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Assessing the Utilization of High-Resolution Two-Field HLA Typing in Solid Organ Transplantation

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Abstract

HLA typing in solid organ transplantation (SOT) is necessary for determining HLA matching status between donor-recipient pairs and assessing patients' anti-HLA antibody profiles. Histocompatibility has traditionally been evaluated based on serologically-defined HLA antigens. The evolution of HLA typing and antibody identification technologies, however, has revealed many limitations with using serological equivalents for assessing compatibility in SOT. The significant improvements to HLA typing introduced by Next Generation Sequencing (NGS) require an assessment of the impact of this technology on SOT. We have assessed the role of high-resolution two-field HLA typing (HR-2F) in SOT by retrospectively evaluating NGS-typed pre- and post- SOT cases. HR-2F typing was highly instructive or necessary in 41% (156/385) of cases. Several pre- and post-transplant scenarios were identified as being better served by HR-2F typing. Five different categories are presented with specific case examples. The experience of another center (Temple University Hospital) is also included, whereby 21% of cases required HR-2F typing by Sanger sequencing, as supported by other legacy methods, to properly address post-transplant anti-HLA antibody issues.

1. Introduction:

In solid organ transplantation (SOT), characterization of Histocompatibility Leukocyte Antigen (HLA) polymorphisms of donors and recipients is essential. HLA matching affects outcomes and long-term graft survival [1, 2]. Given the high level of HLA polymorphism and shortage of available organs, current clinical practice uses immunosuppression to compensate for compromised HLA matches [1], leading to significant loss of grafts (40%) over a 10-year period [2].

Histocompatibility assessment requires knowledge of the recipient's anti-HLA antibody profile and recipient's and donor's HLA determinants to prevent allograft rejection due to memory and/or primary alloimmune response to mismatched donor HLA antigens [3]. Accurate characterization of antibody profiles is most commonly addressed using solid phase antigen immunoassays, despite their technical limitations [4-7]. HLA typing methods have evolved over the last five decades to address the need for accurate and thorough definition of HLA determinants in compatibility assessments. Laboratories commonly perform initial low- to intermediate-resolution typing [Serology, Sequence Specific Primers (SSP), sequence-specific oligonucleotides (SSO)], with subsequent high-resolution (HR) typing (Sanger sequencing, combination of legacy methods) as needed. HR typing is defined in the literature as the determination of a set of alleles encoding the same protein sequence for the region of the HLA molecule called the antigen recognition site and excluding alleles that are not expressed as cell-surface protein [8]. Identification based solely on the antigen recognition site or domain (ARD) allows for differences in other parts of the molecule. Given that the whole molecule is not defined, ambiguities result upon HR typing.

HLA typing for SOT has been primarily focused on characterization of the ARD. Recently, next-generation sequencing (NGS) has revealed the benefits of characterizing additional segments of the HLA molecule (non-ARD). The Children's Hospital of Philadelphia (CHOP) Immunogenetics laboratory has used NGS as the primary molecular method for HLA typing for approximately five years, initially to support the stem cell transplant program, and more recently for SOT programs. Given that NGS can provide HLA typing results up to the fourth-field level, the two–field level can be achieved directly without ambiguities [9]. In this report, we refer to this level of resolution that

describes the totality of the known protein elements of the HLA molecule, as high-resolution at two-field level (HR-2F) (e.g. HLA-B*07:02) [10].

CHOP cases were retrospectively reviewed over a two-year period and categorized by pre- or post-transplant scenarios in which HR-2F typing was relevant and necessary. Examples from each category are presented. Cases from an adult program at Temple University Hospital (TUH) were included. These cases, initially typed by SSO, were further analyzed by Sanger sequencing and other legacy methods to achieve HR-2F for post-transplant donor-specific-antibody (DSA) assessment.

2. Materials and methods

2.1. Study population

This retrospective study includes kidney, heart, lung, liver, and hand transplant cases from 2016 and 2017 at the CHOP Immunogenetics Laboratory, and lung, kidney and kidney/pancreas transplant cases from 2010-2017 at the TUH Histocompatibility laboratory.

