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## Quarterly



Second Quarter 2016

Volume 40, Number 2

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# Table of Contents

## American Society for Histocompatibility

## Features

### SCIENTIFIC COMMUNICATIONS

- The Illusion of Quality: Taking Good Quality Processes and Making Them Great . . . 21  
Frederick G. Strathmann, PhD, DABCC (CC, TC)

- Genome-Wide Genetic Variation and Hematopoietic Cell Transplant Outcome . . . . 26  
John A. Hansen, MD

- C1q Binding Donor-Specific Anti-HLA Antibodies  
Occurring Late After Kidney Transplantation. . . . . 30  
Christina Savchik  
Rosanne Scandaliato  
Peter Masiakos  
Adriana Colovai, PhD

## Departments

### EDITORIAL

- From the Editor-in-Chief . . . . . 2  
President's Column . . . . . 4  
My Life in HLA . . . . . 5  
My Way to HLA. . . . . 9  
Current Literature . . . . . 11  
Technology Reports . . . . . 13  
Regional Workshop Recap. . . . . 17  
Key Stats About the 2016 Annual Meeting . . . . . 18  
ASHI Staff Chart. . . . . 40

### COMMITTEE ACTIVITIES

- ARB Update . . . . . 42  
ABHI Update. . . . . 43

### ASHI QUARTERLY CONTINUING EDUCATION QUIZ . . . . . 36

## Editor's Note

Individuals interested in submitting articles for the ASHI Quarterly should observe the following requirements:

- All articles must be submitted via e-mail in Microsoft Word format
- All articles must be double-spaced

Article submissions should be forwarded to:

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# ASHI Quarterly

## American Society for Histocompatibility and Immunogenetics

## Volume 40, Number 2

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# From the Editor-in-Chief

James M. Mathew, PhD



In this issue of the ASHI *Quarterly* we have the usual complement of scientific articles, current literature review, "My Life in HLA" series recollection, Technical report series article and committee reports. Unlike in the last two issues where we had a common theme, the subject matter in the scientific articles of this issue is varied.

In the first scientific article entitled "The Illusion of Quality: Taking Good Quality Processes and Making Them Great," Dr. Strathmann of University of Utah makes a theoretical analysis of the practical problem of Quality Control (QC) in the clinical laboratory. The rapid advancement of laboratory medicine has made it essential that we take a new look at QC, as laboratories may be operating "under an 'illusion of quality' rather than on a 'foundation of quality' supported by data and metrics." He suggests that the "2 Standard Deviation Trigger Rule" be replaced with a scheme wherein "Standardized Troubleshooting Guides" are utilized for QC reevaluations. He goes on to recommend that the data on ranges for assay values be continuously updated to improve quality performance.

The second article by Dr. John Hansen of the University of Washington, Seattle, is an in-depth review of the genome-wide variation and its effect on hematopoietic cell transplant (HCT) outcome. He defines genetic polymorphism as the cumulative variations that occur in single nucleotide polymorphisms (SNPs) plus structural insertions and deletions (indels) in the population. As a result of this polymorphism, "everyone with the exception of genotypically identical twins has a unique genetic constitution." Then, he demonstrates the power of genomics in characterizing genetic diversity and relationships among populations giving specific examples. He also reviews how genome-wide association studies (GWAS) have demonstrated that SNPs contribute to both health and disease. Coming to the central theme of the article, he describes how the degree of graft versus host disease (GVHD) correlates with the measure of disparity measured in terms of mismatches in MHC, minor histocompatibility antigens, and indels. He concludes by stating that there is a need for larger adequately powered GWAS to measure HCT outcomes.

The third scientific article is by Dr. Adriana Colovai and colleagues from Montefiore Medical Center from Bronx, New

York, studying the occurrence of C1q binding DSA late after kidney transplantation in a large group of patients. All patients had a negative CDC crossmatch at the time of transplantation. But 19% developed post-transplant DSA with 8% being C1q binding. Further analysis demonstrated C1q+ DSA is associated with younger patient age, deceased donor transplantation and higher cPRA values, resulting in higher incidence of chronic AMR/transplant glomerulopathy. C1q+ DSA were predominantly directed to the donor mismatched HLA-DQ antigens. They concluded that long-term monitoring of DSA after kidney transplantation is warranted to identify patients at risk for antibody mediated allograft injury.

The articles that come under the editorial section include the President's Message from Dr. Anat Tambur, recollections of an HLA pioneer Dr. Roy First in the My Life in HLA series, a How I found my Way to HLA article by Christopher Do of UCSF, and the review of Current Literature by Dr. Siva Kanangat of Rush University Medical Center on the significance of MHC-gamma region in bone marrow/hematopoietic stem cell transplantation.

The new Technical Reports series that we have initiated in the last issue of the *Quarterly* is successfully continued. Drs. Rene Duquesnoy and Marilyn Marrari of University of Pittsburgh have given us the first of a Technical Trilogy on "Application of HLA Epitope-Based Matching in the Clinical Transplant Setting." Please read it, enjoy it and then wait for the next installment.

I continue to encourage the members of our Society, especially technologist members, to write on such topics as case studies that led to changes in procedures; modifications in assays that increase reliability, sensitivity, specificity, etc.; how you achieve efficiency in what you do; a review article of a topic that interests you, such as how dialysis centers organize sample collection; how coordinators manage/organize multiple cases of deceased donors; tips for surviving being a coordinator, and so on. Please send your submissions to Walter Herczyk at WHerczyk@giftolifemichigan.org or to me at james-mathew@northwestern.edu.

Also, I invite the members of ASHI to contribute scientific articles derived from your expertise, review articles or commentaries on significant topics, technical reports based on your experiences, synopsis of meetings and conferences you attend, or to share your reminiscences for the My Life in HLA series.

*Please send your submissions to Walter Herczyk at  
[WHerczyk@giftolifemichigan.org](mailto:WHerczyk@giftolifemichigan.org) or to me at  
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# President's Message

Anat Tambur, DMD, PhD



My dear fellow ASHI members,

We are entering the sunny part of the year and I hope everyone has great plans for the summer.

We had a busy few months and I am happy to provide you with some details. Dr. Amy Hahn had worked wonders in her role as interim editor of *Human Immunology*. In only a few short months she was able to handle the huge backlog that crippled the journal (thank you to all reviewers who helped in this process). Amy had done that in her gentle, polite, yet fierce manner and we are all indebted to her for helping rescue our society's journal. THANK YOU AMY!

In April I had the pleasure of visiting with our European colleagues during the EFI meeting at the beautiful island of KOS. The scientific program covered a wide range of topics relation to histocompatibility and immunogenetics and the social program, in the Greek spirit, was phenomenal. I greatly enjoyed the friendly hospitality and had a chance to spend a few hours with Prof. Elissaveta Naumova, current president of EFI. Unfortunately, Rhonda Holdsworth, president of APHIA, was not able to join us, and Elissaveta and myself discussed in length plans to bring the societies in closer collaborative spirit, working on joint projects including the summer school, PT, QAS, as well as additional potential projects that we are in the process of developing.

A significant part of our time was dedicated to discussions regarding the upcoming international histocompatibility and immunogenetics workshop, headed by Marcelo Fernandez-vina and Dolly Tyan. One of the harder-working bees for this endeavor is Tamara Vayntrub, and the photo at right shows Elissaveta, Tamara, and me hard at work making plans for this exciting workshop.

Another exciting meeting in the formulation stages is geared toward our members who are considering the implementation of next generation sequencing (NGS) technology in their laboratory. Please be tuned to near future announcements. The plan is to allow our members to compare the different platforms side by side, hear from the experience of laboratories that had gone through the motions of testing different platforms and making different decisions based on their laboratory size (from large to small) and relevant cost effectiveness consideration. We believe this will be a great service for laboratories that are currently in the early steps of choosing the best workflow for their own program as well as those that consider introducing NGS into routine testing within the next year.

I also attended the American Transplant Congress (ATC) that took place this year in Boston, Massachusetts. I am happy to report that the educational day symposium proposed by ASHI was very well received. We had over 250 people in attendance—mostly clinicians. The program was divided into two sessions. The first was geared more toward the basic science of the HLA system, including presentations from Annette Jackson, Medhat Askar, Elaine Reed, and Howie Gebel. The second session was about going from the bench to the bedside, with presentations from pathologists and nephrologists emphasizing the role of HLA antibodies in assigning the right diagnosis and determining appropriate treatment. Parmjeet Randhawa, Rene Rodriguez, Michael Mengel, and John Friedewald all presented. We have already received very positive feedback and are looking to continue our collaboration with the AST in future years.

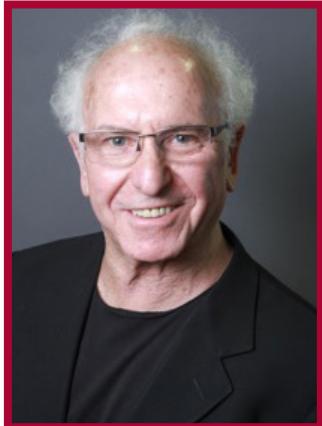
In September we will have our annual ASHI meeting. Please explore the program carefully. This year we will have an exciting addition on Monday, Sept. 26: an immunogenetics basic science symposium. The program was carefully crafted to include cutting edge basic science presentations and feature the most prominent scientist in immunogenetics. Kudos to Elaine Reed, Peter Parham, and other colleagues who worked hard to put this program together for our members. Please note that this special day requires separate registration. The annual meeting will follow immediately at the heels of this special day and I am sure you will be hearing soon from the program chair about the outstanding plan he and his planning committee have put together to enlighten us.

Looking forward to seeing you all in St. Louis!



# My Life in HLA: A Clinician's Perspective on 50 Years of Tissue Typing

M. Roy First, MD



My first exposure to transplantation was as a medical student in Johannesburg, South Africa, in 1966. Kidney transplantation had been started in South Africa only a few years earlier. At that time, with the rare exception of identical twin transplants, the results of transplantation were poor, with one-year survival rates approximating 50-60%. However, long-term dialysis was scarcely available; thus, the high risk of transplantation

was felt to be acceptable. However, there were a few exceptions – the occasional patient did very well on an immunosuppressive regimen of azathioprine and prednisone. It was these notable exceptions that determined the next 50 years of my career.

Back in those days, tissue typing was quite unsophisticated. Donor recipient selection was based on ABO blood group compatibility, matching of four antigens (two HLA-A and two HLA-B antigens), and a negative crossmatch based on the complement-dependent cytotoxicity (CDC) assay. In the era of the '60s and '70s, the only effective treatment for the anemia of end-stage renal disease (ESRD) was blood transfusions which resulted in a large number of highly sensitized patients on the list. This, together with the relatively unsophisticated crossmatch techniques at that time, resulted in a hyperacute rejection rate of around 5%.

## HLA Typing and Development of Histocompatibility Testing

The discovery of the first human major histocompatibility complex (MHC) antigen was made in 1958. Since then, there has been a rapid growth in the knowledge of the genetic structure and biologic function of the MHC, known in man as the human leukocyte antigen (HLA) system. The MHC is a series of closely linked genes found in humans on chromosome number 6. The MHC genes and the cell surface protein molecules encoded by the MHC play a critical role in T cell recognition, and function as antigens during transplantation. Dramatic advances in knowledge of the HLA genes have come about through the organization of an international group of expert investigators willing to work together. The exchange of reagents, antisera, and cell panels resulted in the standardization of the definition of antigens and established a common nomenclature. The first HLA workshop involved scientists from several countries and

proved to be a seminal event, providing an impetus for more extensive collaborations in the following years. In addition to providing a mechanism for exchanging reagents, the international HLA workshops promote new technology and disseminate both reagents and technical skills worldwide. This has been an invaluable resource for stimulating immunogenetics research and facilitating rapid translation of new technology and knowledge to patient care. Key advances from these workshops are described in the following text.

The first histocompatibility testing workshop was organized by Bernard Amos and held in his laboratory at Duke University in Durham, North Carolina in June 1964. Participants included 23 pioneering investigators, who were only able to study six sera consisting of 1 mL each. It was at this workshop that Paul Terasaki introduced the microcytotoxicity test for serologic typing, and described the first positive leukocyte antibody crossmatch test associated with hyperacute renal graft rejection. The second histocompatibility workshop in 1965 focused on mixed lymphocyte culture (MLC) techniques, while the third workshop in 1967 evaluated HLA-typing in renal transplantation.

The fourth international histocompatibility workshop in 1970 introduced a third locus (HLA-C), and the sixth workshop in 1975 was the first to include the use of D region homozygous typing cells to study determinants of the HLA-D locus. At this workshop, numerous reports were made of HLA antigens that were expressed on B cells but not on T cells. These antigens were termed "Ia-like" or "D region associated B cell antigens," and their description paved the way for the future description and characterization of the HLA-DR locus and the DR series of leukocyte antigens. The seventh international histocompatibility workshop in 1977 further defined the serological study of the newly-described Ia (Immune-associated) determinants and the relationship between the Ia specificities and Dw determinants defined by MLR and Dw homozygous typing cells. The eighth workshop in 1980 allowed the HLA-DR locus to be more clearly defined and provided additional clarification of the newly defined MB and MT specificities, which, in subsequent workshops, were shown to be encoded by genes of the HLA-DQ locus. This workshop also dealt with the relationship between histocompatibility testing and transplantation outcome and studied the relationship between HLA and susceptibility to disease. In the ninth workshop in 1984, the HLA-DP locus, with six specificities, was formally described based on cellular typing using T cell clones.

The tenth histocompatibility workshop in 1987 focused on the standardization of assays used to characterize HLA genes and their products at the molecular, genetic, biochemical, and cellular level. This workshop was the first to implement DNA-based HLA typing with the restriction fragment length polymorphism (RFLP) Southern blot methodology using a standard panel of cDNA probes for HLA class I and class II, thereby establishing a method for DNA typing of HLA. The eleventh workshop in 1991 saw the widespread introduction of polymerase chain reaction (PCR) technology with sequence specific oligonucleotide probe (SSOP) hybridization as an HLA-DNA typing method. The thirteenth histocompatibility workshop in 2002 marked the introduction of virtual DNA analysis, and the sixteenth workshop in 2012 focused on characterization of donor specific antibodies (DSA). The seventeenth histocompatibility workshop is scheduled to be held in San Francisco in 2017.

As a result of this remarkable international collaboration, 16 workshops have been held in 12 different countries. This has led to the achievement of a highly sophisticated, state-of-the-art tissue typing system, which has benefited hundreds of thousands of patients around the globe.

