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Letters to the Editor

# Regarding "Recipients Receiving Better HLA-Matched Hematopoietic Cell Transplantation Grafts, Uncovered by a Novel HLA Typing Method, Have Superior Survival: A Retrospective Study"

Carolyn K. Hurley[1], Stephen Spellman[2,*], Jason Dehn[3], Juliet N. Barker[4,5], Steven Devine[2,3], Marcelo Fernandez-Vina[6], Michael Gautreaux[7], Brent Logan[8], Martin Maiers[2], Carlheinz Mueller[9], Miguel-Angel Perales[4,5], Neng Yu[3], Joseph Pidala[10]

[1] *Department of Oncology, Georgetown University, Washington, DC*
[2] *Center for International Blood and Marrow Transplant Research, Minneapolis, Minnesota*
[3] *National Marrow Donor Program, Minneapolis, Minnesota*
[4] *Adult Bone Marrow Transplantation Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, New York*
[5] *Department of Medicine, Weill Cornell Medical College, New York, New York*
[6] *Histocompatibility, Immunogenetics & Disease Profiling Laboratory, Department of Pathology/Blood Center, Stanford University School of Medicine, Palo Alto, California*
[7] *HLA/Immunogenetics Laboratory, Wake Forest School of Medicine, Winston-Salem, North Carolina*
[8] *Center for International Blood and Marrow Transplant Research and Institute for Health and Equity, Division of Biostatistics, Medical College of Wisconsin, Milwaukee, Wisconsin*
[9] *ZKRD (German National Bone Marrow Registry), Ulm, Germany*
[10] *Blood and Marrow Transplantation and Cellular Immunotherapy, H. Lee Moffitt Cancer Center, Tampa, Florida*

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*To the Editor:*

The retrospective study by Mayor et al. [1] drew the attention of the National Marrow Donor Program (NMDP) Histocompatibility Advisory Group to the impact of third-generation DNA sequencing to evaluate HLA matching of unrelated donors and their recipients. The technology, sequencing of long, individual DNA fragments using a Pacific Biosystems platform, allows long-range phasing to define allelic sequences for all or most of each HLA gene. The term "ultra-high resolution" was used by the authors to describe the ability of the technology to provide the nucleotide sequence of all exons that encode the expressed extracellular domains of the mature protein and, in the case of HLA class I genes, the noncoding regions. The aim of the study, as stated in the abstract, was to "explore the extent of diversity outside of the ARD [antigen recognition domain] and to determine the impact of this diversity on transplant outcome." The conclusion of this study stated in the discussion is that "polymorphism in previously uncharacterized regions of the classic HLA genes affects VUD-HCT [volunteer unrelated donor hematopoietic cell transplantation] outcomes." Unfortunately, the arguments for this conclusion over-reach the actual results. Because over 98% of the 810 discrepant pairs already differ in the ARD of 1 or more loci, it is unlikely that the relevance of non-ARD mismatches could be derived from this dataset.

The study evaluates HLA matching in 891 transplantations occurring between 1996 and 2011. During this time the methods and reagents used for HLA matching evaluation [2] evolved from interrogating short segments of 1 (class II) or 2 (class I) exons with sequence-specific oligonucleotide probes to applying a more comprehensive but potentially unphased evaluation of the nucleotide sequences using the Sanger method, all in the face of an ever-increasing number of HLA alleles. The exons evaluated for matching were those encoding the most highly polymorphic region of the HLA protein that interacts with bound peptide and T cell and natural killer cell receptors, the ARD, a common focus for HLA typing in most outcome studies. When the 891 pairs were re-evaluated for HLA matching based on the phased sequences of their ARD in the Mayor et al. retrospective study, many pairs were discovered to be more mismatched than originally anticipated. For example, in their Supplementary Table S1, of the 99 pairs originally believed to be 12/12 matched based on matching of the ARDs, 23 were discovered to be mismatched after the third-generation sequencing. This observation is not new; discrepancies observed in the level of matching were noted when donor–recipient pairs transplanted through the NMDP were retrospectively typed using methods that are not "ultra-high resolution" [3]. Although DNA sequencing is now the gold standard for matching