2.2. Categorization of cases

At CHOP, both pre- and post-transplant cases were reviewed, grouping the most frequent types of cases into four pre- and one post-transplant scenarios. Given that HR-2F typing at TUH is primarily performed for post-transplant DSA assessment, only post-transplant cases were reviewed.

2.3. HLA typing by NGS, SBT, SSO, and SSP

At CHOP, NGS-based HLA typing was performed as previously described [9] using HoloType HLA™ kits (Omixon, Inc. Budapest, Hungary), and sequenced on an Illumina MiSeq (Illumina, San Diego, CA.). Sequence data were analyzed with Target™ (Omixon) and NGSengine™ (GenDx, Utrecht, the Netherlands), and final genotype was assigned after comparison of outputs from the two programs. Typing of DRB3, 4 and 5 was performed using NGS, as described above and/or LABType® SSO typing kits (One Lambda, Canoga Park, CA). At TUH, initial typing was performed with LABType® SSO (One Lambda). HR-2F typing was performed either by Sanger Sequence-Based Typing

(SBT) using SeCore SBT sequencing kits (One Lambda) or SSP (Micro SSP; One Lambda). All assays were performed according to the manufacturer's recommendations.

HaploStats (http://www.haplostats.org), a web application provided by the National Marrow Donor Program for HLA typing analysis and HLA haplotype frequency was used to predict HR-2F results when unavailable.

2.4. Anti-HLA antibody detection by solid phase immunoassays

At CHOP, Class I and Class II antibody screens were performed with LABScreen® Single Antigen beads (SAB) or LABScreen Single Antigen Supplemental® beads (One Lambda), and run on LABScan 100 flow analyzer (One Lambda). At TUH, antibody characterization was also performed by LABScreen® Single Antigen beads (One Lambda). Phenotype bead and FlowPRA assays (One Lambda) were performed to investigate suspected false positive specificities. Both centers used a positive cut-off of 1,500 mean fluorescence intensity (MFI). Sera were treated with ethylenediaminetetraacetic acid (EDTA) (10 and 5 mM at CHOP and TUH, respectively).

3. Results:

Three hundred eighty-five (139 pre- and 246 post-transplant) and 562 post-transplant cases were processed in the CHOP and TUH laboratories, respectively.

Among the CHOP cases, HLA typing was either instructive or necessary in 54 (14%) pre-transplant and 102 (27%) post-transplant cases. Cases were categorized according to the challenge presented (Table 1). The first four categories pertain to pre-transplant evaluation challenges: 1. Non-self, allele-specific antibodies, 2. Apparent autoreactive anti-HLA antibodies, 3. Crossmatch interpretation, and 4. Prioritization of multiple living kidney donors. The fifth category pertains to post-transplant evaluation of donor-specific antibodies (DSA).

Category 1: Pre-transplant allele-specific antibodies

Of 54 pre-transplant cases requiring HR-2F typing, 12 consisted of patients with serologic or low-resolution typing. Antibody characterization through SAB revealed reactivity against an allele within the same allele group designated by the patient's low-

resolution typing. Such apparent self-reactive antibodies require HR-2F typing of patients to distinguish antibodies targeting self or non-self-determinants.

Case 1.1. *HR-2F typing provides the patient's exact HLA type and characterizes reactive antibodies as allele-specific and non-self.* A 3-year-old male heart transplant candidate had a class I and II Calculated Panel Reactive Antibodies (cPRA) of 74% and 68%, respectively. Class II specificities included DRB1*13:03 (MFI=2,561), DRB1*13:01 (MFI=1,632), which were unexpected since the patient possessed DR13. NGS-based typing identified the patient as DRB1*13:02, and a supplemental SAB with DRB1*13:02 was negative (MFI=657). Reactivity to DR13 was therefore considered allele-specific (DRB1*13:01 and DRB1*13:03) and not against the self-antigen DRB1*13:02.

