Tuesday, October 2, 2018
2:30 PM - 4:00 PM
Scholar Award Abstract Session

OR1

INVISIBLE ORGANS MADE BY GENETIC ENGINEERING TO TURN OFF MHC PRIOR TO ALLOGENEIC TRANSPLANTATION PREVENT A PRO-INFLAMMATORY CYTOKINE RESPONSE IN THE RECIPIENT

Constanca Figueiredo, Marco Carvalho Oliveira, Chen Chen-Wacker, Klaus Hoeffler, Mark Kuehnel, Katharina Jansson, Axel Haverich, Gregor Warnecke, Rainer Blasczyk, Hannover Medical School, Hannover, GERMANY.

Aim: HLA and minor antigen mismatches are the main causes of allograft rejection and graft failure. We have shown in mice and rats that MHC silenced cells and tissues are protected against immune rejection. We also demonstrated in porcine lungs that SLA expression can be turned off in a complex vascularized organ. In this study we evaluated the effect of MHC I silencing prior to allogeneic lung transplantation (Tx) in an established porcine Tx model by monitoring the cytokine response during the first 12 weeks after Tx with immunosuppression given only in the first 4 weeks. **Methods:** SLA I was permanently silenced during normothermic ex vivo perfusion with lentiviral vectors encoding short hairpin RNAs targeting b2m (n=2). A lentivirally transduced non-specific shRNA was used in the control lung Tx group (n=3). NanoLuc was used as a reporter gene in both groups. In each transplant experiment both donor lungs were genetically engineered with one lung being transplanted and the other lung used for quality control. Levels of b2m mRNA and SLA were quantified by RT-PCR and flow cytometry. SLA downregulation of endothelial cells was advisedly designed to not exceed 70%. Cytokines were monitored every second day after Tx and weekly after the post-operative day (POD) 7 by multiplex technology. **Results:** Already 1h after Tx the serum levels of IL-1b, IL-6 and IL-8 increased significantly in all animals by up to 0.263, 1.370 and 0.497 pg/ml, respectively. On POD 1, the cytokine secretion in the SLA silenced group decreased to pre-transplant levels whereas those of the control group remained significantly elevated ($p<0.01$). On POD 14, levels of IL-12 increased significantly by up to 0.286 pg/ml in the control group whereas it remained at pre-transplant levels in the SLA silenced group. In addition, levels of IL-2, IL-10 and TNF-a increased exclusively in animals with SLA expressing lungs while it was undetectable in animals with SLA silenced lungs. Due to severe rejection none of the control group animals reached the end of the monitoring period whereas all recipients of the SLA silenced lungs could be monitored over the entire period. **Conclusions:** These data strongly indicate that grafts silenced for MHC I are immunologically invisible supporting that MHC I and II silencing may successfully combat the burden of rejection and immunosuppression.

OR2

LIGATION OF HLA CLASS II MOLECULES PROMOTES ENDOTHELIAL CELL PERMEABILITY THROUGH ACTIVATED SRC AND ERK SIGNALS

Fang Li[1], Nicole Valenzuela[1], Rebecca Sosa[1], Enrique Rozengurt[2], Elaine F. Reed[1], [1]UCLA Immunogenetics Center, Los Angeles, CA, [2]UCLA Digestive Diseases Research Center, Los Angeles, CA.

Aim: Microvascular inflammation, increased vascular permeability, and accumulation of intravascular mononuclear cells are characteristics of acute (AMR) and chronic (cAMR) antibody-mediated rejection. In solid organ transplantation, donor specific antibodies (DSA) are associated with AMR, and early or late graft loss. However, the mechanisms underlying how DSA against HLA class II (HLA II) activate the endothelium and mediate inflammation and vascular permeability are poorly understood. **Methods:** We utilized recombinant Class II Transactivator (CIITA) sub-cloned with pAd/PL-DEST vector to achieve expression of HLA II on vascular endothelium, and then evaluated the effects of HLA II antibodies (anti-HLA II) on endothelial cell (EC) permeability. Immunoblotting was used to determine the activation of signaling pathways and confocal microscopy was used to determine vascular endothelial - Cadherin (VE-Cad) phosphorylation and internalization. **Results:** Ligation of HLA II by anti-HLA II activated Src and ERK, induced stress fiber formation, and increased EC permeability. Anti-HLA II also stimulated VE-Cad phosphorylation at Tyr685 as well as its internalization, thereby