## Crossmatching

Crossmatch tests are used primarily for transplant candidates to assess the suitability of a potential donor. They are also used for platelet refractory patients and bone marrow candidates with aplastic anemia who may have developed anti-HLA antibodies. The crossmatch procedure is the most important test performed in the HLA laboratory in solid organ transplant recipients. Patients who have circulating antibodies that recognize HLA antigens on a donor organ or tissue may experience rapid and irreversible destruction of the graft upon transplantation (hyperacute rejection). Hyperacute rejections are rare today because of the sophisticated crossmatch techniques that have been developed to ensure that such reactions do not occur. Even low levels of antibodies may damage the grafts, so more sensitive antibody tests are used for crossmatching and for identifying the risk of immune antibody-mediated damage. Crossmatch tests use the complement-dependent cytotoxicity test and flow cytometry to detect antibodies that react with donor HLA antigens prior to transplantation. Tests are performed separately using donor T and B cells to indicate the likely target of the reaction as HLA-A, -B, -C or HLA-DR, -DP, or -DQ differences, respectively, between the donor and recipient.

## Complement-Dependent Lymphocytotoxicity

Complement-dependent cytotoxicity (CDC) identifies the most important antibodies in the crossmatch test – those responsible for hyperacute rejection of grafts. A positive crossmatch due to IgG antibodies directed against HLA-A, -B, -C, -DR-DP, or -DR-DQ antigens is a clear contraindication to transplantation because of the high risk of hyperacute rejection. Lymphocytotoxicity crossmatches are reported as positive or negative depending upon the percentage of donor cells killed by the recipient serum in the presence of complement and a vital dye. A positive B cell crossmatch, when it is due to anti-HLA antibodies, also carries a high risk of antibody-mediated damage and transplantation would not be recommended.

## Flow Cytometry

Flow cytometry is the most sensitive test for the detection of antibody even when present at levels that are not detected by lymphocytotoxicity tests. The flow cytometry test detects IgG antibodies. Positive crossmatches detected only by flow cytometry suggest the presence of preformed antidonor HLA antibodies (donor-specific antibody/DSA) and carry a risk of accelerated rejection and damage to the graft. These sensitive crossmatches should be interpreted in terms of the patient's sensitization history. Flow cytometry results are reported as positive or negative based upon the median channel shift caused by the binding of a specific antibody. A key factor determining the significance of the result is the cut-off value used to determine a positive test. These are not applied uniformly between centers and those that apply a very low cut-off value will increase sensitivity at the expense of specificity. Currently, many centers run 20 known negative sera using a cut-off for a positive crossmatch of two to three times the standard deviation (SD) above the mean channel fluorescence of the normal sera controls.

## Virtual Crossmatch

Crossmatch results can now be accurately predicted when the patient's antibody specificities have been identified using recombinant single HLA antigen bead technologies and the potential donor HLA type is known. Thus, offers of deceased donor kidneys and other organs can be avoided when a positive crossmatch can be predicted. This permits importation of organs over greater distances without the fear of a positive crossmatch and provides an advantage to those disadvantaged by sensitization.

## Endothelial Cell Crossmatch

Endothelial cells constitute the first contact point between the transplanted organ and the recipient's immune system. Endothelial cell crossmatching (ECXM) provides testing for antibodies to non-HLA antigens only expressed on endothelial cells prior to transplantation. Antibodies reactive with donor endothelial cell antigens have been implicated in cases of humoral rejection when no anti-HLA antibodies can be detected. The endothelial cell crossmatch can identify antibodies that may increase the risk of antibody-mediated rejection before or after transplantation.

## Clinical Applications

The methods used to test for HLA antigens and antibodies have evolved over time and become more sophisticated. In the beginning, serology and cellular-based assays such as the complement-dependent cellular lymphocytotoxicity (CDC) assay were used to determine HLA antigens and anti-HLA antibodies. Now, antigens are molecularly defined through DNA analysis, and anti-HLA antibodies are detected using flow cytometry, solid-phase, single-antigen bead immunoassays, such as Luminex. These assays can also be used to detect non-HLA antibodies. The reliability of DSA as a biomarker for the risk of hyperacute rejection has essentially eliminated this event following kidney transplantation.

## Desensitization

Desensitization protocols emerged in the late 1990s to deal with the increased number of sensitized wait-list patients. Pre-existing sensitization against HLA antigens with DSA leads to protracted waiting times and an increased risk of antibody-mediated rejection (AMR) after transplantation. Based on the highly sophisticated crossmatch techniques currently available, pre-transplant desensitization can effectively achieve a negative crossmatch, thereby allowing for successful transplantation of a number of potential recipients who would have previously been excluded from transplantation. Desensitization protocols consist of conditioning regimens using IVIG, and/or anti-CD20 (rituximab), and IVIG in combination with plasma exchange (plasmapheresis).

Similar desensitization techniques have now also allowed for ABO incompatible kidney transplants between some recipients and living donors. The option of having a living donor with a different blood type has thus reduced the waiting time for some recipients who need a kidney transplant.

## Kidney Allocation

A new kidney allocation scheme (KAS) was implemented Dec. 3, 2014 and is based on the kidney donor profile index (KDPI) and the estimated post-transplant survival (EPTS) of the patient in an effort to balance equity and utility. In accordance with the new policy, zero-antigen-matched kidneys can be given to patients with a low cPRA, but sensitized patients receive many more points under the new KAS than they used to receive under the old allocation policy.

Most of the worldwide data on deceased-donor transplantation have been based upon random assignment of cases without regard to HLA incompatibility, and the importance of lesser degrees of matching has only been determined after transplantation. Regional organ sharing is a strategy designed to improve transplant survival by increasing the frequency of partially matched grafts, although the results are less dramatic, compared with the six-antigen-matched cases.

## Conclusions

What does the transplant physician want and expect from the tissue typing lab? In the 1970s, hyperacute rejection occurred in approximately 5% of recipients. This was emotionally devastating to the recipient, the recipient's family, and to the transplant team. One of the worst events was hyperacute rejection of a living donor kidney. In those times, the most important service that the lab could provide was a sensitive crossmatch result that avoided this outcome; however, unfortunately, this was not always the case. Happily, this is now a very rare event. The beneficial effect of HLA matching on long-term survival has been clearly established. Moving ahead, what the transplant physician would like to see are methods to optimize the immunological status of the recipient allowing for minimization of immunosuppression, but at the same time preventing the development of broad sensitization.

In order to facilitate successful transplantation of the highly sensitized patient, high resolution HLA typing and precise quantitation of HLA antibodies are essential. The tremendous advances in tissue typing that have occurred over the past 50 years have increased the safety and long-term outcomes for solid organ transplant recipients. Recent advances have already provided access to transplantation for many potential recipients who would otherwise spend prolonged time on the waiting list. Further development of sophisticated computerized algorithms and technological advances offer the possibility of even greater success in the future for the utilization of this scarce resource.

## Acknowledgements

My sincere thanks to Dr. Anat Roitberg-Tambur for reviewing this article and for her helpful suggestions.

*The discovery of the first human major histocompatibility complex (MHC) antigen was made in 1958. Since then, there has been a rapid growth in the knowledge of the genetic structure and biologic function of the MHC, known in man as the human leukocyte antigen (HLA) system. The MHC is a series of closely linked genes found in humans on chromosome number 6.*



## THE UNIVERSITY OF MICHIGAN HEALTH SYSTEM

The Department of Pathology at the University of Michigan Health System in Ann Arbor, Michigan is seeking an MD, MD PhD, or PhD to serve as Director for the Histocompatibility Laboratory.

The University of Michigan Histocompatibility Laboratory is an internationally recognized, high volume, ASHI-accredited laboratory providing services for one of the largest solid organ transplant programs in the country, in addition to supporting the Hematopoietic Cell Transplantation (HCT) program. The laboratory offers a full range of histocompatibility procedures to support the needs of our University of Michigan patients as well as national and international clients. The Director will collaborate with laboratory management team to facilitate pre-analytic, analytic and post-analytic phases of laboratory testing while also identifying innovative opportunities to add value to care.

The department of pathology at the University of Michigan is among the top programs in the country serving not only the world class providers and patients at the University of Michigan Health System (UMHS) but also numerous patients served through the MLabs outreach division. Ours is one of the most highly ranked pathology

residencies in the country, with multiple subspecialty fellowships including an opportunity for a fellowship program in histocompatibility and immunogenetics. Basic, translational and clinical research accounts for over \$33 million dollars in research expenditures with additional internally funded projects reflecting a strong departmental and institutional commitment to innovation and patient care.

Ann Arbor offers a quality of life and amenities found in few other comparably sized communities. In 2011 alone, Ann Arbor was ranked #2 on *U.S. News and World Report's* list of the "10 Most Educated Cities," #6 on *Forbes Magazine's* list of the "25 Best Cities for Retirement," and #1 on *Bizjournals.com's* list of the "Smartest Big Cities," just ahead of Cambridge and Berkeley.

Candidates must have completed a Transplant Immunology and Immunogenetics fellowship, be board certified by the American Board for Histocompatibility and Immunogenetics (ABHI), and be an accredited Histocompatibility Laboratory Director by the ASHI-DTRC. Appointment will be at a level consistent with the successful candidate's experience and track record of accomplishments in service, education and academic activities.



Interested candidates should submit their curriculum vitae, a cover letter detailing experience and future interests, and the names and addresses of three individuals who could provide letters of recommendation to:

**David F. Keren, M.D.**  
**Director, Division of Clinical Pathology**  
**Department of Pathology**  
**University of Michigan Medical School**

1301 E. Catherine Street, SPC 5602  
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# How I found my Way to HLA

Christopher Do



I have to admit that I entered the field of HLA entirely by accident. When I was growing up, my ideal life plan was to be bitten by a radioactive spider so that I could save lives. In reality, my interest in science and the way the world works led me to pursue degrees in molecular biology and economics. After graduation, there were so many opportunities that I found myself jumping from job to job, trying to find something that felt right. I had originally planned to pursue a career in research and never thought I would end up in a clinical HLA lab a few years later. I did everything from working at a biotech company to teaching under-privileged kids at an independent charter school. I even considered a research associate position at the Federal Reserve Bank of San Francisco that was sent my way by a former economics professor. At the biotech company I didn't feel a direct connection to helping real people or improving lives. Teaching was extremely rewarding and finding unique ways to inspire children was very fun, such as using Iron Man and Bruce Banner as science career role models. However, I developed an itch to get my hands back on a pipette and get back in a laboratory. Eventually I received the opportunity to take a laboratory support position at the UCSF Immunogenetics and Transplantation Laboratory (ITL), which marked my first step into the vast field of HLA.

As I worked at the UCSF ITL, I developed an appreciation for HLA that I did not have before. Even though I started off by only supporting the lab through validating temperature monitoring systems and writing safety procedures, I watched and observed the CHTs and CHSs as they worked. The passion they had for patient care was inspiring. The diligence that complex assays were completed with was incredibly admirable. From an academic standpoint, all the HLA discoveries both in the past and recent history truly piqued the curious scientist in me. My interest in my colleagues' work led me to learn much more about HLA in a short period of time. Although I did not perform any clinical testing, I picked up many of the techniques and scientific principles that were used in the lab. My appreciation for and knowledge of HLA continued to grow as I validated new instruments and assays, updated and revised SOPs, and attended educational lectures. This led me to jump at an opportunity to participate in the UCSF ITL CHT training program and take the ABHI examination. Through the training program I learned an enormous amount about the science, principles, and history of HLA. I also received

hands-on instruction on clinical assays for HLA/KIR typing, antibody screening, and crossmatching. After the CHT training program and ABHI examination, I became a full-fledged CHT at the UCSF ITL in July 2014.

Although I've had only been in the field of HLA for a short period of time, it is clear that HLA is a fast-moving field that is consistently in a state of flux. From the CHT training program, I learned that B cells used to be isolated using nylon wool and straws. Today, we use magnetic microbeads for B cell isolation. Serological typing has been replaced with SSP typing, SSO typing, and sequencing based typing. The UCSF ITL is now using next generation sequencing in combination with other typing techniques to improve accuracy, resolution, and speed. Sensitivity of crossmatching has greatly improved from complement dependent cytotoxic crossmatches to flow cytometric crossmatching. Today, the UCSF ITL uses virtual crossmatching to determine histocompatibility for transplantation.

When I'm asked the standard interview question, "What is your greatest strength?" my answer is my ability to learn scientific principles and pick up hands-on skills quickly. A career in HLA complements my greatest strength perfectly. I find myself constantly training and researching to keep sharp with new cutting edge technology and techniques. Current established techniques and protocols are always scrutinized and revised to optimize speed, efficiency, and accuracy of results. It's an amazing feeling to know that I'll always be learning new things and be intellectually stimulated with my career in HLA. Even at this moment, the UCSF ITL is validating a C3d antibody screening assay and moving to next generation sequencing. All members of my laboratory regularly attend Georgetown lectures and other continuing education events, which are great opportunities to learn even further through new articles and findings in the field of HLA. Many other laboratory careers lead to positions that perform the same set of tests with very few changes from year to year. But a career in HLA is never stagnant or humdrum due to the constant discoveries and changes in HLA.

It's a tiny bit embarrassing to confess, but sometimes I lie in bed not able to fall asleep because I think of possible changes and improvements that can be implemented in HLA. Typing resolution, accuracy, and speed increases all the time and more loci will be typed in the future for transplant matching. Perhaps even minor histocompatibility complexes will be typed along with the major histocompatibility complexes. When the speed of high resolution sequencing is fast enough, we might even see deceased donors providing bone marrow donations in addition to solid organ donations. Antibody screening techniques will need to cover more alleles and loci just to keep up with typing

improvements. I also envision the use of liquid handling systems to perform crossmatches in the future. The UCSF ITL already implements a 96-well format for flow crossmatching so we are just a short step away from semi-automation or even full automation. The field of HLA may also move toward testing the expression levels of cell surface antigens to supplement typing, antibody, and crossmatch data when providing clinical recommendations.

Aside from continual advancements in the field, a career in HLA is extremely gratifying because we are improving the health of patients. Patient care is the first priority for everyone here at the UCSF ITL. The fruits of our labor are not the test results from HLA testing, whether it is HLA typing, crossmatching, antibody screening, or STR testing. The figurative fruit of our labor is aiding with a successful transplant or providing accurate post-transplant monitoring to improve the health of our patients. Although I do not personally interact with any patients, the most rewarding aspect of my career in HLA is knowing that I am making a positive difference in patients' lives. A UCSF nephrologist once brought one of his patients to speak with our laboratory and to thank us personally for our work that helped make the kidney transplant possible. Another patient appeared in a video on YouTube to speak about his experience with his lung transplant at UCSF. It's an indescribable sensation when I know I had a hand in changing a patient's outlook for the better. The best way I can explain it is that good feeling you get when you help out a stranger by removing the toilet paper stuck from their shoe or when you hold the door for a guy who is carrying seven grocery bags, except multiplied by 1000! I take great joy in knowing that that the direct result of my work in HLA enriches the lives of patients.