Case 1.2. *Addressing the complexities of high PRAs and proper reporting of unacceptable antigens (UA) to United Network for Organ Sharing (UNOS).* Sera from a highly sensitized 16-year-old male (100% cPRA, both class I and class II) kidney transplant candidate showed strong reactivity (MFI=15,121) against only DRB1*04:02 (not against other DR4 allele-specific beads), while the patient was DRB1*04:08 positive. At the time, we were unable to list DRB1*04:02 as an UA in U-Net. It would have either been reported as DR4 or not reported at all, risking a positive crossmatch, should an offer be accepted. Given the complexities of our multiethnic population in the United States, using DRB1*04 allele frequencies derived from individual populations to determine which allele to report to UNOS may be misleading. This case reveals the limitations of our reporting systems, which have been outpaced by HLA typing technologies. The ability to report UAs at the HR-2F level would have prevented the described scenario. As of this writing, DR4 alleles may be listed as UAs, and therefore HR-2F typing would permit assignment of DRB1*04:02. However, this ability remains absent for several other HLA antigens.

Category 2: Identification of possible anti-self HLA antibodies

Antibodies may represent self-reactivities as defined by HR typing of the patient. Among 54 pre-transplant cases, 17 were placed in category 2 (Table 1). Again, the question becomes whether these antibodies are truly directed against self or artifacts of SAB causing false positive reactivities [11]. Characterization of antibody specificities facilitates decision-making to guide patient management.

Case 2.1. *The perceived autoantibody is due to falsely reactive beads.* A 6-year-old male kidney transplant candidate had reactivity to DRB3*02:02 (MFI=12,476) on SAB. NGS typing revealed that he was also DRB3*02:02. Given the strong reactivity, a crossmatch was performed using surrogate donor cells homozygous for DRB3*02:02. The result was negative, suggesting the DRB3*02:02 SAB reactivity was likely a false-positive, consistent with previous reports [11]. Recognizing technical limitations of these assays is critical for proper assessment of anti-HLA antibodies. HR-2F typing of the patient (and surrogate donor for the mock crossmatch) enabled better characterization of reactivity for SAB.

Case 2.2. *Identification of a self-reactive antibody.* A 20-month-old female lung transplant candidate had multiple class II antibodies, including DRB1*07:01 (MFI=8,005). HR-2F typing revealed she was DRB1*07:01 and DRB1*13:02, indicating the antibodies in question were against self-antigen. Although an auto-flow crossmatch was negative, a B-cell flow crossmatch using DRB1*07:01, DRB1*10:01 positive surrogate donor cells was weakly positive. No other donor specificities were identified. Therefore, the specificity detected by SAB was considered significant and posed a potential risk with transplanting a DRB1*07:01 positive graft. The reason(s) these antibodies developed and their biologic role remain unclear.

Category 3: Facilitate the interpretation of crossmatch results.

HR-2F typing aided interpretation of crossmatch results in 15 cases (Table 1).

Case 3.1. *Based on HR-2F typing, a positive crossmatch may be attributed to non-HLA antibodies.* An 18-year-old female received a living-related donor kidney transplant from her father in 1999. No DSA was detected post-transplant, but she developed chronic allograft nephropathy from an unknown etiology and was relisted for a second kidney transplant. Crossmatch testing evaluating her sibling as a potential donor was significant for a weak positive B-cell flow crossmatch and CDC crossmatch, the latter of which was reduced by dithiothreitol (DTT) treatment. A T-cell crossmatch was negative. The B-cell results were unexpected given the absence of any detected anti-HLA antibodies in the patient's sera. The CDC crossmatch results also suggested the presence of IgM antibodies because reduced reactivity was observed with DTT treatment.

The patient's father's HLA typing, previously determined by low-resolution methods at another institution, was reported as A2, A31, B35, Cw4, DR4, DR14, DR52, DR53, DQ3, and DQ7. NGS-based typing determined the patient's sibling to be A*26:01, A*31:15, B*38:01, B*35:05, C*13:03, C*04:01, DRB1*04:02, DRB1*04:11, DR53 and DQA1*03:01, DQB1*03:02. In the context of the family's haplotypes, the typing results suggested that the father was A*31:15 and DRB1*04:11 or DRB1*04:02. Given the absence of A*31:15 and DRB1*04:11 in the standard SAB panel, antibodies against these antigens could have been missed. Both the flow and CDC T-cell crossmatches between the patient and the sibling were negative, suggesting the patient did not have anti-HLA antibodies to A*31:15. No reactivity to DRB1*04:11 (MFI=0) was observed on a supplemental SAB panel. This data suggests the weak crossmatch reactivity between the patient and her sibling was likely due to non-HLA antibodies. Without HR-2F typing and further testing with supplemental SABs, the interpretation of the B-cell crossmatch would have remained unclear. The patient was transplanted with a deceased donor and has been reported to be clinically well at one-year post-transplant.