disassembling intercellular junctions. Class II mediated stress fiber formation and EC permeability was abrogated by pharmacological inhibition of ERK (U0126), Src (PP2) or MLC_2 (ML-7). Both pharmacological and siRNA inhibition of Src, but not ERK, inhibited class II-induced phosphorylation of VE-cCd at Tyr685 and consequent VE-Cad internalization**. Conclusions:** In conclusion, our data show that HLA class II DSA promotes actin stress fiber formation via a Src/ERK/MLC_2 dependent pathway. Further, class II crosslinking with antibodies induces rapid endocytosis of VE-Cad via Src-dependent tyrosine phosphorylation of VE-Cad, thereby disrupting the endothelial barrier function and contributing to vascular permeability.

OR3

DEVELOPMENT OF HLA DE NOVO DONOR SPECIFIC ANTIBODY IS ASSOCIATED WITH PREFORMED NON-HLA AUTOANTIBODIES AND LUNG TRANSPLANTATION REJECTION

Aiwen Zhang[1], Rui Pei[2], Dawn M. Thomas[1], Yuchu Sun[3], John McMichael[1], Jeffrey Allen[1], Elizabeth Winn[1], Karen Seifarth[1], Marie Budev[4], [1]Allogen Lab, Cleveland Clinic, Cleveland, OH, [2]Thermo Fisher Scientific, Waltham, MA, [3]Avalon University School of Medicine, Youngstown, OH, [4]Cleveland Clinic, Cleveland, OH.

Aim: Autoantibodies have been implicated in the chronic rejection process after Lung Transplantation (LuT), but it is less certain if preformed non-HLA autoantibody (pAuAb) is the pre-LuT determinant for De Novo Donor Specific Antibody (dDSA) development. This study aims to investigate the relationship of pAuAb to dDSA fomation and the clinical impact of dDSA to LuT. **Methods:** 124 recipients underwent LuT during 10/2012 -2/2014 at our center were retrospectively analyzed for dDSA (Mean Fluorescence Intensity ≥ 1000). pAuAbs were detected among a subgroup of 118 patients using LABScreen™ Autoantibody (Thermofisher, panel coverage is listed in Table 1a). Cut-offs for pAuAbs were set up using 85% reference background values, which were calculated of lower than 85% population from a group of random selected non-transplanted and non-transfused individuals. Acute Cellular Rejection (ACR), Antibody Mediated Rejection (AMR) were recorded to assess LuT outcomes. **Results:** 71 out of 124 (57%) recipients were identified with dDSA. Among dDSA recipients, Class I only, Class II only, and combined Class I and Class II were 16 (23%), 30 (42%), and 25 (35%), respectively. 53 (75%) of total dDSA was from DQ. pAuAbs to Angiotensinogen and Vimentin were clearly related to the formation of dDSA ($p<0.001$), while pAuAbs to FLRT2, CD36, and TubulinA1B were also associated with the generation of dDSA ($p<0.05$) (Table 1a). Total class II and DQ dDSAs were relevant to the occurrence of ACR, while total Class I and total Class II dDSAs, as well as DQ dDSA were significantly related to incidence of AMR (Table 1b). **Conclusions:** The significant relevance of multiple pAuAbs to the formation of dDSA may provide a new perspective for evaluating the possibility of pAuAbs in eliciting dDSA at pre-LuT, which may help to reduce the risk of ACR and AMR related negative impact to LuT.