As a newcomer to HLA, I can't wait to see what my future in HLA holds. I look forward to the innovations in the future of HLA and I can only imagine what the field will look like five, 10, or even 20 years from today. I also know that in those five, 10, and 20 years, I'll be helping patients and improving lives every single day. A career in HLA provides me with a unique mix of satisfying hands-on bench work, stimulating continuing education, and fulfilling patient care. I found that my accidental step into HLA led me on an exceptionally lucky path to the remarkable career I have now. I never would have guessed the magnitude of the field of HLA before I took the lab support position at UCSF ITL. I have a cousin in middle school who asked me about my job since he never heard of CHTs in his school. After explaining all about clinical laboratory scientists, transplantation, and how my job fits into the healthcare picture, my cousin commented, "So basically you help save lives, like Superman?" I could only reply, "More like Ironman, with science!"

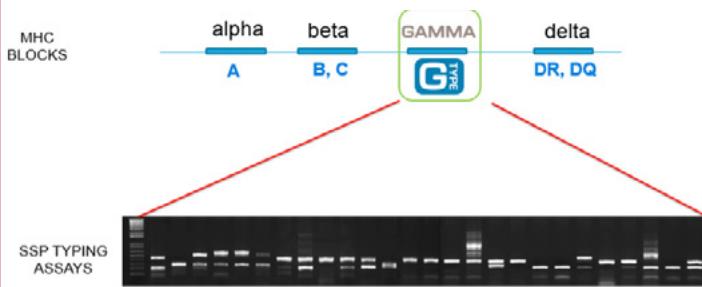
*As I worked at the UCSF ITL, I developed an appreciation for HLA that I did not have before. Even though I started off by only supporting the lab through validating temperature monitoring systems and writing safety procedures, I watched and observed the CHTs and CHSs as they worked. The passion they had for patient care was inspiring. The diligence that complex assays were completed with was incredibly admirable.*

# Current Literature Review

## *Significance of MHC-Gamma Region in Bone Marrow/Hematopoietic Stem Cell Transplantation*

Siva Kanangat, PhD, D(ABHI)

As we all know, the current HLA matching for unrelated BMT/HSCT is based on high resolution HLA matching at HLA-A, B, C, DR; or A, B, C, DR, and DQ; or A, B, C, DR, DQ, and DP at the allelic level depending on individual transplant programs. This HLA loci matching does not consider the minor Histocompatibility Antigens (mHA) mismatch between the donors and recipients. The HLA-Loci belong to different blocks on the MHC Block on the short arm of Chromosome 6 (i.e., HLA-A: alpha block; HLA-B, -C: beta block; HLA-DR, -DQ, -DP: delta block). In addition there is a Gamma Block (GB) between the beta and delta block as shown below. It appears that there are several Single Nucleotide Polymorphisms (SNPs) in this region. And this GB has several immune response-associated genes that could play a major part in BMT/HSCT despite allele-to-allele match in unrelated donor BMT/HSCT.



The major concern in fully matched related and unrelated donor BMT/HSCT is the issue of Graft vs Host Disease (GVHD) of varying degrees, some of which could be fatal and some of which could be very debilitating. This could be partly due to the mismatch in mHA. However, previous investigations by Petersdorf et al<sup>1,2</sup> had emphasized the importance of haplotype matching using elaborate procedures in unrelated donors, which is not performed in the current way of typing unrelated donors in most histocompatibility testing laboratories.

Petersdorf's studies led to the identification of several SNPs in the Gamma Region.<sup>1,2</sup> In a study presented at the BMT-Tandem Meeting, Medhat et al<sup>3</sup> presented data that showed GB SNP mismatch is associated with a higher risk of severe acute GVHD, a finding that is independent of the HLA and MICA and various other clinical risk factors. They examined 25 GB SNPs in 236 HCT recipient/unrelated donor pairs transplanted at their center between 200-2010 that included AML (n=89), ALL (41), MDS (34), NHL (28), and other diagnoses. All donor/recipient pairs were typed by high resolution typing of HLA A, B, C, DRB1, DQB1, and DPB1 loci, as well as for MHC class I related chain A (MICA). GB SNP typing was performed by sequence specific

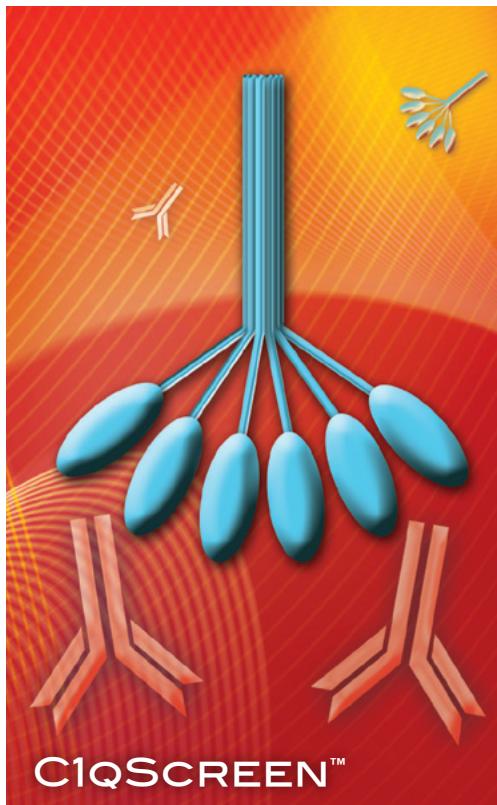
primers. They examined SNPs c.2918+98G, c.3316C, and c.4385C (reference sequence C4A NG\_011638.1) and were found to be associated with severe GVHD. The data showed that SNP mismatch was associated with increased risk of severe GVHD in univariable analysis (HR 2.43, 95% CI 1.32-4.47, P=0.004), while no significant differences among SNP match and mismatch pairs regarding HLA match (10/10 vs. <10/10, 8/8 vs. <8/8, DPB1), MICA match, and patient, disease, or transplant characteristics was observed. Based on the multivariate analysis using Fine and Gray regression, the authors suggest that avoiding GB SNP mismatch can potentially reduce the risk of severe acute GVHD.

Studies conducted at our institution on 52 recipient/donor pairs who received HSCT at our institution showed that 10% of related (2/22), 100% haploidentical (4/4), and 65.4% of unrelated (17/26) recipient/donor pairs were GB mismatched. Therefore, unrelated recipient/donor pairs are 7 times more likely to harbor GB-mismatch compared to related pairs. With the confounding effect of HLA-DP mismatch, unrelated pairs are 1.7 times more likely to have GB-mismatch. A limited clinical outcome study on these 52 patients showed that GB-mismatch had a higher incidence of grade 2-4 acute GVHD (20.5% v 13%; p=0.044) and chronic GVHD (28.4% v 16.3%; p=0.048). Multivariate regression showed that GB-mismatch is associated with higher transplant related mortality (p=0.020) and a trend for severe acute GVHD (HR 2.450, 95% CI 0.96-6.22; p=0.060) after controlling for donor type.<sup>4</sup>

There are not many published data on the GB typing and the effect on BMT/HSCT outcome or GVHD. The information from Petersdorf et al's published data, data from Medhat et al presented at BMT/HSCT Tandem Meeting 2016, and our own data indicate that GB typing between donor/recipient pairs can be an additional marker for superior donor selection for better BMT/HSCT outcome in terms of GVHD/graft survival and reduced transplanted related mortality (depending on the cohorts studied). More retrospective and prospective studies with long-term follow up are needed. Significance: GB typing is an important diagnostic tool in addition to HLA matching for BMT/HSCT.

*See references on page 24*

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# Technology Reports: Application of HLA Epitope-Based Matching in the Clinical Transplant Setting

## Part I: Antibody Reactivity with Epitopes

Rene J. Duquesnoy, Marilyn Marrari

### Introduction

HLA matching at the epitope level offers new approaches to identify compatible transplant donors including acceptable mismatches for sensitized patients. Molecular modelling of HLA alleles and amino acid sequence comparisons have permitted structural descriptions of epitopes reacting with HLA antibodies. This article describes some basic concepts about HLA epitopes and how they need to be considered in the analysis of HLA antibody reactivity patterns of sera aimed to determine mismatch acceptability for sensitized patients.

HLA antibodies recognize epitopes that correspond to structurally defined eplets (i.e., small configurations of polymorphic amino acid configurations on HLA molecules). Several reviews provide more details.<sup>1,3</sup> Eplet information requires HLA typing at the four-digit allele rather than antigen levels.<sup>4</sup> The International HLA Epitope Registry ([www.epregistry.com.br](http://www.epregistry.com.br)) has structural descriptions of repertoires of HLA-ABC, DRDQDP, and MICA epitopes including those that have been verified with informative antibodies. The HLAMatchmaker website ([www.HLAMatchmaker.net](http://www.HLAMatchmaker.net)) has several eplet matching and epitope-based antibody analysis programs and is an extensive resource of publications, PowerPoint slides, and tutorials about HLA epitopes.

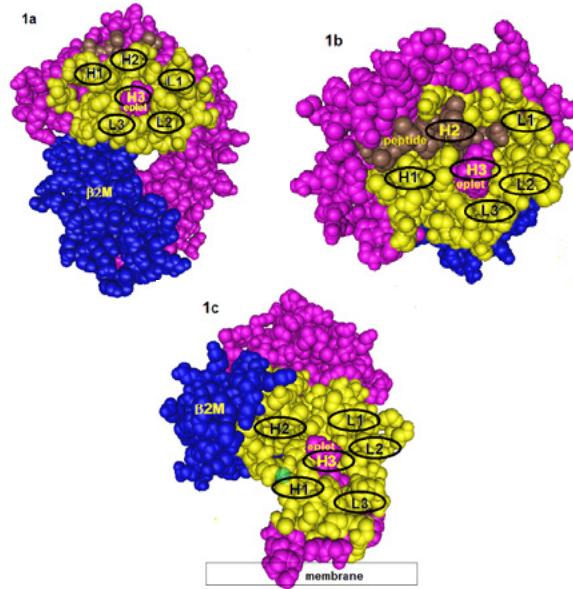
This article has three components. Part I focuses on basic aspects of monospecific (or monoclonal) antibody reactivity with HLA epitopes. Part II in the 2016 third-quarter issue of *ASHI Quarterly* will deal with epitope specificity analyses of HLA antibodies in sera from sensitized patients and the determination of mismatch acceptability. Part III in the 2016 fourth-quarter issue will address the control of antibody responses to HLA epitopes and how to identify permissible mismatches.

### Basic Aspects of Antibody Reactivity with HLA Epitopes

Epitope-based determinations of mismatch acceptability for HLA alloimmunized patients requires a basic understanding of how antibodies interact with epitopes. Antibody binding to protein epitopes occurs through six Complementarity Determining Region (CDR) loops, three of them – H1, H2, and H3 – are on the immunoglobulin heavy chain and L1, L2, and L3 are on the light chain. Each loop interacts with a small set of amino acid residues within the so-called structural epitope and CDR-H3, which binds to the so-called functional epitope (or hot spot) in a central location, has a dominant role in determining antibody specificity. Eplets are considered to be equivalent to functional epitopes.

Figure 1 shows hypothetical models of eplets in three different locations on class I molecules and in context with corresponding structural epitopes the possible contact sites for the CDRs of antibody. These models are based on the general concept that structural epitopes on protein antigens have about 15-25 contact residues distributed over a surface of 700-900 square Ångstroms. Two eplets are readily antibody-accessible on the upper domains of the HLA molecule (Figures 1a and 1b) but an antibody to an eplet on the membrane-proximal domain (Figure 1c) might interact with only solubilized but not with lymphocyte membrane-bound HLA molecules where such eplets may not be readily antibody-accessible. Would such epitopes be clinically significant?

**Figure 1. Three Models of Structural HLA Class I Epitopes**



The centrally located eplet interacts with CDR-H3 of antibody and other configurations (in oval circles) make contact with other CDRs on heavy chain (H1 and H2) and light chain (L1, L2, and L3).

Let us first consider the scenario illustrated in Figure 1 that a monoclonal HLA antibody recognizes through its CDR-H3 a specific eplet. Other eplets in the same sequence location or eplets with the same residues but in a different sequence position will generally not react with that antibody. For instance, consider position 62. Certain antibodies are specific for the 62GE eplet (on A2, B57, and B58 alleles), others recognize 62LQ (on A29 alleles and A\*43:01), and still others are specific for 62EE (on A23 and A24 alleles and A\*80:01), etc. In other words, these eplets determine structurally distinct epitopes uniquely identified by specific antibodies.

However, there can be exceptions if an eplet in the same sequence position has a residue difference but is still structurally similar to the eplet that has induced a specific antibody. As an example, the well-known Bw4 epitope can be described by residues in sequence positions 80, 82, and 83 on the molecular surface. The 82LR eplet has two very closely nearby residues: 80I shared by one group of Bw4 alleles such as A\*24:02, B\*38:01, and B\*51\*01 and 80T shared by a second group of Bw4 alleles including B\*13:01 and B\*44:02. Now let us consider the specific antibody response to Bw4 induced by an allele that has 82LR and 80I such as A\*24:02 or B\*49:01 and testing in Ig-binding assays with single allele beads reveals a monospecific antibody.

**Table 1. Effect of the Presence of 80I and 80T on 82LR-Specific Antibody Reactivity Patterns**

Panel	Allele	Case 1		
		Residue	Immunizer	Case 2
			A*24:02	B*49:01
	A*23:01	80I	10483	12601
	A*24:02	80I	8916	14744
	A*24:03	80I	8966	14899
	A*25:01	80I	11850	8041
	A*32:01	80I	11570	13091
	B*15:13	80I	8736	6132
	B*15:16	80I	10384	6967
	B*38:01	80I	12522	11071
	B*49:01	80I	11240	13172
	B*51:01	80I	8448	6403
	B*51:02	80I	11258	9178
	B*52:01	80I	10274	7570
	B*53:01	80I	10994	9049
	B*57:01	80I	10461	9968
	B*57:03	80I	10132	9236
	B*58:01	80I	8695	8115
	B*59:01	80I	8664	7745
	Mean±SD		10211±1275	9881±2866
	B*13:01	80T	11531	700
	B*13:02	80T	12031	2651
	B*27:05	80T	7684	3690
	B*37:01	80T	6007	2251
	B*44:02	80T	12112	1753
	B*44:03	80T	12149	1860
	B*47:01	80T	9968	605
	Mean±SD		10212±2468	1930±1082
	Other Alleles		<150	<100
				<100

There are three reactivity patterns at the eplet level (Table 1). Case 1: all Bw4 alleles show comparably high MFI values regardless of the presence of 80I or 80T. Case 2: all 80I-carrying alleles show high MFI values whereas the 80T-carrying alleles have significantly lower MFI values although their reactivity can still be considered positive.