Case 3.2. *Optimization of virtual crossmatch using HaploStats and retrospective HR-2F typing of the donor assesses actual risk due to the presence of anti-HLA antibodies**:*** A 13-year-old female lung transplant candidate had antibodies solely against the DQ6 dimer DQA1*01:03/DQB1*06:01 (MFI=2,082). An offer was made for a DQ6-positive organ, for which no HR-2F typing was available. According to HaploStats, the most likely haplotype with DQ6 based on the donor's typing was A3-B7-C7-DR15-DQ6, ranked second in the Caucasian population. The DQ6 for this particular haplotype was DQB1*06:02. A virtual crossmatch with the potential donor was negative and the organ was accepted. Retrospective NGS-based typing confirmed the donor to be DQA1*01:02/DQB1*06:02, which was not the dimer to which the patient's sera demonstrated reactivity. Post-transplantation, reactivity to DQA1*01:03/DQB1*06:01 persisted with MFIs ranging from 2,000 to 4,200. Based on the aforementioned testing, however, she has been reported as DSA-negative and has been clinically well at two years post-transplant. This case illustrates the advantages of using a combination of resources, including HaploStats and HR-2F typing, to optimize donor selection and patient management. Concordance data between HaploStats and actual NGS

genotyping of donors are shown in Table 2. It should be noted that HaploStats does not include HLA typing information for DQA1, DPA1, and DPB1 loci.

Category 4: Identify the best match in living donation

Networks of living donors, developed recently to facilitate kidney donor selection, would be better served by HR-2F typing of donor/recipient pairs. Such information permits matching at the epitope level and risk stratification of recipient-donor pairs. Furthermore, for sensitized patients, HR-2F typing of alternative living donors would enable a comparative and proper risk assessment based on HLA allelic profiles. NGS played a critical role in assessing alternative donors for ten living donation cases (Table 1).

Case 4.1. *HR-2F typing allows for optimal selection of a living donor.* An 11-year-old male kidney transplant candidate had five potential living-unrelated donors. The patient's anti-HLA antibody screen was negative with concordant negative donor crossmatches. In this setting, which donor should be selected? Table 3 shows the HR-2F typing of the patient and potential donors. Matched alleles are indicated in bold. The compatibility of each patient-donor pair was evaluated based on the mismatches at the antigen, HR-2F, and epitope level (Table 3). The antigen and HR-2F level mismatches indicated donor 3 to be the best choice. At the epitope level, donor 1 is the best choice with the least number of mismatched epitopes. Of note, 22% of the mismatched epitopes were from the non-ARD segments of the HLA molecule (Table 3).

Case 4.2. *Based on the patient's anti-HLA antibodies profile, HR-2F typing of alternative living donors enables proper donor selection.* A 17-year-old female kidney transplant candidate had anti-HLA antibodies against DQ2 (DQA1*05:01/DQB1*02:01; MFI=5,653). Her parents were evaluated as possible donors, both of whom possessed DQ2. NGS-based typing identified her father as DQA1*05:01/DQB1*02:01, the dimer to which the patient's serum demonstrated reactivity. However, her mother possessed the DQA1*02:01/DQB1*02:02 dimer, for which all of the patient's historic sera had negative reactivity. The patient's mother was chosen as the preferred donor. Post-transplantation, the patient's sera continued to show fluctuating reactivity against DQA1*05:01/DQB1*02:01 (MFIs 2,800-7,800) but no reactivity against the DQA1*02:01/DQB1*02:02 dimer. She was reported as DSA-negative, with reportedly stable kidney function at three years post-transplant.

Category 5: Facilitate bead selection to monitor DSAs.

Post-transplant, it is necessary to detect antibodies developed against mismatched HLA alleles on the graft. DSA can be characterized appropriately if the incompatible HLA alleles of the graft are known and represented by the SAB panel used for detection. Among post-transplant cases, 102 of 385 CHOP cases and 118 of 562 TUH cases required HR-2F typing of donors to assess apparent DSA (Table 1).