Table 1a. Association of Preformed Autoantibodies to the Development of HLA De Novo Donor Specific Antibody

*Target Antigens of Autoantibodies	Total (**n=118)	***dDSA Positive (n=73)	dDSA Negative (n=45)	p value
LG3	4 (3.3%)	4 (5.5%)	0 (0%)	> 0.05
IFIH1	15 (12.7%)	12 (16.4%)	3 (6.7%)	> 0.05
ENO1	12 (10.2%)	10 (13.7%)	2 (4.4%)	> 0.05
FLRT2	33 (28.0%)	28 (38.4%)	5(11.1%)	< 0.05
Myosin	14 (11.9%)	9 (12.3%)	5 (11.1%)	> 0.05
PTPRN	43 (36.4%)	31 (42.5%)	12 (26.7%)	> 0.05
Angiotensinogen	40 (33.9%)	34 (46.6%)	6 (13.3%)	< 0.001
CD36	28 (23.7%)	25 (34.2%)	3 (6.7%)	< 0.05
Vimentin	34 (28.8%)	29 (39.7%)	5 (11.1%)	< 0.001
TubulinA1B	32 (27.1%)	27 (37.0%)	5 (11.1%)	< 0.05

*LG3---C-terminal fragment of perlecan
IFIH1---Interferon induced helicase domain 1
FLRT2---Fibronectin leucine transmemberane protin 2
PTPRN---Protein tyrosine phosphatase receptor type N
** Six out of 124 patients missing
*** De novo Donor Specific Antibody

Table 1b. The Association of HLA De Novo Donor Specific Antibody and the Rejections after Lung Transplantation

dDSAs		ACR (n=40)	p value	AMR (n=20)	p value
Class I	Positive (n=41)	13 (32%)	>0.050	11 (27%)	0.044
	Negative (n=83)	27 (33%)		9 (11%)	
A	Positive (n=26)	8 (31%)	>0.050	5 (19%)	>0.050
	Negative (n=98)	32 (33%)		15 (15%)	
B	Positive (n=23)	7 (30%)	>0.050	7 (30%)	>0.050
	Negative (n=101)	33 (33%)		13 (13%)	
Class II	Positive (n=55)	24 (44%)	0.026	15 (27%)	0.006
	Negative (n=69)	16 (23%)		6 (9%)	
DR	Positive (n=8)	3 (4%)	>0.050	3 (38%)	>0.050
	Negative (n=116)	37 (32%)		17 (15%)	
DQ	Positive (n=53)	24 (45%)	0.013	14 (26%)	0.016
	Negative (n=71)	16 (23%)		6 (8%)	

OR4

HIGH RESOLUTION HAPLOTYPE ANALYSES OF CLASSICAL HLA GENES IN FAMILIES WITH MULTIPLE SCLEROSIS

Kazutoyo Osoegawa[1], Lisa E. Creary[2], Kalyan Mallempati[1], Sridevi Gangavarapu[1], Stacy Caillier[3], Jill Hollenbach[4], Jorge Oksenberg[3], Marcelo Fernandez-Vina[2], [1]Stanford Blood Center, Palo Alto, CA, [2]Department of Pathology, Stanford University School of Medicine, Palo Alto, CA, [3]UCSF School of Medicine, San Francisco, CA, [4]Univ. of California San Francisco School of Medicine, San Francisco, CA.

Aim: HLA alleles are observed in specific haplotypes because of linkage disequilibrium between particular alleles. Multiple sclerosis (MS) has been associated with HLA genes and haplotypes. Our goal was to identify candidate HLA gene alleles and haplotypes that are susceptible or protective to MS. **Methods:** We applied a high-throughput, high-resolution NGS method to type 11 HLA loci in 481 trio families comprising of 962 unaffected parental controls and 481 children diagnosed with MS. We developed an in-house computer program named HaplObserve that builds haplotypes by comparing offspring and parents HLA genotypes from nuclear families. HLA haplotypes were built