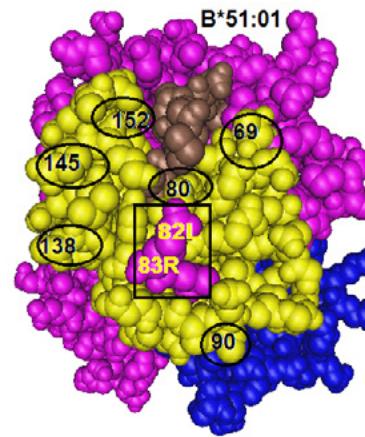
The 80T versus 80I residue difference represents the so-called Landsteiner type of serological cross-reactivity whereby slightly different but structurally related eplets react with the same antibody.

Case 3 represents a reactivity pattern whereby a Bw4 antibody reacts only with 80I-carrying alleles. This indicates a dominant role of 80I over 82LR and the HLA Epitope Registry lists 80I as an antibody-verified epitope. One can also expect three possible reactivity patterns of Bw4 antibodies induced by 80T-carrying alleles such as B\*44:02. Other structurally related class I and class II eplets exhibit also the Landsteiner type of cross-reactivity but this is beyond the scope of this article.

As illustrated in Figure 1, the structural epitope has other amino acid configurations that interact with the remaining CDRs of antibody. An important consideration is the so-called affinity maturation process during the antibody response whereby DNA regions corresponding to CDRs undergo mutations, which increase antibody affinity with the structural epitope. As an example, let us consider for Figure 1a just one loop, CDR-L2, which has increased binding with a given amino acid configuration in the structural epitope of the immunizing allele.

Two possible configurations can be considered. One comprises only monomorphic residues shared between all eplet-carrying alleles in the panel and the epitope recognized by antibody corresponds solely to a single eplet. Second, this configuration has polymorphic residues and only alleles that share such residues with the immunizing allele are antibody-reactive. Such residues can be identified as critical contact sites for antibody. Accordingly, these epitopes can be defined by eplets paired with other polymorphic residue configurations (including eplets) in other sequence locations within a 15 Ångstrom radius of the eplet, the presumed dimension of a structural epitope.

**Figure 2. Molecular Model of Critical Contact Sites for Various Bw4 Antibodies**



Position 80 is close enough to 82LR to be contacted by the same CDR of antibody.

**Table 2. Reactivity Patterns of Antibody-Verified Bw4 Epitopes Recorded in the International HLA Epitope Registry**

Case	Reactive Epitope	Reacting Bw4+ alleles*	Residues on Nonreactive Bw4+ Alleles
1	82LR (with 80I or 80T)	All Bw4	-
2	80I (80ILR)	A23,A24,A25,A32,B38, B49,B51,B52,B53,B57, B58,B59,B63,B77	80T
3	80TLR	B13,B27,B37,B44,B47	80I
4	82LR+90A	All, except A25	90D (A25)
5	82LR+144QR	All, except A24 and B13	144KR (A24) 144QL (B13)
6	82LR+145R	All, except B13	145L (B13)
7	82LR+145RA	All, except A25 and B13	145RT (A25) 145LA (B13)
8	82LR+138T	Only HLA-B alleles	138M (HLA-A)
9	82LR+138M	Only HLA-A antigens	138T (HLA-B)
10	80I+69TNT	B38,B49,B51,B52, B53,B59,B77	69AHS (A23,A24, A25,A32) 69ASA (B57,B58, B63)
11	80I+90A	A23,A24,A32,B38,B49, B51,B52,B53,B57,B58, B59,B63,B77	90D (A25)
12	80I+151RE	B49,B51,B52,B63,B77	151HV (A24) 151RE (A25) 151RV (A23,A32, B38,B53, B57,B58)

\* 82LR-carrying antigens: A23, A24, A25, A32, B13, B\*27:05 (not B\*27:08), B37, B38, B44, B47, B49, B51, B52, B53, B57, B58,

The variety of Bw4 antibody responses provides useful illustrations (Table 2). There are Bw4 antibodies whose reactivity are influenced by residue polymorphisms in surface positions 69, 90, 138, 145, and 152; all of them are within a radius of 15 Ångstroms of 82LR (Figure 2). It should be noted each critical contact residue configuration is always present on at least one allele of the antibody producer and this suggests an autoimmune component of the antibody response to a mismatched eplet.<sup>5</sup>

Some CDRs can make contact with peptides bound to the groove and it is possible that peptides serve as critical contact sites with antibody such CDR-H2 as illustrated in Figure 1b. Indeed, it has been reported that certain HLA antibodies are peptide-dependent.<sup>6,7</sup>

For many protein antigen-antibody complexes there is a certain level of permissiveness for residue substitutions in critical contact areas and this applies also for HLA epitopes. Certain residue substitutions in the structural epitope have a moderate effect on

an allele's reactivity with antibody (i.e., the MFI values are lower but still considered positive), whereas others are inhibitory to the level of very weak or no reactivity of specific eplet-carrying alleles. Such alleles might be considered as epitope-based acceptable mismatches.

## Class II Epitopes

Epitopes on DRB alleles often correspond solely to eplets; examples are 16Y on DRB1\*08 and \*12 alleles and the high-frequency 25R on all DRB alleles except DRB1\*07:01 and DRB4. Only a few DRB epitopes have been defined by eplet pairs and this might be due to monomorphic residue nature of the DRA chain. Several immunogenic DRB1 eplets are also on alleles encoded by DRB3, DRB4, and/or DRB5. Prime examples are the 4Q eplet shared between DRB1\*07, DRB1\*09, and DRB4\* alleles and the immunogenic 96EV eplet shared between DRB1 and DRB5 alleles.<sup>8</sup> The HLA Epitope Registry lists distinct antibody-verified eplets on DRB3/4/5 and these loci are important in class II antibody responses.

DQ and DP encode for heterodimers of A and B chains which are both polymorphic and have distinct eplets, many of which have been antibody-verified and recorded in the HLA Epitope Registry. DQ plays a dominant role in the antibody responses to class II mismatches. Such antibodies are specific for eplets on DQA and DQB chains. Many immunodominant DQB eplets correspond to the original DQ1-9 serological specificities and several eplets have numerical subscripts. For instance, DQ1 corresponds to 52PQ, which actually represents two eplets, 52PQ and 84EVI, which cannot be distinguished using the Luminex panel alleles with the DQ5 and DQ6 "splits" of DQ1. As another example, DQB1\*02:01 and 02:02 have a unique 45GE, that represents three spatially separate configurations: 45GE, 55LL, and 71KAVR. Eplets with subscripted numbers should not be considered as eplet pairs but reflect multiple configurations shared by groups of Luminex alleles. Common DQA eplets are 40GR, on DQA1\*04, \*05, and \*06 alleles and 47KHL uniquely on DQA1\*02:01.

There is an emerging concept that some DQ epitopes are defined by residue configurations involving both chains. This suggests that epitope-based matching should consider the DQ heterodimer rather than the individual chains alone.<sup>9</sup>

DP mismatching involves generally fewer epitopes on DPB and especially on DPA; immunogenic eplets such as 84DEAV and 55DE are present on large groups of DPB chains. It should be noted that certain 55DE-specific antibodies react also with 57DE on DRB1\*11 alleles suggesting an interlocus cross-reactivity.

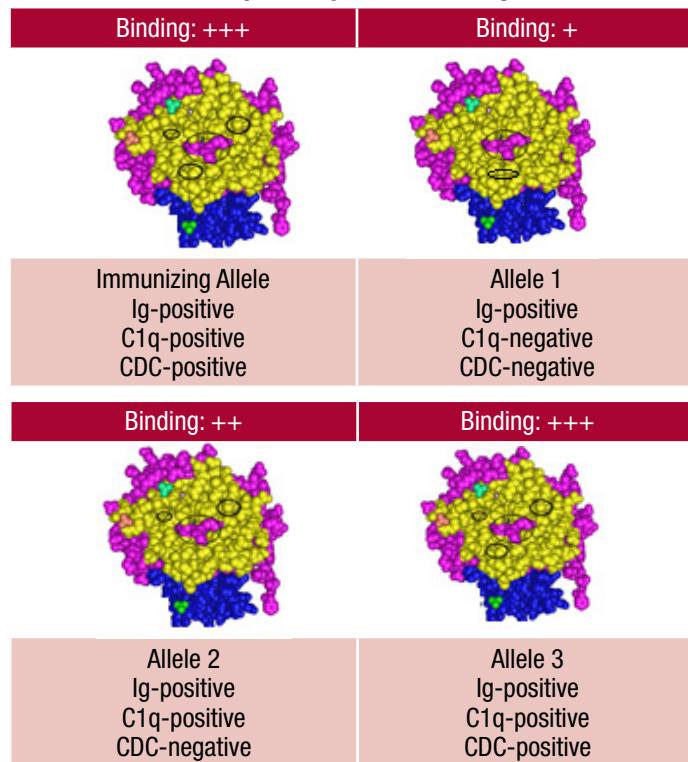
## Technique-Dependent Epitope Specificities of HLA Antibodies

HLA antibodies are tested with panels using different techniques, from immunoglobulin (Ig) and complement component C1q binding to isolated HLA molecules attached to microbeads, to flow cytometric binding on lymphocytes and complement-dependent lymphocytotoxicity. For the same monospecific antibody, these methods may give different results regarding epitope specificity determinations. This can only be explained with basic concepts about the strength of antibody binding to an epitope and the release of so-called free energy.

C1q-binding and complement-dependent lymphocytotoxicity are sequential events which depend on the formation of the antibody-epitope complex. C1q-binding requires a conformational change of the antibody molecule thereby exposing the C1q receptor on the Fc part. This step requires a sufficient amount of free energy released upon binding of antibody to epitope. The initiation of the complement cascade leading to cytotoxicity depends on a conformational change of the bound C1q followed by the formation and activation of antibody-bound C1qrs complex; this process requires an additional amount of free energy released upon antibody-epitope complex formation.

In other words, it is all about free energy release, which depends on the binding strength of all CDRs interacting with the contact sites of the structural epitope of different alleles (Figure 3).

**Figure 3. Structural Modeling of HLA Epitope-Carrying Alleles with Different Reactivities in Ig-Binding, C1q-Binding and CDC Assays**



The sizes and numbers of the circles reflect the binding strengths of the CDRs with the different parts of the structural epitope.<sup>10</sup>

It is well known that IgG1 and IgG3 type antibodies can fix complement very well and that IgG2 and IgG4 generally do not. Let us consider a monospecific IgG1-type antibody that reacts with an eplet on the immunizing allele in all three assays. Suppose the eplet-carrying alleles of the panel do not react equally well with in Ig-binding, C1q-binding and cytotoxicity assays as illustrated in Figure 3. As previously described,<sup>10</sup> the identification of relevant residues within the structural epitope may offer an explanation but how can this be determined?

You will need to download molecular HLA crystal structures, preferably the immunizing allele, and the Cn3D (version 4.3) molecular modelling program from the [NCBI website](#). Cn3D has a “Help” heading to show you how to use the program. Under “Select” use the Pick Structures command to select the chains and peptide for a single HLA structure. Under “Style” select under Rendering Shortcuts the Space Fill command. Then, under Coloring Shortcuts, select the Molecular command and this will show the HLA molecular model normally used in HLA epitiology. You can turn the model around on the screen. Now go to the Sequence/Alignment Viewer and select on the correct chain the residues of the eplet. Go back to “Select” and click on Select by Distance. Enter 15 Ångstroms and deselect “other molecules only.” The model shows now lots of yellow residues and they are highlighted in the Sequence Viewer. For analysis of the structural epitope select only those highlighted residue positions with polymorphic and locus-specific residues. For each eplet-carrying allele in the panel enter the residues in these positions into a spreadsheet and this will result in the overall map of a structural epitope. A comparison of the amino acid residue differences between positive and negative alleles may provide information which configurations in the structural epitope are involved with binding to antibody.

The HLA Epitope Registry has residue tables for several structural epitopes and we plan to come up with a complete list.

## Conclusions

Part I offers some basic information how HLA antibodies react with epitopes. Although the eplet is considered an essential component of an HLA epitope, it is apparent that the specific reactivity of HLA antibodies measured in various assays depend on interactions with other amino acid residues within the context of structural epitopes. In other words, HLA epitopes can have quite complex structures. A major goal of HLA epitope matching is to identify those epitopes that have been experimentally verified with informative antibodies. The HLA Epitope Registry has already a record of antibody-verified epitopes for each locus but the list is still incomplete. We are working very hard to add new ones and we need help from more histocompatibility laboratories. Let us know if you have sera with interesting antibody reactivity patterns that might recognize new HLA epitopes.

See references on page 24

# Recapping the ASHI Regional Workshop in Long Beach

The Renaissance Long Beach welcomed 103 attendees to ASHI's Regional Education Workshop 1 in Long Beach, California. We kicked off the meeting with keynote speaker, Jon Kobashigawa, MD, and enjoyed drinks and California cuisine throughout the night. We had two packed days of education; touching stories from a donor's mother, Tani Penland, and a kidney and pancreas transplant recipient, Jame White; and ended with a faculty debate on the pros and cons of cytotoxicity. Nine vendors presented to the group and were available to attendees in the vendor room. We also had an educational lunch symposium sponsored by One Lambda, A Thermo Fisher Scientific Brand. After each day of sessions, Long Beach offered many dining options that were within walking distance for attendees. We hope attendees had a wonderful time in Long Beach and we look forward to seeing you again soon!

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## Regional Education Workshop 2

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## An Attendee's Perspective

By Cecelia Villa

Attending the ASHI regional workshop was an exciting experience with great information and opportunities to network within the histocompatibility community. I was able to meet with professionals from all across the nation and from various lab backgrounds. It was incredibly interesting to hear their stories and compare different lab techniques and how they function as a whole. It also served as a great resource for any future contact I might need as a tech within this community. The speakers were knowledgeable and eager to answer questions. The presentations were full of useful information relevant to a tech in the lab but broad enough to encompass all labs and lab staff. I highly recommend an ASHI regional workshop as a resource for information, and believe it is a crucial learning tool for any new technologist joining the field. I am incredibly thankful for the ASHI travel award that made this experience possible.