Case 5.1. *HR-2F typing enables selection of proper SABs for monitoring DSA.* A 9-year-old female received a heart transplant in 2008. The low-resolution HLA typing of the donor (Table 4) indicated mismatches in the host vs. graft direction for A1, A2, B7, B52, DR15, and DR51. No assignment was made at DQ because the patient typed as DQ6 and the donor typed as the broad antigen DQ1 or at DP where no donor typing was available. Thus, a total of 6 mismatches were identified. To better characterize the donor, NGS-based typing was performed. The donor's HLA-C locus included an additional allele, C*12:02, not detected serologically. In addition, the donor was found to be heterozygous for DR15 and DQ1 (DRB1*15:01, DRB1*15:02, DQB1*06:01 and DQB1*06:02); the patient was DQB1*06:03. An additional mismatch at DP was also discovered, for a total of 11 mismatch assignments for post-transplant DSA monitoring (Table 4). This particular case demonstrates improved DSA assessment, which is critical for understanding downstream complications and proper intervention strategies.

Case 5.2. *HR-2F typing of the donor allows for proper monitoring of patient's anti-DQ antibodies when multiple DQ dimer beads are positive.* An 18-year-old female received a kidney transplant in 2008. The donor was DQ9 positive and the patient developed antibodies against DQ9 in 2013. Initially, only the low-resolution HLA type was available for both patient and donor. The DQ9 dimer DQA1*03:02/DQB1*03:03, was assigned for DSA monitoring given that it had the highest MFI (12,417) among the different DQ beads. In the absence of HR-2F typing of the donor, we did not want to underestimate the risk of graft rejection by reporting lower MFIs. The patient was asymptomatic, but given the high MFI value, the clinical team considered modification of immunosuppression. Prior to any changes in patient management, HLA typing by NGS was performed and identified the donor as DQA1*02:01/DQB1*03:03. The MFI value of the corresponding beads was 2,126, significantly lower than the initially reported bead (DQA1*03:02/DQB1*03:03). The updated DSA correlated better with the patient's clinical status and her immunosuppression was not adjusted.

Case 5.3. *HR-2F typing facilitates bead selection when donor's alleles are not represented by available SAB panels.* A 4-year-old male underwent a living related donor kidney transplant in 2017. Mismatched antigens (shaded) were identified (Table 5). The SAB panel does not have beads to represent B*35:08 or DRB1*13:21. Epitope analysis identified shared mismatched eplets between B*35:01 and B*35:08, and between DRB1*13:03 and DRB1*13:21. Therefore, beads for B*35:01 and DRB1*13:03 were used to monitor B*35:08 and DRB1*13:21 DSAs. HR-2F typing enabled epitope analysis to provide a better assessment of the antibody profile for post-transplant DSA assessment.

4. Discussion

This report is a partial description of the benefits derived from characterization of HLAs at the HR-2F level. HR-2F typing is instructive, addresses HLA testing limitations, and is important in both pre- and post-transplant settings.

The five categories described illustrate the utility of HR-2F. In the pre-transplant setting, HR-2F typing provided relevant information for optimizing donor selection. The first and second categories pertain to discernment of allele-specific, possible anti-self antibodies, or artifacts in the setting of bead reactivity through accurate characterization of anti-HLA antibodies. Assessment of these alternatives requires HR-2F typing of patients. Mock crossmatches for further confirmation of suspected anti-self HLA antibodies also require HR-2F typing of surrogate donors. The dependency of laboratories on bead assays necessitates an understanding of their technical limitations, particularly false positives due to denatured HLA antigens [6, 7]. While these assays (Phenotype and FlowPRA beads) are helpful for confirming or ruling-out SAB false positives, mock crossmatches, if possible, allow for a more thorough and credible evaluation of bead reactivity [5, 12, 13].