from the trio families using HaplObserve that also allows tracing HLA allele/haplotype transmissions from the parents to the offspring. We dissected the extended haplotypes generated into smaller haplotypes and alleles, then explored associations with MS using standard transmission disequilibrium test (TDT) and multiallelic TDT analyses. **Results:** We built a pipeline to systematically analyze HLA alleles and various segments of haplotypes using standard TDT and multiallelic TDT tests. Standard TDT and multiple allelic TDT showed that the DRB5*01:01:01~DRB1*15:01:01:01 haplotype was significantly predisposing (standard TDT: $p < 2.2e-16$). We identified that DRB1*01:01:01~DQB1*05:01:01:03 (standard TDT: $p = 8.63e-06$), C*04:01:01:01~B*35:01:01:02 ($p = 0.001645$) and A*02:01:01:01~C*03:04:01:01~B*40:01:02 ($p = 0.008318$) haplotypes were protective. Our analysis indicated that DRB1*11:01:01:01 ($p = 0.001738$), B*27:05:02 ($p = 0.0004531$), B*38:01:01 ($p = 0.0031953$) and C*07:04:01:01 ($p = 0.004126$) were protective. **Conclusions:** We have shown the suitability of the HaplObserve to efficiently build haplotypes from NGS data in a large number of families, as well as the efficacy and power of TDT analysis for MS association studies. By comparing statistical significance of various haplotype segments, we were able to fine-map MS candidate alleles/haplotypes while eliminating false signals resulting from 'hitchhiking' alleles.

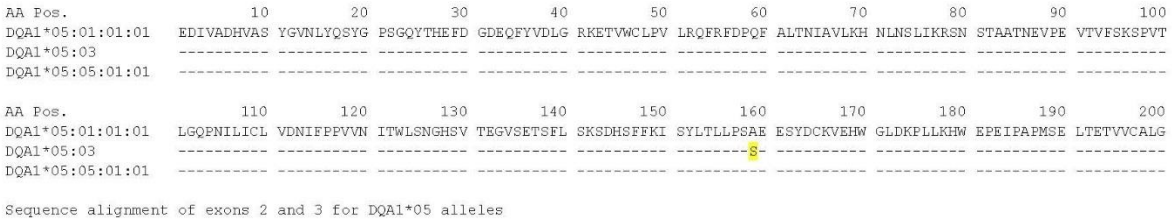
Tuesday, October 2, 2018
4:30 PM - 6:00 PM
Abstract Session I: Solid Organ Pre/Post - Transplant Testing I

OR5

DONOR SPECIFIC ANTIBODY (DSA) ASSESSMENTS USING HLA P GROUPS: THE DEVIL IS IN THE DETAILS

June Jones[1], Annette M. Jackson[1], Donna P. Lucas[1], Maria Bettinotti[2], [1]Johns Hopkins University, Baltimore, MD, [2]Johns Hopkins University School of Medicine, Baltimore, MD.

Aim: Use of HLA P groups for reporting purposes and DSA assessments for potential donors has been steadily increasing. P groups for HLA class II encompass exon2 sequences and capture the polymorphic rich peptide binding domain that serves as the primary target for alloantibody. Nevertheless, we have observed multiple sera exhibiting HLA-DQA1 or HLA-DPB1 reactivity to polymorphic sites outside of exon 2 and confirmed this reactivity using cell adsorptions. **Methods:** HLA antibodies were assessed using Lifecodes single antigen; Immucor and LabScreen Single Antigen; One Lambda. Sera were pretreated to remove interference using Melon Columns™ (ThermoFisher). HLA typing was performed using rSSO LabType, One Lambda and confirmed using NGS TruSight HLA, Illumina. 10th international workshop B cell lines were used for adsorptions. **Results:** We present a representative example in Figure 1. Patient #1 typed as a DQA1*05:05/DQB1*03:19 yet her sera reacted with the DQA1*05:03/DQB1*03:01 bead with a median fluorescence intensity of 11,000. Sequence alignments illustrate an amino acid difference between DQA1*05:03 and DQA1*05:05 at position 159. There is also one amino acid difference between DQB1*03:01 and DQB1*03:19 but the DQA1*05:05/DQB*03:01 bead reactivity was negative, negating the possibility that the antibody was directed at HLA-DQB1. Serum adsorptions using a DQA1*05:03/DQB1*03:01 cell line showed an 84% reduction in the DQA1*05:03/DQB1*03:01 bead reactivity and a concomitant 3% reduction in reactivity with the DQA1*03:01/DQB1*03:01 bead. These data show that the serum reactivity with the DQA1*05:03/DQB1*03:01 bead was directed toward the HLA-DQA1 protein, which differed from the recipient by a single polymorphic difference outside of exon 2. **Conclusions:** We show evidence of sensitization to a polymorphism outside of exon 2 implying the potential danger of using P group designations when performing virtual crossmatch assessments or unrelated stem cell donor searches for patients with complex HLA sensitization.