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* Correspondence and reprint requests: Stephen R. Spellman, MBS, Center for International Blood and Marrow Transplant Research, 500 5th St N, Minneapolis, MN, 55401.
*E-mail address:* sspellma@nmdp.org (S. Spellman).
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unrelated donor and recipient pairs, the Mayor et al. study does not assess whether the third-generation sequencing platform they used is more accurate than other sequencing strategies in current use. This would have required parallel testing, not a retrospective analysis.

The major aim of the study, to explore HLA diversity outside the ARD and its impact on outcome, is limited by the small number of pairs that carry only non-ARD exon mismatches (n = 3) or only intron mismatches (n = 13). These are the only relevant mismatches revealed by the ultra-high resolution testing method. The fact that only a few pairs carried variation outside the ARD confirms previous reports evaluating unrelated donor pairs transplanted through the NMDP network [4]. The data on the impact on survival of the 13 intron mismatches shown in their Figure 1C is the basis for the statement in the figure legend: "The 12/12 UHR [ultra-high resolution] HLA-matched pairs have significantly higher survival probabilities than patients who received either a 12/12 coding DNA sequence (CDS) match (ie, where there is the presence of only intronic or untranslated region mismatches) or any other HLA mismatch." The text accompanying this figure provides a *P* value (.02) for the comparison between the 12/12 ultra-high resolution (A) and all other mismatch group (C). The *P* value for 12/12 ultra-high resolution versus 12/12 CDS (B) (intronic differences only, n = 13) is not shown in the figure or provided in the text and so provides no support for any claim (as made in the title and main text) of a survival benefit between A versus B or B versus C. The significant observation is apparently the high error rate of the original typing, with 29.1% of the pairs changing matching status. Thus, the question of whether non-ARD variation impacts outcome must wait until a larger cohort of these mismatches (with the ARD matched) are identified before their impact on outcome can be assessed separately from ARD mismatches.

Previous studies of the role of HLA matching at the level of the ARD in large unrelated donor–recipient cohorts have demonstrated that mismatches in the ARD of HLA-A, -B, -C, and -DRB1 lead to a significant decrease in survival with a drop of 10% for each mismatched locus [5–8]. ARD mismatches at the lower expression HLA loci (HLA-DQB1, -DPB1, -DRB3/4/5) have an impact on survival in pairs already mismatched at the 8/8 level [9] and/or have an impact based on the presence of non-permissive mismatches [6,10]. Mismatches at the ARD form a predominant component of the Mayor et al. study where they are merged into a single category for the analysis. Of the 810 mismatched pairs noted in their Supplementary Table S2, 796 (98%) carry ARD mismatches. Thus, it is likely that the decreased survival noted is largely because of the impact of ARD mismatches, an observation that has been previously reported.

In summary, the conclusion reached by the Mayor et al. study regarding the relevance of non-ARD mismatches on outcome is not warranted based on the data provided. This is concerning because this publication encourages requirements for additional matching of regions not yet shown to be relevant and, for many transplant programs, ultra-high resolution testing that is not yet readily available in their laboratories. This may create confusion and, if not inhibit transplants that occur today based on recommended HLA matching, could delay the identification of donors and delay transplantations where time is of the essence. Additional studies on the role of non-ARD mismatches in contemporary transplant populations are necessary. The Center for International Blood and Marrow Transplant Research has initiated a study evaluating ultra-high resolution matching in a cohort of nearly 6000 recent (2000 to 2017) transplants. The results of this study and others will provide more clarity and guidance on the relevance of ultra-high resolution matching in VUD-HCT. The recommendations on unrelated donor selection from the NMDP Histocompatibility Advisory Group are described in *Blood* [11,12]

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