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# The Illusion of Quality: Taking Good Quality Processes and Making Them Great

Frederick G. Strathmann, PhD, DABCC (CC, TC)

It is an exciting time in laboratory medicine as scientific advances are prevalent in nearly every area of the laboratory including direct to patient microtainer collections for routine screening,<sup>1</sup> bacterial identification using time-of-flight mass spectrometry,<sup>2</sup> and an increased emphasis on the measurement of proteins by mass spectrometry.<sup>3</sup> Automation has taken laboratory efficiencies to new heights, and auto-verification of generated results has reduced the need for technologists to manually review results from automated analyzers, and high complexity assays using mass spectrometry. While the number of advances and publications emerge in these areas at an impressive rate, the discussions surrounding quality control (QC), standardization and best practices are given far less attention. Several factors naturally contribute to this imbalance in publications including a failure to understand that additional quality assurance (QA) is needed or interest in the area of quality control by the field, an emphasis on novelty and not always demonstrable quality by journals and sometimes a general lack of appreciation of the impact that poorly optimized processes may have on laboratory quality. For example, a review of literature in the area of traumatic brain injury biomarkers revealed a lack of QC or even validation data in the majority of high profile publications attempting to integrate research or clinical findings into the mainstream.<sup>4</sup>

Although all laboratories that conduct clinical testing under CMS and accrediting organizations like the College of American Pathologists (CAP) and ASHI are required to have a QC policy in place, the details of the policy are left to the laboratory director. Numerous organizations, independent consultants, and software companies provide guidance, tools, and help with laboratory quality processes, but many laboratories continue to use antiquated approaches to QC. Patient medians are one of the newer entries to laboratory quality metrics,<sup>5</sup> although means are still widely used.<sup>6</sup> QC practices are the key to a successful laboratory operation, but often they do not get as much attention as the testing they are designed to maintain. Although there is an acute need for quality practices to evolve along with testing, it is surprising to find that many laboratory quality plans are not fully developed as needed. Many laboratories operate under an “illusion of quality” rather than on a “foundation of quality” supported by data and metrics. Given the widespread use of computers, software, and web-based tools, several outdated aspects of older QC processes are used routinely. As examples of outdated practices, the rest of this article will explore a few common practices that have outlasted their utility including trigger rules, poor troubleshooting habits, and inappropriate QC ranges set on anything but instrument performance. These outdated practices can cause an otherwise high-quality laboratory to have mounting frustration, inability to balance workloads, and a staff that feels fragmented and unsure of best practices (Figure 1).

Good quality practices are well known but may not be fully implemented when turnaround time, efficiency, and costs continue to exert their influence on laboratory decision making. One of the foundational resources in laboratory quality, *Basic QC Practices*<sup>7</sup> by Dr. James Westgard, is the template on which many of the presented ideas that follow are based. This and other resources cover aspects of statistical QC including an explanation of various rule combinations, good practices in QC commutability, and parallel processing of QC samples with patient samples. Instead of focusing on many aspects of laboratory quality, including the statistical QC rules that are available, it is more important to start with a focus on the one pervasive rule that stands in the way of a modern QC plan: the 2SD Trigger Rule.

## The 2SD Trigger Rule and Why it Needs to Go—Eventually

A laboratory may use the 2SD Rule rule as a “trigger” for investigation of subsequent rule violations. The threshold would typically be set at  $\pm 2$  standard deviations from the target and it is designed to reduce the time and effort that is required to investigate all applied statistical rules for every QC result. In the era before widespread software and computer availability it was necessary to reduce the time technologists spent in reviewing QC data. However, even with the time savings inherent with a trigger rule in place, the flaw in this plan is that an imprecision rule stands in the way of interrogation of rules designed to identify bias, not imprecision (Table 1). For example, the 4-1s rule identifies four QC points greater than one standard deviation away from the mean in the same direction and is a valuable indicator of bias. The 10x rule provides much needed constant correction that can keep small biases from becoming significant biases. However, with a 2SD trigger rule that requires violation prior to investigation of subsequent rules, it is possible that numerous 4-1s and 10x rule violations are missed on a routine basis. In test systems where calibration is performed on a frequent basis, these small calibration biases that are missed may lead to considerable inaccuracy and increased total error in the measurement system.

The removal of a “trigger” rule is a fairly straightforward change that can be made in any laboratory. Removal of this trigger rule will allow more rule violations to be found and a better approach to quality. However, it might be predicted that removal of a trigger rule might invariably lead to an exponential increase in the number of rule violations, batch failures and a laboratory in chaos. This concern is what may keep many laboratories from removing the trigger rule or cause some of the laboratories that do remove the trigger rule to promptly put it back in place. But just like with any change, preparation is the key to success.

## Standardized Troubleshooting Guides: The Key to Survival in a Post-Trigger Rule World

To overcome the concern associated with increased rule failure, the laboratory needs a standardized plan for the process change. One area where standardization is not typically well implemented is in how quality failures are investigated. The troubleshooting that is conducted is often only as good as the technologist performing it. Every laboratory has a mixture of seasoned and novice technologists, making consistency quite challenging to achieve in many areas. Years of experience can lead a technologist to notice that something isn't right with results. It is very important to take the best practices for troubleshooting and generate detailed algorithms associated with each failure type and train the staff. Often both good and bad examples of troubleshooting will be identified in standardizing processes. Putting forms in place that provide a clear start to finish path for each technologist, and each rule failure, will ensure both accurate patient results and that technologists can learn to identify and rectify the issues. Documentation of the problem can lead to identification of aberrant processes in the laboratory in many areas, including reagent preparation, storage or use. After troubleshooting has taken place, technologists may find a creative outlet in applying new problem solving ideas that can be reviewed by managers. In this manner, new troubleshooting ideas can be tested and eventually incorporated while standard strategies continue to be followed.

There are many benefits, when standardized troubleshooting processes with proper documentation are in place. First, the laboratory will more easily be able to retrospectively investigate issues that arise no matter how far in the past the concerns are raised. For example, in the event of a failed proficiency test challenge, a detailed list of steps taken the day of the failure can be used as part of the overall investigation. Second, careful tracking of quality issues can be taken to identify problem assays, calibrator issues, or precision problems that may track with new reagent lots, new staff or new/old instruments and maintenance. Third, should an issue arise where clinical judgment is required for patient result reporting, the knowledge that the technologist followed a standard set of steps can provide confidence for any decision.

## Tightening Ranges to Match the Data: The White Whale of QC

Once appropriate rule combinations and associated troubleshooting guides are in place, a time will come when tightening acceptance ranges is suitable. Similar to removal of a trigger rule, tightening ranges before a strategy for dealing with failures is in place, may lead to problems. Tighter ranges may result in a higher rate of failure, unless the laboratory knows how to deal with those issues. Tightening ranges can result in better overall control of the associated assays. How to tighten can be challenging, but in most situations the more data to set ranges, the more likely they will reflect reality in the laboratory. Unless QC targets change significantly, data across different lots can be used to help strengthen the established ranges. Too little data can

result in ranges that may be too tight or loose. Long timeframes allow for reagent lot changes, operator differences, and all unforeseen variability to be part of the performance expectations.

The data that range updates are based on can vary considerably depending upon how failures are handled. Laboratories can choose to reject QC values or even discard QC. Generally, rejected QC results are incorporated into summary statistics, while discarded QC results may be treated as if they never happened. Best practice is to keep the QC results incorporated, if patient results were reported (e.g., a 10x failure resulting in a warning only) but to discard and remove the QC results, if the batch or affected patient results are not reported (e.g., R4s or when one control result is to one side of the mean by 2SD and the next is 2SD on the opposite side of the mean). All QC results that were used to report patient results are incorporated into the summary statistics and will keep the targets and ranges true to in-control laboratory performance. If only the passing QC results were used, the targets and ranges would not reflect true laboratory performance and excessive failures may occur. If failed QC data associated with non-reported patient results are included, the targets and ranges may be incorrectly set, resulting in poor quality performance (Figure 2).

It is important to note that the process of updating ranges is never over. The initial adjustment will enable the data to produce higher quality results, but eventually the ranges will again appear too wide. With continuous investigation of subtle biases based on 10x and 4-1s failures, the targets should stay in place, any shifts or trends will indicate a developing problem. QC ranges, targets, and processes should be optimized so investigation of "trouble" is easily categorized as an analytical problem and never rises to the level of a clinical problem. The laboratory should be confident that the QC strategy will alert staff to a potential problem before true clinical concerns have emerged.

## Final Thoughts

It is important to maintain an emphasis on proper laboratory QC processes. As laboratory workflows become increasingly complex, the retrieval and visualization of quality data becomes a greater concern. Many laboratorians are actively engaged in QC review, either in real-time as the results are generated or during retrospective weekly and monthly reviews. It is easy to see that QC practices must continue to evolve to meet the demands of higher complexity testing, while not overlooking the added quality time and complexity technologists have to deal with. With increases in automation, less time is spent reviewing data, and there is increased scrutiny by outside agencies regarding laboratory quality and test utilization. It is important to consider how quality data are reviewed, how problems are found and how we investigate identified problems. Retrospective reviews of data will need to give rise to prospective identification of problems. How a "good" result is classified, will need to be based on more than a single value or a few parameters, it will need to incorporate a wide array of internal and external laboratory information. Much like how N-of-1 clinical trials aim to simplify the identification of true

treatment impact,<sup>8,9</sup> it is important that processes and available tools evolve to provide a foundation of quality to ensure accurate test results and patient safety.

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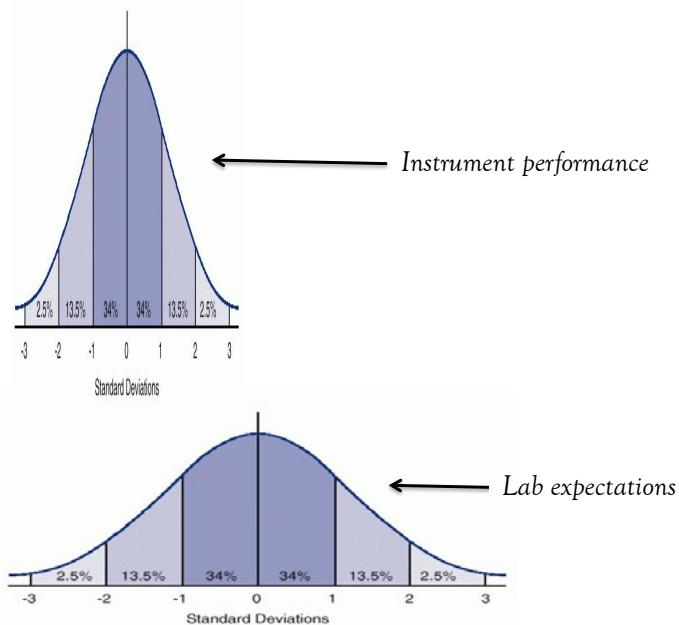
**Figure 1. When Quality Processes are Not Optimized the Laboratory Morale Can Suffer**

Poor performing assays  
 Assays not working well  
 too busy  
 Solving problems individually  
 Lack of staffing  
 procedural inflexibility  
 short on time  
 pulling long hours  
 short term solutions  
 Instruments not functioning properly  
 very rushed  
 limited amount of automation  
 Personal opinion  
 always very rushed

**Table 1. Descriptions of Commonly Used Multirules and the Type of Errors Detected**

Rule	Explanation	Error Detection
10x	10 consecutive results on one side of the mean	Systematic error
4-1s	4 consecutive results 1 standard deviation away from the mean to the same side of the mean	Systematic error
2-2s	2 results greater than 2 standard deviations away from the mean to the same side of the mean	Systematic error
1-3s	1 result greater than 3 standard deviations away from the mean	Random error
R-4s	2 consecutive results greater than 2 standard deviations away from the mean on opposite sides of the mean	Random error

**Figure 2. Importance of Appropriate QC Range Settings**



QC ranges set based on instrument performance are ideal for optimal control of assay performance. QC ranges set wider than observed performance will allow for imprecision and bias to go unnoticed and can negatively impact testing.

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# Genome-Wide Genetic Variation and Hematopoietic Cell Transplant Outcome

John A. Hansen, MD

Allogeneic hematopoietic cell transplantation (HCT) from a normal healthy HLA matched donor is a potentially lifesaving therapy for many patients with otherwise incurable inherited and acquired diseases, but non-major histocompatibility (MHC) genetic differences between donor and recipient are responsible for inducing strong T-cell mediated alloimmune responses that put the recipient at risk of graft-versus-host disease (GVHD), a complication that represents a major cause of morbidity and mortality following HCT.<sup>1</sup>

## Human Genetic Diversity and Population Structure

Genetic polymorphism occurs across the genome in the form of single nucleotide polymorphisms (SNPs) or structural polymorphisms including insertions and deletions (indels). Human genetic polymorphism is so extensive that virtually everyone with the exception of genotypically identical twins has a unique genetic constitution. Whole-genome sequencing over the last decade has been performed for more than 1000 subjects who globally represent the major human racial groups.<sup>2</sup> The 1000 genomes project has identified more than 10 million SNPs to date, accounting for one SNP on average every 300 nucleotides.<sup>3</sup> SNP frequencies and SNP-defined haplotypes vary substantially from one population to another. With the advent of the 1000 genomes database,<sup>4</sup> analytical methods and bioinformatics have been developed for detailed characterization of human diversity including methods for measuring genetic distance between populations and detecting population admixture, a phenomenon that can confound and mislead genetic association studies.

As an example of the power of genomics for characterizing genetic diversity we used a principal component analysis (PCA) to characterize genetic ancestry for a cohort of HCT recipients. The power of this approach for distinguishing individual subjects according to primary genetic origin is illustrated in Figure 1A. Using the first two principal components, the majority of subjects cluster with one of three nodes representing European Caucasian (left upper), African (right lower), and Asian (right upper). Each sample in the plot is color coded according to self-reported racial and ethnic origin including "unknown." The samples falling between the main nodes represent individuals of mixed racial origin. Using statistical criteria minimizing admixture, the PCA analysis can be used to identify genetically homogenous samples, for example as illustrated in Figure 1B the identification of a homogenous subset of European ancestry subjects for the purpose of an association analysis. By adjusting population structure in this way we are able to avoid the confounding effect that unknown population admixture can have on an association analysis, which may lead to spurious results.<sup>5</sup>

## Genome-Wide Functional Variation Affecting Phenotype and Risk of Disease

There is a large class of genome-wide genetic polymorphisms that have been shown to be functional variants that modulate phenotype and risk of disease by enhancing or suppressing gene function including genes involved in immune response and immunity. Generally, these genetic variants can operate at several levels by regulating the level of gene expression, translation, and transcription, inducing alternate splicing and encoding amino acid substitutions, all of which might alter gene and cell function. Genome-wide association studies (GWAS) have demonstrated that SNPs contribute to both health and disease sometimes as risk factors for immune mediated disease and autoimmunity. For example, more than 100 SNPs have been identified genome-wide that contribute to risk of inflammatory bowel disease (IBS). A comprehensive and searchable catalog of disease associated SNPs is maintained by the [National Human Genome Research Institute \(NHGRI\)](#) and the [European Bioinformatics Institute \(EMBL-EBI\)](#).