Sequence alignment of exons 2 and 3 for DQA1*05 alleles

	Target Bead Phenotype		
Adsorbing Cell Phenotype	DQA1*05:03/ DQB1*03:01	DQA1*05:05/ DQB1*03:01	DQA1*03:01/ DQB1*03:01
None	11,000	600	20,800
DQA1*05:03/DQB1*03:01	1,800 (84%)	0 (100%)	21,500 (3%)

Patient #1 phenotype: DQA1*05:05/DQB1*03:19 . Data are presented as MFI values (%difference).

A.M. Jackson: *3. Speaker's Bureau; Company/Organization; Thermo Fisher.* ***M. Bettinotti:*** *3. Speaker's Bureau; Company/Organization; ThermoFisher.*

OR6

SUCCESSFUL DECEASED DONOR KIDNEY TRANSPLANTATION OF HIGHLY SENSITIZED CANDIDATES ACROSS POSITIVE CROSS MATCH AND STRONG DONOR-SPECIFIC HLA-DP ANTIBODIES WITHOUT DESENSITIZATION

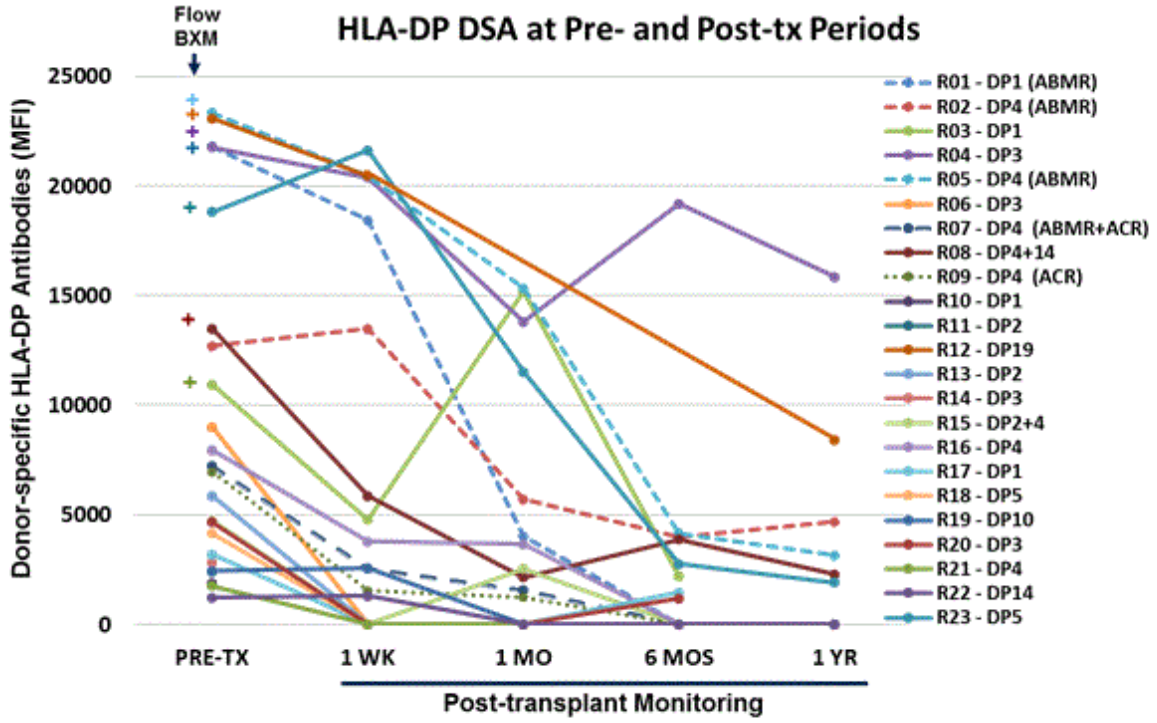
Raja Rajalingam[1], David Gae[1], Zoltan G. Laszik[2], Kelly J. Cunniffe[1], John Roberts[3], [1]Immunogenetics Transplantation Laboratory, Department of Surgery, University of California San Francisco, San Francisco, CA, [2]Department of Pathology, University of California San Francisco, San Francisco, CA, [3]Department of Surgery, University of California San Francisco, San Francisco, CA.