The third category relates to crossmatch interpretation. HR-2F typing enables the attribution of positive results to HLA or non-HLA antibodies. Additionally, in the absence of timely HR-2F typing for deceased donors, combined use of SAB, HaploStats, and HR-2F typing of patients optimizes the virtual crossmatch (Case 3.2). Our retrospective

NGS typing of deceased donors reveals a high concordance (92.5%) between NGS typings and HaploStats prediction based on available intermediate-level typings (Table 2), providing some degree of assurance that this approach is safe. While concordance for HLA-A, B, C, and DQB1 is between 93% to 96%, the concordance for DRB1 is lower (84%). Furthermore, our reported concordances for different ethnic groups may be biased due to our small sample sizes. As such, until HR-2F typing of deceased donors can be performed with shorter turnaround time, the described approach is viable for predicting crossmatch outcomes, with relative confidence but also caution.

HR-2F typing is informative for living donation evaluations. For non-sensitized patients with multiple potential donors, the rank order of preferred donors changes based on HR-2F typing and criteria for matching (Case 4.1). Epitope matching has been reported to be a better criterion of compatibility compared to others [14-16]. HR-2F typing is necessary for epitope analysis, which is based on complete and unambiguous protein sequences of specific alleles [17, 18]. Of all mismatched epitopes identified in Case 4.1, 22% were from non-ARD portions of the HLA molecule. This finding highlights the importance of reporting all protein segments in international ImMunoGeneTics (IMGT) information system so that appropriate comparisons can be made between HLA alleles for epitope analysis. HR-2F typing also optimizes donor selection in paired-exchange networks. HR-2F typing of prospective donors enables better assessment of antibodies that may be directed against donor antigens to facilitate donor selection (Case 4.2). The same approach may be applied to highly sensitized patients with difficulty receiving transplant offers. Recent progress suggests that these patients may have growing opportunities for transplant with epitope analysis, through better characterization of recipient antibody profiles and identification of potentially permissive mismatched donors [19].

Although immunosuppression enables the prevention and management of T-cell-mediated rejection, the humoral alloresponse remains a major source of late graft loss [20]. While prospective HR-2F typing of a deceased donor is presently not feasible, retrospective post-transplant HR-2F typing can be useful for identifying the appropriate SAB bead for detecting and monitoring of DSA (Case 5.1 and 5.2). Anti-DQ antibodies, predominantly in the post-transplant setting and implicated in antibody-mediated

rejection and allograft loss, are particularly challenging to properly characterize [21-26]. In such cases (Case 5.2), HR-2F typing enables proper bead selection for DSA monitoring, which can influence patient management. Furthermore, when particular antibody specificities are excluded from the SAB panel (case 5.3), epitope analysis can provide alternative “surrogate” specificities for monitoring. Prerequisite to this approach is HR-2F typing of the donor.

Our study suggests that the most frequent utilization of HR-2F typing in SOT is for post-transplant monitoring of DSA (26.5% at CHOP and 20.9% at TUH). The combined experience of both programs suggests that in over 20% of cases, HR-2F typing would be necessary for proper monitoring of DSA.

Additional questions associated with proper assessment of DSA are of a quantitative nature. A common epitope recognized by a single antibody can be present on multiple beads. This unavoidable reactivity pattern must be recognized and accounted for, given that the MFI values corresponding to a single bead may not accurately represent the amount (titer) or affinity of an antibody. HR-2F typing and epitope analysis can help with the data interpretation by identifying common epitopes on different beads responsible for the reactivity patterns. This rather artificial reduction of MFIs must be accounted for in the virtual crossmatch, as cell surfaces in a crossmatch have only one or two alleles that will react with a particular anti-HLA antibody, resulting in a positive reaction. A recent report by the Sensitization in Transplantation: Assessment of Risk (STAR) working group [3] brings this phenomenon of “epitope sharing” to our attention and recommends having a mechanism in place for its detection.

Our study shows that HR-2F typing permits better donor-recipient compatibility assessment and post-transplant DSA monitoring in 41% of cases. Each lab had different conceptual approaches for typing. CHOP routinely performs NGS for both the pre- and post-transplant cases, irrespective of whether there is a perceived need for HR-2F, a decision based primarily on the superiority of typing data obtained by NGS, and secondarily on operational and financial considerations. TUH lab pursues HR-2F retrospectively and specifically for post-transplant DSA assessment. These different approaches may explain the different percentages reported by CHOP and TUH for all

cases (41% and 20.9%, respectively) but very similar percentages for the post-transplant scenarios (26.5% and 20.9%, respectively), where HR-2F is useful for DSA assessment. It, therefore, appears that the practice of routinely performing NGS, which regularly generates HR-2F, facilitated the resolution of a significantly larger number of cases as compared to practices in which HR-2F typing is not routinely obtained.