## Measuring Genome-Wide Matching Between Donor and Recipient

Genetic variation results in the mismatching of nucleotides between transplant donor and recipient. One approach to measuring donor-recipient matching is to define "identity by state" (IBS) for all SNP genotypes scoring each as 0, 1, or 2 according to the number of shared alleles between donor and recipient (Table 1). The reproducibility of the DNA array generated SNP genotypes was verified by an analysis of blinded replicate samples that showed a mean correlation of  $0.976 \pm 0.015$  when comparing results for 134 replicated samples (Table 2). The correlation of average IBS scores among sibling pairs ( $0.422 \pm 0.037$ ) was significantly greater than the average IBS scores between paired unrelated individuals ( $0.237 \pm 0.007$ ).<sup>6</sup> The frequency distribution of IBS scores for unrelated pairs, HLA identical sibling pairs, and related donor-recipient pairs mismatched for HLA are illustrated in Figure 2. The mean IBS scores between paired related and paired unrelated individuals are significantly different reflecting as expected for sibling pairs the shared inheritance of approximately 50% of the parental genomes, whereas paired unrelated individuals have a significantly lower IBS score reflecting the multiple generations and multiple recombination events that have occurred since the unrelated donor and recipient shared a common ancestor. The greater diversity between unrelated donor-recipient pairs is further reinforced by the global reach of the unrelated donor search process, which crosses geographic and ethnic boundaries to achieve HLA matching.

## Correlation of Genome-Wide Disparity and HCT Outcome

Genome-wide SNP-defined genetic disparity was coded according to the presence in the recipient of one or two alleles that are not present in the donor (the GVHD metric). The average GVHD disparity score across the genome is greater for unrelated donor-recipient pairs (0.346; CV, 0.022) compared to HLA matched related donor pairs (0.168; CV 0.346) (Table 3). When the GVHD disparity score is correlated with transplant outcome there is a significant association observed between the genome-wide SNP disparity score and risk of grade 3-4 severe acute GVHD (HR=1.27, p=0.002) and risk of gut GVHD (HR=1.31, p=0.008) in HLA matched sibling donor HCT. No significant association of the GVHD disparity score with GVHD was observed among HLA matched unrelated donor transplants presumably due to the minimal variation in the average GVHD disparity score among unrelated individuals (Table 3).

## Assessment and Impact of Genome-Wide Disparity in HCT

One measure of patient/donor disparity is the GVH vector, which measures alleles present in the patient but not the donor. For our preliminary analysis, we calculated the mean genome-wide GVHD disparity between a patient and donor using all post-QC autosomal SNPs in either cohort I or II. This disparity is greater for MURD compared to MRD pairs. However, the coefficient of variation (CV) is much greater for MRD compared to MURD pairs (Table 4) confirming previous observations.<sup>34</sup> We evaluated cGVHD, grade 3-4 aGVHD and aGVHD of the gastrointestinal tract (Gut) using a time-to-event analysis with the mean GVH disparity as the independent variable. We observed a significant signal for aGVHD3-4 and Gut for MRD cases (Table 4).

## Genetic Variation in Genes Encoding Histocompatibility Antigens

The impact of this genetic variation is very different depending on whether the polymorphism occurs within the major histocompatibility complex (MHC) or effects the function of non-MHC genes. Within the MHC, sequence differences among HLA genes can translate to amino acid substitutions in HLA antigens that are capable of inducing strong alloimmune responses leading to graft rejection or GVHD. However, a strong alloimmune response can also be generated by non-MHC genetic disparity as demonstrated by the occurrence of clinically significant acute and chronic GVHD in HCT recipients transplanted from an HLA identical sibling. Certain of these non-MHC genetic differences encode amino acid substitutions in cellular peptides that are processed within the cell and presented on the cell surface to T-Cells by MHC molecules that define a class of alloantigen known as minor histocompatibility antigens (mHA).<sup>7</sup>

The number of functional mHA is not known but informed estimates project as many as several hundred.<sup>6</sup> Individually, mHA have little apparent effect, but in aggregate the alloimmune response to mHA can be substantial. The strongest effect of mismatching for histocompatibility antigens, however occurs whenever there is mismatching within HLA genes, even when mismatching results in disparity for a single class I or class II HLA allele, a difference that is known to increase the overall incidence and severity of acute GVHD and the incidence and duration of chronic GVHD.<sup>8,9</sup>

## Structural Variants and Alloimmunity

A common gene deletion polymorphism can have a major impact on the alloimmune response. If the structural variant occurs in the donor as a homozygous deletion, the absence of a gene product in an otherwise normal individual would negate thymus-mediated negative selection and allow the individual to recognize the antigen normally encoded by deleted gene, but now absent, to be recognized as a non-self foreign antigen. The presence of the wild type in a HCT recipient could elicit a strong immune response. The relevance of structural variation to HCT was demonstrated by a study several common deletion mutations showing that the risk of acute GVHD was greater (OR=2.5; 95%CI, 1.4-4.6) when donor and recipient were mismatched for a homozygous deletion of *UGT2B17*, a gene that normally encodes multiple histocompatibility antigens including at least three different T-cell epitopes and at least one serologically defined epitope.<sup>10,11</sup>

## Non-MHC Genetic Variation and HCT Outcome

Preliminary data from several candidate gene studies have shown that genetic variation can affect the risk of GVHD, opportunistic infection, and mortality.<sup>12-19</sup> Results of these studies however have not been consistent most likely due to the limited statistical power that can be achieved with relatively small study cohorts of 100-500 transplants. Nevertheless, there are clear differences in patient outcomes that are not attributable to donor type or HLA matching. In the absence of other clinical or environmental risk factors that might be responsible for adverse outcomes, the evidence strongly suggests that genetic differences contribute to treatment success and failure. There remains a great need to resolve the apparent differences in the scientific record with adequately powered studies, and an unmet need to apply GWAS technology to test with unbiased genome-wide scope the hypothesis that genetic variation in recipient and donor genomes controls the function of genes, pathways, and cells in ways that affect HCT outcomes.

## Summary

The discovery of markers and functional variants associated with HCT outcome will have significant implications for future translational research aimed at improving risk assessment and directing mechanistic research. The HLA complex is an established model system for demonstrating how an effective genetic analysis and application to treatment planning can be translated into improved HCT outcomes. An understanding of HLA-matching data has contributed significantly to multivariate statistical models that risk-stratify patients and accurately predict transplant outcomes.<sup>20,21</sup> The discovery of previously unrecognized functional variants defining genes and pathways associated with GVHD, organ toxicity, relapse, and opportunistic infection will have significant impact on mechanistic translational research aimed at developing novel targeted therapies for controlling GVHD, facilitating tolerance, and improving supportive care for the HCT patient.

**Table 1. Classification of SNP Genotypes Illustrating the Concept of Donor/Recipient Genetic Identity by State (IBS)**

Identity by State (IBS)				
Donor genome	A/A	C/C	G/C	A/A
Recipient genome	T/T	C/G	G/C	A/A
IBS score	0	1	2	2

SNP genotypes generated genome-wide by DNA sequencing or high-density DNA arrays.

**Table 2. Mean Genome-Wide SNP-Defined IBS Scores for Paired Related and Unrelated Samples\***

Relationship between paired samples (n)	Mean IBS score (SD)*
Replicate samples (134)	0.976 (0.015)
Sibling samples (322)	0.422 (0.037)
Unrelated samples (726)	0.237 (0.007)

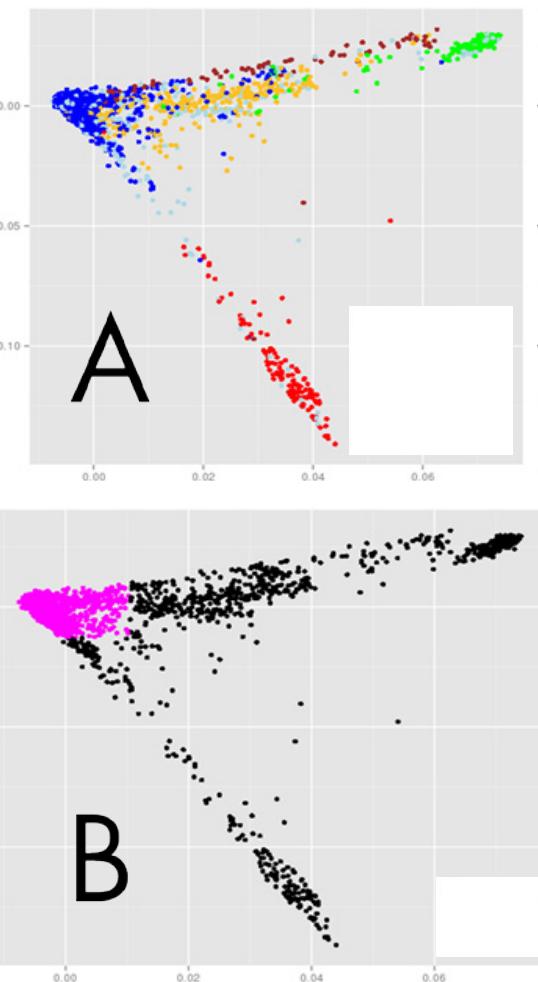
\* Computed for 440,792 informative SNP genotypes.

**Table 3. Genome-Wide Analysis of Patient/Donor Disparity and Transplant Outcome (p-value/hazard ratio)**

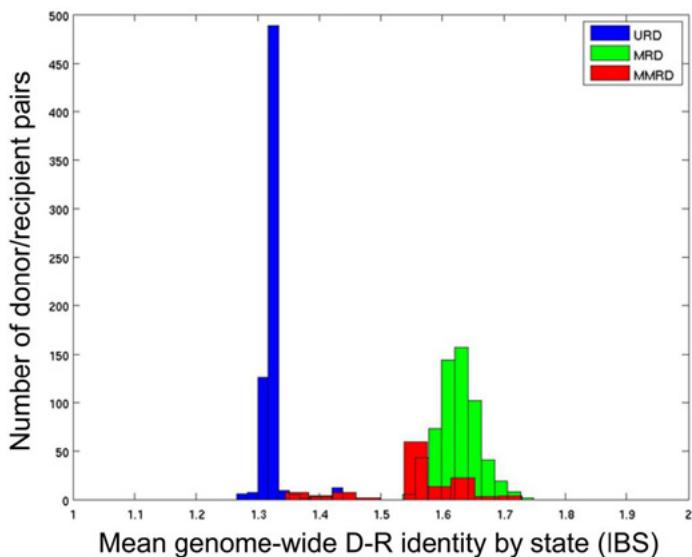
Genome-wide disparity <sup>1</sup>	Donor Type (HLA Matched)	
	Related Sibling	Unrelated
Mean (coefficient of variation)	0.1677 (0.085)	0.3462 (0.022)
Transplant outcome	p-value/hazard ratio	p-value/hazard ratio
Acute GVHD, grade 3-4	0.002/1.27	0.29/1.03
Gut GVHD	0.008/1.31	0.60/1.02

<sup>1</sup> Genome-wide SNP-defined disparity for the GVHD metric. Unrelated donors were allele matched with recipients for HLA-A, B, C, DRB1, and DQB1. <sup>2</sup> Hazard ratio (HR) values represent the interquartile range (IQR) of disparity within each patient group.

**Figure 1. Principal Component Analysis of Genetic Diversity and Ancestry**



(A) Self-identified genetic ancestry; and  
(B) genetically defined European ancestry

**Figure 2. Frequency Distribution of Average Genome-Wide SNP Variation Classified as Identity by State (IBS)**

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# C1q Binding Donor-Specific Anti-HLA Antibodies Occurring Late After Kidney Transplantation

Christina Savchik, Rosanne Scandaliato, Peter Masiakos, Adriana Colovai, PhD

## Abstract

In this study we analyzed the prevalence of complement binding donor-specific antibodies (DSA) occurring late after kidney transplantation (median time 7 years). The patient population included 405 kidney transplant recipients who received transplantation at our center from 1980 to 2009 and had functioning allografts. All patients had a negative CDC crossmatch at the time of transplantation. Patients' sera were tested using the IgG and C1q Single Antigen Bead (SAB) assay. SAB results and historical donor HLA typing data were used to assign donor specific antibodies (DSA). Out of 405 patients, 77 (19%) displayed DSA and, among these, 33 (8%) had C1q+ and 44 (11%) had C1q- DSA. C1q+ DSA were associated with younger patient age, deceased donor transplantation, and higher cPRA values. More than half of the C1q+ DSA were directed to the donor mismatched HLA-DQ antigens. MFI values obtained with the IgG SAB assay predicted the C1q binding capacity of DSA ( $p=0.03$  and  $p=0.02$  for class I and class II DSA, respectively). There was a strong association between C1q+ DSA and incidence of chronic AMR/transplant glomerulopathy, which occurred in 51% of patients with C1q+ DSA, 25% of patients with C1q- DSA and only 6% of patients without DSA ( $p<0.001$ ). These results indicate that long-term monitoring of DSA after kidney transplantation is warranted in order to identify patients at risk for antibody mediated allograft injury.

## Introduction

Donor specific anti-HLA antibodies (DSA) increase the risk of antibody mediated rejection (AMR) and graft loss in kidney allograft recipients.<sup>1-4</sup> Introduction of highly sensitive and specific techniques for detection of anti-HLA antibodies, such as the Single Antigen Bead (SAB) assay, increased our ability to identify sensitized patients, define their immunologic risk before transplantation and select the most suitable donor for each transplant recipient.<sup>5-7</sup> Several studies have reported an association between DSA levels measured as mean fluorescence intensity (MFI), flow-cytometry (FC) cross-match channel shift values, and development of AMR.<sup>8-11</sup> However, the conventional SAB assay is limited in its ability to distinguish complement fixing from non-complement fixing anti-HLA antibodies. The development of the C1q assay, which measures the capacity of anti-HLA antibodies to bind the complement subunit C1q, aimed to fill that gap.<sup>12</sup> C1q fixation is the first step in the activation of the classical complement cascade.<sup>13</sup> Thus, the functional feature added into the C1q assay may help to better predict the clinical significance of DSA detected post-transplantation.

Several studies have assessed the incidence of C1q binding anti-HLA antibodies after renal transplantation. Tyan and colleagues reported higher rates of allograft loss, and acute and chronic AMR in renal transplant recipients with C1q-binding DSA.<sup>12,14</sup> Loupy et al reported that, in a large cohort of 1016 patients, those who developed C1q-binding DSA within a year after transplantation had lower 5-year allograft survival and higher AMR rates compared to patients with non-C1q-binding DSA.<sup>15</sup> However, few studies have analyzed the prevalence of C1q-binding DSA occurring late after transplantation.<sup>16,17</sup> Here we report the prevalence of C1q+ DSA in a population of 405 kidney transplant recipients at 7 years median time post-transplantation. We also report on the relationship between C1q+ DSA and incidence of antibody mediated rejection.

## Materials and Methods

### Study Population

Our study population included 405 patients with functioning allografts transplanted at our center from Jan. 1, 1980 to May 1, 2009. This group represents 18% of 2297 patients who received renal transplantation during this interval. Patients who lost their grafts, expired, or were lost to follow up were excluded due to lack of serum samples. The current study was approved by Montefiore Medical Center's Institutional Review Board.

