogel, S. Spellman, F. Claas, M. Oudshoorn, HLA mismatches that are identical for the antigen recognition domain are less immunogenic, Bone Marrow Transplant (2018).
[57] R. Thomas, C.L. Thio, R. Apps, Y. Qi, X. Gao, D. Marti, J.L. Stein, K.A. Soderberg, M.A. Moody, J.J. Goedert, G.D. Kirk, W.K. Hoots, S. Wolinsky, M. Carrington, A novel variant marking HLA-DP expression levels predicts recovery from hepatitis B virus infection, J. Virol. 86 (2012) 6979–6985.
[58] S. Kulkarni, V. Ramsuran, M. Rucevic, S. Singh, A. Lied, V. Kulkarni, C. O'hUigin, G.S. Le, M. Carrington, Posttranscriptional Regulation of HLA-A Protein Expression by Alternative Polyadenylation Signals Involving the RNA-Binding Protein Syncrip, J. Immunol. 199 (2017) 3892–3899.
[59] N. Vince, H. Li, V. Ramsuran, V. Naranbhai, F.M. Duh, B.P. Fairfax, B. Saleh, J.C. Knight, S.K. Anderson, M. Carrington, HLA-C Level Is Regulated by a Polymorphic Oct1 Binding Site in the HLA-C Promoter Region, Am. J. Hum. Genet. 99 (2016) 1353–1358.
[60] E.W. Petersdorf, T.A. Gooley, M. Malkki, A.P. Bacigalupo, A. Cesbron, T.E. Du, G. Ehninger, T. Egeland, G.F. Fischer, T. Gervais, M.D. Haagenson, M.M. Horowitz, K. Hsu, P. Jindra, A. Madrigal, M. Oudshoorn, O. Ringden, M.L. Schroeder, S.R. Spellman, J.M. Tiercy, A. Velardi, C.S. Witt, C. O'hUigin, R. Apps, M. Carrington, HLA-C expression levels define permissible mismatches in hematopoietic cell transplantation, Blood 124 (2014) 3996–4003.
[61] E.W. Petersdorf, M. Malkki, C. O'hUigin, M. Carrington, T. Gooley, M.D. Haagenson, M.M. Horowitz, S.R. Spellman, T. Wang, P. Stevenson, High HLA-DP expression and graft-versus-host disease, N. Engl. J. Med. 373 (2015) 599–609.
[62] M. Israeli, D.L. Roelen, M. Carrington, E.W. Petersdorf, F.H. Claas, G.W. Haasnoot, M. Oudshoorn, Association between CTL precursor frequency to HLA-C mismatches and HLA-C antigen cell surface expression, Front. Immunol. 5 (2014) 547.
[63] G.A. Hoff, J.C. Fischer, K. Hsu, S. Cooley, J.S. Miller, T. Wang, M. Haagenson, S. Spellman, S.J. Lee, M. Uhrberg, J.M. Venstrom, M.R. Verneris, Recipient HLA-C haplotypes and microRNA 148a/b binding sites have no impact on allogeneic hematopoietic cell transplantation outcomes, Biol. Blood Marrow Transplant 23 (2017) 153–160.

[64] G. Kaur, S. Gras, J.I. Mobbs, J.P. Vivian, A. Cortes, T. Barber, S.B. Kuttikkatte, L.T. Jensen, K.E. Attfield, C.A. Dendrou, M. Carrington, G. McVean, A.W. Purcell, J. Rossjohn, L. Fugger, Structural and regulatory diversity shape HLA-C protein expression levels, Nat. Commun. 8 (2017) 15924.
[65] G. Schofl, K. Lang, P. Quenzel, I. Bohme, J. Sauter, J.A. Hofmann, J. Pingel, A.H. Schmidt, V. Lange, 2.7 million samples genotyped for HLA by next generation sequencing: lessons learned, BMC Genomics 18 (2017) 161.
[66] V. Marx, Next-generation sequencing: the genome jigsaw, Nature 501 (2013) 263–268.
[67] C. Kollman, S.R. Spellman, M.J. Zhang, A. Hassebroek, C. Anasetti, J.H. Antin, R.E. Champlin, D.L. Confer, J.F. DiPersio, M. Fernandez-Vina, R.J. Hartzman, M.M. Horowitz, C.K. Hurley, C. Karanes, M. Maiers, C.R. Mueller, M.A. Perales, M. Setterholm, A.E. Woolfrey, N. Yu, M. Eapen, The effect of donor characteristics on survival after unrelated donor transplantation for hematologic malignancy, Blood 127 (2016) 260–267.
[68] D.L. Confer, L.K. Abress, W. Navarro, A. Madrigal, Selection of adult unrelated hematopoietic stem cell donors: beyond HLA, Biol. Blood Marrow Transplant 16 (2010) S8–S11.