A recent review by Hurley and Ng on reassessment of the resolution required for clinical testing asserts that clinical decision-making should be focused on the ARD and not the rest of the molecule [27]. That commentary stands in contrast to our assertion that HR-2F is meaningful for clinical practice, as HR-2F invokes concepts of the whole HLA molecule. While the authors acknowledge that variation outside of the ARD can impact different aspects of HLA and affect immune responses, they conclude that “at present, there is insufficient data to support extending typing to include regions outside of the antigen recognition domain in clinical decision making.” Our current work suggests otherwise. A good proportion of cases in this report (around 40%) required HR-2F. For many alleles that are characterized only for the ARD in IMGT, the definition of HR and HR-2F will indeed coincide. In genotype combinations involving alleles characterized only for the ARD, HR-2F typing would not offer significant advantage. However, for many other alleles with unique sequences that go beyond the ARD, the HR and HR-2F are distinct typings. Forty-nine percent (5,457/11,181) of unique class I proteins have characterized sequences that extend beyond exon 2 and 3, and 31% (1,211/3,914) of unique class II proteins have sequences that extend beyond exon 2 (IMGT/HLA database version 3.34). Of note, as more partially sequenced HLA alleles are completed over time, and as more new alleles are reported in IMGT, the likelihood for generating ambiguities will increase. The major advantage of HR-2F, derived through NGS, is the elimination of almost all ambiguities (unambiguous typing for class I at 99.9% and class II at 94%; internal data, details not shown). Eliminating ambiguities through HR-2F benefits our practice by facilitating analysis, adding confidence to HLA callings, providing clarity, influencing reporting, and improving communications. Above all, it enables epitope mapping, a direction our field moves towards. Such benefits are irrespective and independent of any clinical relevance these non-ARD components may have.

Nevertheless, it may be argued that for all our presented cases, HR, and not necessarily HR-2F, would suffice, given an effective way to manage ambiguities in our interpretive schemes. The question remains, however, as to why our laboratories must be subjected to data complexity and reporting uncertainties when there are methods to simplify and add certainty to HLA type reporting. Continued practice of HR-2F typing will inevitably reveal additional benefits, as recently reported in a preliminary study on DSA against the α2 domain of DQα chain, a non-ARD region [28]. Such reports pose the dangers of using P group designations (i.e. focusing on the ARD only) when performing virtual crossmatches or stem cell donor selection for patients with complex sensitizations. One may argue that such events are rare, but it is only after we reliably assess these phenomena with available technologies that the frequency of these events can be determined.

A distinction should be made between the ability to effectively characterize the entire sequence of HLA genes by NGS and the clinical utility of this information. Our position is that clinical practice is better served when HLA typing is performed at the HR-2F level. We further propose that NGS is the most efficient methodology to obtain HR-2F, while acknowledging that presently not all of the information obtained by sequencing entire HLA genes can be utilized. As long as this approach is cost-effective and operationally acceptable, the relevant HR-2F data reflecting HR-2F can be used presently, with any additional information readily available for retrospective applications. A recent issue of Human Immunology dedicated to the significance of non-ARD of HLAs may be a prelude to the future clinical relevance of NGS (https://www.sciencedirect.com/journal/human-immunology/vol/80/issue/1).

Realizing the importance of HR-2F level typing for SOT, will most likely promote the use of NGS. As NGS is implemented for routine clinical work, it will facilitate a better understanding of alloresponses to HLA mismatches, with eventual application to pre- and post-transplant compatibility issues and patient management. The confluence of technical developments and improvements materialized the last several years will most likely translate to better outcomes for our patients in the near future.

Disclosures

D. Monos is a consultant to, and owns options in Omixon. D. Monos, D.Ferriola and J.L.Duke receive royalties from Omixon. The other authors of this manuscript have no conflicts of interest to disclose.

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