Prior to transplantation, anti-HLA antibodies were tested using complement dependent cytotoxicity (CDC). DSA were identified using solid-phase assays only post-transplantation. In this cross-sectional study, DSA prevalence was determined using the conventional SAB and the C1q assays in sera collected in 2012-2013 (3-30 years post-transplantation).

### Detection of Serum Anti-HLA Antibodies

Serum anti-HLA antibodies were identified using commercially available Luminex SAB (LABScreen, One Lambda Inc, Canoga Park, CA), according to the manufacturer's instructions. Phycoerythrin (PE) - conjugated anti-human IgG was used as the detection antibody. Sera found to contain antibodies directed to any of the donor HLA-A, B, C, DR and DQ antigens were further analyzed using LabScreen beads and PE-conjugated anti-human C1q antibody (One Lambda Inc, Canoga Park, CA). The same SAB batch was used to detect pan-IgG and C1q binding anti-HLA antibodies in any given serum. Although the C1q assay can detect IgG as well as non-IgG antibodies, C1q+ DSA identified in this study were primarily IgG because the sera tested with the C1q assay were selected based on the presence of IgG-DSA. Anti-

HLA antibody profiles were analyzed using the Fusion software (One Lambda Inc.). Cutoffs were set at 1000 MFI and 500 MFI for PE-IgG and PE-C1q assays, respectively. cPRA (calculated panel reactive antibody) was determined using the UNET cPRA calculator. For each patient, we recorded the number, specificities, and MFI values of the DSA. DSA level for HLA antigens represented by more than one bead in the single antigen panel was defined as the highest MFI value observed within that bead group.

### ***HLA Typing and Cross-Matching***

HLA typing was performed by serologic and DNA (sequence specific primers) methods. Typing of HLA-A, B, DR and DQ was available for all donor-patient pairs. HLA-C typing was available in 70% of donors. CDC was the primary cross-match method. All patients had a negative T and B cell CDC cross-match prior to transplantation.

### ***Allograft Biopsies***

Allograft biopsies were performed when clinically indicated by worsening kidney function and/or proteinuria. Biopsies were scored using the Banff criteria.<sup>18</sup> Acute and chronic AMR were diagnosed per Banff 2009 classification.<sup>19</sup> Transplant glomerulopathy (TGP) patients displaying DSA were diagnosed as chronic AMR regardless of C4d staining results. In the absence of DSA, patients with TGP features yet negative C4d staining were diagnosed as TGP alone.

### ***Statistical Analysis***

Patient characteristics in DSA-, C1q- DSA+, and C1q+ DSA+ patient groups were compared using either anova or chi square tests as appropriate. Class I and class II DSA MFI values between the C1q- DSA and C1q+ DSA groups were compared using the Student's t-test. Results are presented as mean (standard deviation), median interquartile range (IQR), or number (percentage) based on the distribution of the data. Data analysis was performed using Stata 11.2 (College Station, TX).

## **Results**

### ***Prevalence, Type, and Levels of C1q-Binding DSA After Renal Transplantation***

In this cross-sectional study, anti-HLA antibodies were tested in 405 patients with long-term functioning kidney allografts at 3-30 years after transplantation (median time 7 years). Out of 405 patients, 77 (19%) displayed DSA and, of these, 33 (8%) had C1q+ DSA and 44 (11%) had C1q-DSA (Figure 1A). The distribution of C1q+ and C1q- DSA in patients who have had functioning grafts for 3-10 years (N=240), 11-20 years (N=131), and >20 years (N=34) is illustrated in Figure 1B. Notably, the highest incidence of C1q+ DSA was seen in the 3-10 years group (11%), while in the 11-20 years and >20 years groups the incidence of C1q+ DSA was only 4% and 6%, respectively. These results suggest that many patients with C1q+ DSA may have lost their grafts by the tenth year post-transplantation and exited the group of patients with functioning grafts.

A total of 101 DSA were identified in 77 patients, with a mean of 1.3 DSA per patient. Out of 101 DSA, 41 had C1q binding capacity. As shown in Figure 2, DSA directed to HLA Class II antigens occurred more frequently than Class I DSA. Notably, C1q+ DSA specific for donor DQ antigens accounted for 58% of all C1q+ DSA found in our patient population. Since donor HLA-C type was not available in all donors, it is possible that the number of DSA directed to HLA-C antigens was underestimated.

To determine whether the MFI values observed with the IgG SAB assay predicted the antibody capacity to bind C1q, we compared the MFI values of C1q+ and C1q- DSA. As shown in Figure 3, the MFI values of C1q+ DSA were significantly higher than those of C1q- DSA (Class I DSA  $7034 \pm 6292$  vs.  $4060 \pm 3150$ , p=0.03; Class II DSA  $9188 \pm 4316$  vs.  $5551 \pm 4332$ , p=0.02).

### ***Clinical Parameters***

The patients' characteristics are presented in Table 1. Based on the DSA status post-transplantation, our patient population was divided into 3 groups: DSA negative (N=328), C1q+ DSA (N=33), and C1q- DSA (N=44). There were no differences in terms of gender, race, and history of previous transplantation between the patients with and without DSA. However, younger recipient age, deceased donor transplantation, and higher cPRA were associated with the presence of C1q+ DSA.

As shown in Table 1, serum creatinine levels were higher in patients with post-transplant C1q+ or C1q- DSA compared to patients without DSA, although the difference did not reach statistical significance (p=0.07). There was no significant difference between the groups in terms of spot urine protein/creatinine ratio. There was a strong association between the incidence of acute or chronic AMR/TGP and the presence of C1q+ DSA (Figure 4). Acute AMR was observed in 15% of patients with C1q+ DSA, 2% of patients with C1q- DSA and 3% of patients with no DSA (p=0.001). Remarkably, the incidence of combined chronic AMR/TGP reached 51% in patients with C1q+ DSA. In contrast, chronic AMR/TGP occurred in only 6% of patients without DSA and 25% of patients with C1q- DSA (p<0.001).

## **Discussion**

In this study, we analyzed the prevalence and clinical significance of C1q+ DSA in a large group of kidney transplant recipients with functioning allografts up to 30 years after transplantation. We found that, at a median of seven years post transplantation, 8% of the transplant recipients had circulating C1q+ DSA. The fraction of patients displaying any DSA, regardless of their ability to bind complement, reached 19%. These results are consistent with the notion that production of DSA remains an active process even many years after transplantation.

About half of the patients with C1q+ DSA had chronic AMR and/or TGP, indicating that C1q+ DSA is a valuable marker for identifying patients at high risk for late antibody-mediated allograft injury. The incidence of chronic AMR and/or TGP in the group with non-complement fixing DSA was lower yet significant (25%), suggesting that C1q- DSA may also have deleterious effects. It should be noted that the capacity of antibodies to bind complement may change over time. Since DSA testing in our study was cross-sectional, it is possible that a DSA with no C1q binding activity at one point in time exhibited such activity at a different time point. Consistent monitoring of DSA after transplantation is needed to better understand the relationship between DSA and chronic rejection.

Loupy et al<sup>15</sup> have reported that circulating C1q+ DSA detected during the first year post-transplantation were strongly associated with graft injury and loss. Our results extend these findings and demonstrate the clinical relevance of C1q+ DSA beyond the first year post-transplantation. It is worth mentioning that the prevalence of C1q+ DSA in our group with functioning grafts 3-10 years post-transplantation, was 3-fold higher (12% vs 4%) than that reported by Loupy et al<sup>15</sup> within the first year post-transplantation.

The use of MFI values obtained with the conventional SAB assay as a surrogate for the complement binding capacity of HLA antibodies is debated.<sup>20,21</sup> Previous studies have shown that MFI values determined with the conventional SAB assay may not correlate with antibody titers and ability to bind C1q.<sup>22</sup> It has been proposed that determination of HLA antibody titers and characterization of IgG isotypes may better predict the cytotoxic activity of DSA. In our study, higher MFI values predicted C1q binding. However, this association is not very strong, suggesting that MFI values should be used with caution. Additional testing, including the C1q assay and/or testing of serum dilutions, is needed to better define DSA strength.

Our study has several limitations. Due to lack of historical serum samples, our population did not include patients who lost their allografts. It is, therefore, likely that the prevalence of C1q+ DSA was underestimated. No data on preformed DSA was available because many patients from our group were transplanted before the implementation of the SAB assay. Finally, our study was cross-sectional and, therefore, did not provide kinetics of DSA development. Despite these limitations, our results strongly support the clinical value of long term monitoring of serum DSA after renal transplantation. In contrast with protocol biopsies, DSA testing is a non-invasive test. Detection of DSA identifies patients at high risk for graft loss despite C4d negativity in allograft biopsies.<sup>24,25</sup> C4d, although specific, lacks sensitivity as an indicator of antibody-mediated injury, particularly of late AMR.<sup>1,23</sup> Furthermore, gene expression studies showed that patients with circulating DSA had intra-graft gene signatures associated with increased immune activity even in the absence of histological features of AMR.<sup>26,27</sup> In conclusion, our results indicate that complement binding DSA are present in the sera of renal transplant recipients many years after transplantation. Thus, long-term monitoring of DSA, in particular C1q+ DSA, is warranted in order to identify patients at risk for antibody mediated allograft injury. The Luminex C1q assay could be a complementary test to the Luminex IgG assay for further

immunological risk assessment of transplant recipients. Patients with C1q+ DSA might require allograft biopsy even in the absence of overt graft dysfunction. This may facilitate early detection of chronic AMR and/or TGP and identification of patients who may benefit from close monitoring or immune intervention.

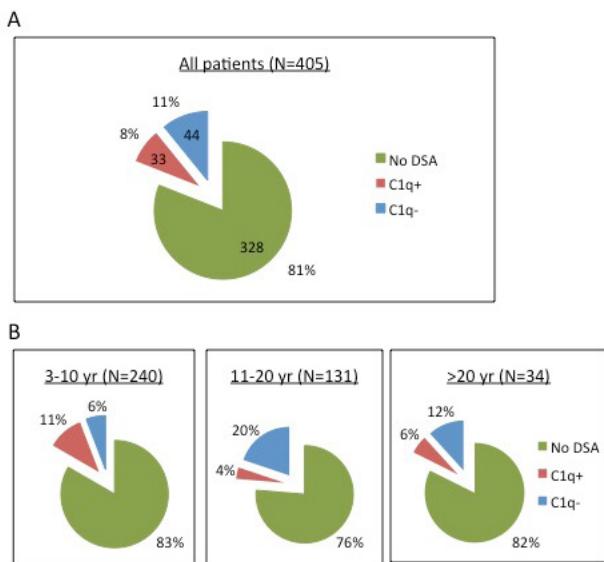
**Table 1. Patient Characteristics**

Variable	No DSA (N=328)	C1q+DSA+ (N=33)	C1q-DSA+ (N=44)	p-value
Mean age at transplant	47±12	39±10	42±12	<0.001
Gender, % female	39	49	48	0.32
Race, % African-American	24	30	18	0.45
Transplant type, % living donor	45	36	61	0.02
Previous transplant, %	9	18	7	0.17
Median time post-transplantation, years (IQR)	7(5-12)	7(5-10)	11(6-15)	0.002
Mean class I cPRA	10±21	24±30	36±35	<0.001
Mean class II cPRA	11±24	58±35	45±29	<0.001
Mean serum creatinine, mg/d	1.6±0.8	1.9±0.9	1.7±1.0	0.07
Spot urine protein/creatinine, g/dl (median (IQR))	0.10 (0.04-0.5)	0.2 (0.08-0.7)	0.2 (0.1-0.675)	0.74

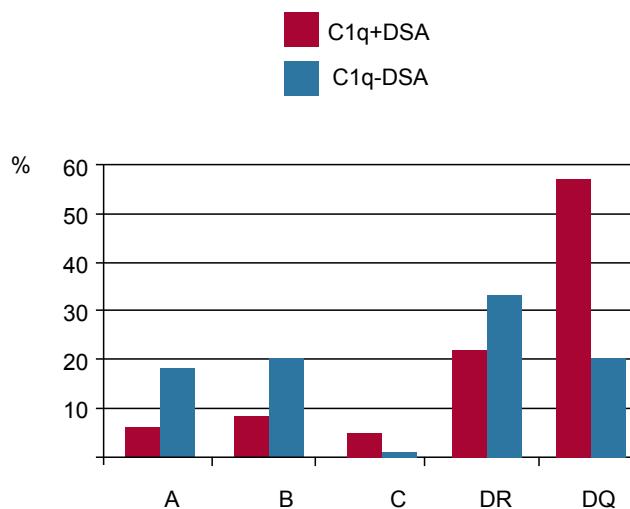
(N=405). Variables shown as mean ±SD or N (%) or as Median (interquartile range). p-values represent comparisons between the 3 groups.

Abbreviations: DSA=Donor Specific Antibody, cPRA=calculated panel reactive antibody, SD=standard deviation, IQR=interquartile range.

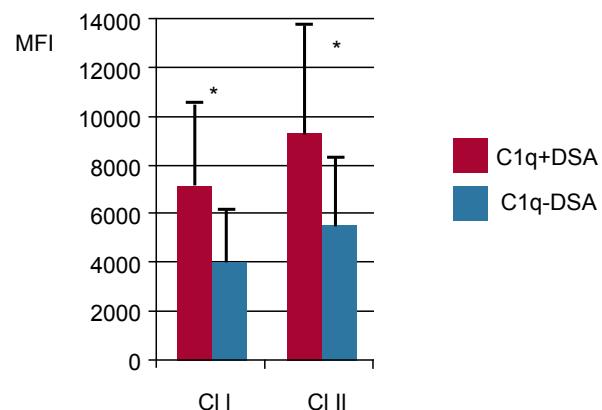
**Figure 1. Prevalence of C1q+DSA and C1q-DSA in a Population of 405 Kidney Transplant Recipients Tested at 7 Years Median Time After Transplantation**



**Figure 2. Specificity of C1q+ DSA and C1q- DSA identified in the study population. The percentages of anti-donor HLA-A, -B, -C, -DR, and -DQ antibodies among C1q+ DSA (N=41) and C1q- DSA (N=60) are indicated.**

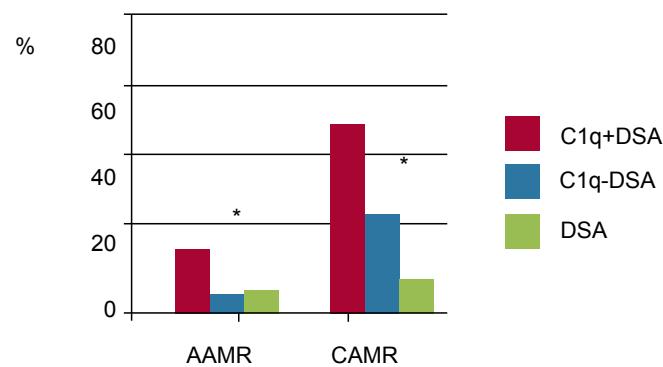


**Figure 3. Mean fluorescence Intensity (MFI) Values and C1q Binding Capacity of the DSA Detected in our Patient Population**



MFI values were determined using the conventional (IgG) SAB assay. p values were calculated using Student's t-test: Class I DSA, p=0.03; Class II DSA, p=0.02.

**Figure 4. Incidence of Acute Antibody-Mediated Rejection (AAMR) and Chronic Antibody-Mediated Rejection (CAMR)/Transplant Glomerulopathy (TGP) in Kidney Transplant Recipients with C1q+ DSA (N=33), C1q- DSA (N=44) or Without DSA (N=328)**



Group comparison p-values: AAMR, p=0.001; CAMR/TGP, p<0.001.

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# 42<sup>ND</sup> ANNUAL MEETING

September 26 - 30, 2016  
St. Louis, MO  
Hyatt Regency St. Louis at the Arch



# ASHI Quarterly Continuing Education Quiz

Quiz Instructions: The multiple choice quiz below is provided as an alternative method of earning CE credit hours. Read each article then select the ONE best answer to each question. The questions are based solely on the content of the article.

Answer all of the questions, and send the quiz answer sheet and fee (if applicable) to the ASHI Executive Office before the processing date listed on the answer sheet. To be eligible for 3.0 credit/contact hours or 0.45 Continuing Education Credits (CECs), ALL questions must be answered, a passing score of 70% must be obtained and the answer sheet must be submitted with fee (if applicable) before the deadline. Quizzes may not be retaken, nor can ASHI staff respond to questions. Allow six weeks for paper processing following the submission deadline to receive return notification of your completion of the CE process. The CE credit will be dated when it is submitted for grading. That date will determine the CE cycle year.

**Quiz may be taken online at [www.ashi-u.com](http://www.ashi-u.com).**

## The Illusion of Quality: Taking Good Quality Processes and Making Them Great

1. Which of the following is a characteristic of an effective QC process?
  - A.QCs are prepared in a similar fashion to patient samples
  - B.Acceptable QC results are kept while all failed QC results are removed
  - C.QCs are prepped ahead of time to ensure they are ready when patient samples will be prepped
  - D.If any QC results are out they are repeated several times before any troubleshooting is conducted
2. Which of the following is a potential metric for tracking quality issues in the analytical phase of testing?
  - A.The number of mislabels identified
  - B.The number of QC failures
  - C.The number of employees that are absent for their shift
  - D.The number of transcription errors for each technologist
3. Which of the following is an acceptable key feature of good QC?
  - A.QC performed when it is most convenient for the laboratory
  - B.QC performed daily but no investigation if a failure occurs
  - C.Using as much QC as possible regardless of the quality of the assay
  - D.QC prepped at the same time as patients

4. Which of the following items should be in place before removing a QC trigger rule?
  - A.Automated QC software
  - B.Trouble shooting guides for each rule failure that will be encountered
  - C.Two lead technologists
  - D.Nothing, just go for it.
5. Which of the following statements is true regarding an effective laboratory QC strategy?
  - A.The more QC failures the worse the laboratory
  - B.The more QC rules in place the higher the laboratory quality
  - C.Quality is overrated and shouldn't be discussed too frequently
  - D.Every QC failure requires investigation and troubleshooting

## Genome-Wide Genetic Variation and Hematopoietic Cell Transplant Outcome

6. Which statements about minor histocompatibility antigens (mHA) are correct?
  - A.The proteins encoded by HLA-A, B or DRB1 alleles can be classified as major or minor histocompatibility antigens depending on their effect on HCT outcome?
  - B.The total number of mHA in the genome is well defined.
  - C.mHA contribute to GVHD in both HLA identical sibling and HLA matched unrelated donor HCT.
  - D.Common variants that result in the deletions of genes can cause GVHD only when present in the recipient.

7. Which statements about histocompatibility antigens are correct?
- A. Matching for HLA and mHA are equally important in the selection of an unrelated donor for HCT.
  - B. Recipients receiving an HCT from a matched related donor do not experience GVHD.
  - C. mHA contribute to GVHD, but there is no evidence to support matching for mHA when selecting an unrelated donor.
8. Both HLA genotypes and non-MHC SNPs are highly polymorphic. Which of the statements are correct?
- A. More than 100 million SNPs have been identified by the 1000 Genomes project.
  - B. A selected panel of genome-wide SNPs provides the most informative data for defining ancestry and racial admixture.
  - C. The best way to control for racial admixture in genetic association studies is to document self-identified genetic ancestry information.
  - D. HLA polymorphisms provide the most informative genetic variation data for defining ancestry and racial admixture.
9. Which of the following statements about mHA are correct?
- A. The risk of acute GVHD in HLA identical sibling HCTs correlates with the SNP-defined donor/recipient disparity score.
  - B. A SNP can generate a mHA only if it causes an amino acid substitution in an expressed protein, and if that variant protein can be recognized by a T-cell.
  - C. There is a greater number of mHA's in related compared to unrelated donor HCT.
  - D. A and B are correct.
10. Genetic variation and immune response. Which statements are correct?
- A. Association studies should document, and control or adjust for the presence of racial admixture.
  - B. HLA genes and non-MHC SNPs have both been to be associated with risk of several immune-mediated diseases such as inflammatory bowel disease, type 1 diabetes, multiple sclerosis and others.
  - C. Certain SNPs are referred to as functional variants because they affect gene expression and cell function, and are associated with alterations in phenotype and disease risk.
  - D. All of the above are correct.

## C1q Binding Donor-Specific Anti-HLA Antibodies Occurring Late After Kidney Transplantation

1. C1q binding DSA are detected early but not late (more than three years) after kidney transplantation.
  - A. True
  - B. False
2. There is a strong association between C1q binding DSA and chronic antibody mediated rejection.
  - A. True
  - B. False
3. The C1q assay is used for detection of:
  - A. Non-HLA antibodies
  - B. Complement binding anti-HLA antibodies
  - C. Non-complement binding antibodies
  - D. Anti-HLA IgG4 antibodies
4. The MFI values of anti-HLA antibodies detected with the conventional Single Antigen Bead (SAB) assay may predict the antibody capacity to bind C1q:
  - A. For anti-HLA class I antibodies only
  - B. For anti-HLA class II antibodies only
  - C. For anti-HLA class I and class II antibodies
  - D. Not for anti-HLA antibodies
5. In this patient population, C1q binding DSA were associated with:
  - A. Younger recipient age
  - B. Deceased donor transplantation
  - C. Higher cPRA
  - D. All of the above
  - E. None of the above



# GENDX JOIN THE EXPERTS

ASHI 2016

## User Group Meeting

*HLA Typing and Beyond – NGS & Chimerism*

Thursday Sept 29

12.30 pm – 2.30 pm

## Teaching Sessions

*Next Generation Sequencing*

Monday Sept 26

8 am – 4 pm

*Chimerism Monitoring*

Monday Sept 26

2 pm – 4 pm

## Reagent & Software Solutions

### Next Generation Sequencing

- NG Sgo®
- NG Sengine®

### Sanger Based HLA Typing

- SBT eXcellerator®
- AlleleSEQ R®
- SBT engine®

### Chimerism Monitoring by qPCR

- KMRtype® & KMRtrack®
- AlleleSEQ R®
- KMREngine®

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Budgeting Oversight  
Staff Development  
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Board & Committee Management

Membership  
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Payment Processing

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Facilitates laboratory inspections, manages the inspector pool and volunteer list, coordinates inspector training  
Staff Liaison -Accreditation Review Board (ARB)  
Staff Liaison Director Training Review and Credentialing (DTRC)

Process travel reimbursements and invoices  
Assist with any ASHI University related issues or questions  
Process ASHI Quarterly quizzes  
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Budget/projections  
Cash flow projections



**Tobie Banscher**

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Database Maintenance

# ARB Update

Myra Coppage, PhD, HCLD

## ARB business

The major news on the ARB front is completion of the re-accreditation process with CMS. The notice of deemed status appeared in the Federal Register publication on April 22. We received the full six-year term on this round!

There will be inspector training available at the annual ASHI meeting this year. It will be a half-day session designed for ACTIVE inspectors and trainees, and is required once every two years. However, for those unable to attend the meeting, there is a new option available. ASHI U now has a series of inspector training modules available under volume 5. They are free to ASHI members. Inspectors who are unable to attend the face-to-face workshops at the annual meetings must complete the Audit Module & ARB Update module every two years as well as three additional modules of their choosing during a two-year period. Inspectors are encouraged to select training modules for which they have limited experience. There are nine modules in the current library, with two more on NGS and chimerism testing on the way.

## Resolution of DPB/DQA Typing

Developments in DP typing and changes to organ allocation policies have resulted in confusing scenarios in the areas of regulatory compliance, result reporting, and proficiency testing for this locus. Historically, HLA type could be neatly divided into areas of low resolution and high resolution with one-field (two digits) results for low and two-field (four+ digits) digit results for CWD high resolution types. DP typing by SSO and RT now yield two-field results and WHO nomenclature has established DP results to two fields with no differentiation for a one-field (except in the cases of DPB1\*02 and \*04) response. What is a solid organ lab with no other apparent need for high resolution accreditation to do?

Labs using SSO and/or RT to define DP, must report those results at two fields (i.e. DPB1\*04:01) when a single antigen or two clearly defined antigens with no ambiguities are determined by testing. If ambiguities are determined, the appropriate G/P group should be identified and any ambiguities must be reported (D.6.2.2.11 and D.6.2.2.12).

For laboratories in this situation, proficiency testing must be reported as a two-field response with the appropriate G/P group, if indicated. Ambiguity strings should be listed in PT comments.

The ARB will apply the same guidelines for labs that may resolve DQA results to two fields. Proficiency testing results can be posted under low or high resolution in Learning Builder (for ASHI accredited labs). In summary, if a lab is determining DP/DQA type by SSO and/or RT and not doing additional testing by sequencing or additional SSP/SSO testing to clearly define discrete alleles, and proficiency testing results at two fields are acceptable, these specific HLA loci will not require additional accreditation under high resolution HLA typing.

Note: UNET does not allow G/P group reporting. Labs are encouraged to attach a document with more detailed HLA typing information (for DP and any other antigens) whenever possible in DonorNet/Attachments.

## HLA Typing Using Multiple Independent Methods

A recent article in CAP Today clarifies the CMS position on PT for tests performed by different methods. It was brought to our attention that this could impact labs that use multiple primary test systems. For example, a solid organ lab might use RT-PCR as a stand-alone test for typing deceased donors, but use SSO/SSP typing for recipients. Another example is labs that use phenotype and single antigen antibody identification alternately. Proficiency testing for these two methods must be performed and reported independently. One solution is for labs to subscribe to separate proficiency programs for each stand-alone method. ARB recognizes that this approach adds cost and complexity to lab operations. Because HLA testing is not among the CMS regulated analytes, CLIA compliance mandates that the methods be tested at least twice per year. Our current recommendation to labs is to divide up the challenges between independent methods. For example, labs that use ASHI PT might use three samples for RT-PCR and two samples for SSO in the first sendout, and reverse that in the second so that each method would have five total challenges over the year.

### Laboratory Cycle Updates

**CYCLE 1:** The interim and onsite applications for laboratories in cycle 1 were due March 1, 2016. The onsite laboratories in this group will be inspected between April 15 and June 15, 2016. Laboratories can expect to receive their re-accreditation letters and certificates in August 2016 pending successful review by the ARB, at the ARB meeting in Salt Lake City.

This is our largest group of labs, including 52 interim laboratories and 30 onsite laboratories.

**CYCLE 2:** Application links for the laboratories in cycle 2 were sent out Friday, April 29. Applications were due July 1, 2016. Onsite laboratories in this cycle will be inspected between Aug. 15 and Oct. 15, 2016.

**CYCLE 3:** Laboratories in cycle 3 were reviewed at the April 2015 ARB Meeting in Phoenix, Arizona. Onsite laboratories in this cycle were inspected between Dec. 15, 2014 and February 15, 2015. Laboratories in this cycle received their new accreditation letters and/or certificates at the end of April 2015.

# ABHI Update

William W. Ward, *PhD, D(ABHI)*, Acting ABHI President

Greetings from the ABHI Board of Directors to all ABHI certificants, friends and colleagues! I'd like to start out by congratulating our newest certificants who successfully completed the CHT and CHS examinations this past March. The next examination will be held in September for both CHA/CHT/CHA and diplomate candidates.

The Board is always looking for ways to make improvements and welcomes suggestions to increase the ease and usefulness of the certification process. As I mentioned in the last update, we will be working with the management company to develop a Web-based application to allow certificants to readily document required continuing education activities and to facilitate the certification renewal process. In addition, we will be increasing the number of opportunities to take the CHA/CHT/CHS examinations from the current two times per year to four times per year, starting sometime in 2017. The current six-month interval between examinations may be a long time to wait for eager staff ready to

demonstrate their level of professional achievement, or for those who may need to retake an examination. We expect to present more information about both of these initiatives at the meeting in St. Louis, Missouri.

Last, I would like to thank the dedicated ABHI Board members, committee chairs, committee members, and our association manager for their tireless behind-the-scenes efforts to maintain ABHI as the preeminent certifying board for the field of histocompatibility and immunogenetics. If you are interested in joining us as a committee member or member of the Board, please contact any one of us via the contact information at [www.ashi-hla.org/ABHI](http://www.ashi-hla.org/ABHI).

Have a great summer!

## Newly Certified Histocompatibility Specialists

Amal N. Al-Gharably Riyadh
Meagan L. Barner Portland, OR
Heather A. Casey Harrisburg, PA
Samantha N. Cooper Oklahoma City, OK
Jamie L. Della Gatta Boston, MA
Tracy L. Fisher Kenosha, WI
Anne M. Halpin Edmonton, AB
Megan E. Jeracki Mountain View, CA
Melissa E. Jerezano Baltimore, MD
Scott Kang Plano, TX
Kara B. Wells Galveston, TX

## Newly Certified Histocompatibility Technologists

Anthony Agyapong Baltimore, MD	Hassan Abdullah Alharbi Ad Dammam
Kelly L. Anderson Newberry, FL	Nisar Baig Rochester, MN
Tiffany R. Baker Jacksonville Beach, FL	John E. Chappelle Houston, TX
Stephanie L. Conklin San Diego, CA	Stephanie Frick Breinigsville, PA
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Emily S. Kibbler Allston, MA	Nichole F. Kim Fullerton, CA
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Starsha V. McGrath Tempe, AZ	Augusto N. Mendiola Portland, OR
Monica G. Monteilh Chandler, AZ	Rae Anna M. Neville Portland, OR
Stefane L. Pufnak Shamokin, PA	Christina Savchik Riverdale, NY
Brian R. Schwartz Archer, FL	Juan J. Segovia San Antonio, TX
Susmita Singh Indianapolis, IN	Deborah Smith Marne, MI
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