Aim: Over 50% of kidney transplant (Ktx) candidates with 100% CPRA display HLA-DP antibodies, which impair the chances of finding a compatible deceased donor (DD) despite national sharing. Herein, we assessed the impact of donor-specific HLA-DP antibodies (DP-DSA) on Ktx outcome. **Methods:** DD Ktxs performed at UCSF (n=569) from 2013-16 were analyzed. Based on pre-tx DSA status, recipients were divided into 3 groups: DSA -ve (n=465), non-DP DSA +ve (n=81), and DP-DSA +ve (n=23). All recipients received standard immunosuppression, and DP-DSA +ve recipients received additional 1 dose IVIG at day-1 post-tx. Grafts were evaluated by 6 mo protocol biopsies (Bx) and cause Bx. HLA antibodies were tested using single antigen bead assay (One Lambda). **Results:** There were more highly sensitized patients (CPRA>97%) and well-matched Ktx in DP-DSA +ve group compared to the other 2 groups (Table). All recipients in DSA -ve and non-DP DSA +ve groups had a -ve T and B cell pronase flow-crossmatch (FXM), while 30% (n=7) of DP-DSA +ve group that had a DP-DSA of >10,000 MFI, displayed a +ve B-FXM (MCS~200; 120 cutoff). There were no hyper acute rejection episodes in all 3 groups. The DP-DSAs were self-decayed overtime post-tx in all 23 recipients transplanted with pre-formed DP-DSAs (Figure). The Bx findings revealed no significant difference in the rate of antibody-mediated rejection (ABMR) or in acute cellular rejection (ACR) between the 3 groups. Two recipients transplanted with DP-DSAs but -ve XMs lost their grafts: one was a 46F re-tx recipient with 100% CPRA, 1/12 HLA-mismatch, DP4 DSA (MFI=6956), lost due to ACR in 4 months; another was 62M, 19% CPRA, 7/12 HLA-mismatch, DP1 DSA (MFI=4165), suffered Polyomavirus nephropathy at 5 months. **Conclusions:** Kidneys can be transplanted across DP-DSAs with no apparent effect on graft survival. This strategy increases the DD Ktx in highly sensitized patients without pre-tx desensitization. The long-term outcome studies are ongoing.

Comparison of deceased donor kidney transplants with distinct pre-transplant DSA

	DP-DSA Positive N=23	Non-DP DSA Positive N=81	DSA Negative N=465	*P* value: DP-DSA pos *vs.* DSA neg	*P* value: DP-DSA pos *vs.* non-DP DSA pos
Female	47.8% (11)	57% (46)	40% (184)	0.5	0.6
Retransplant	65.2% (15)	42% (34)	14% (67)	<0.0001	0.0918
CPRA <20%	8.7% (2)	19% (15)	49% (229)	0.0003	0.4209
CPRA 20-79%	4.3% (1)	16% (13)	27% (125)	0.0303	0.2692
CPRA 80-97%	13% (3)	21% (17)	10% (47)	0.9195	0.58
CPRA 98-100%	73.9% (17)	43% (35)	11% (51)	<0.0001	0.0181
HLA-A,B,C,DR,DQ mismatch ≤5	60.9% (14)	30% (24)	30% (140)	0.0004	0.0124
C4d Posivive	13% (3)	10% (8)	5% (24)	0.2514	0.9588
ABMR	13% (3)	7% (6)	3.4% (16)	0.0541	0.4105
ABMR+ACR	4.3% (1)	2% (2)	2% (8)	0.9042	0.8175
ACR	4.3% (1)	5% (4)	7% (32)	0.9453	0.6633

Data are expressed as % and number (n) of transplants.



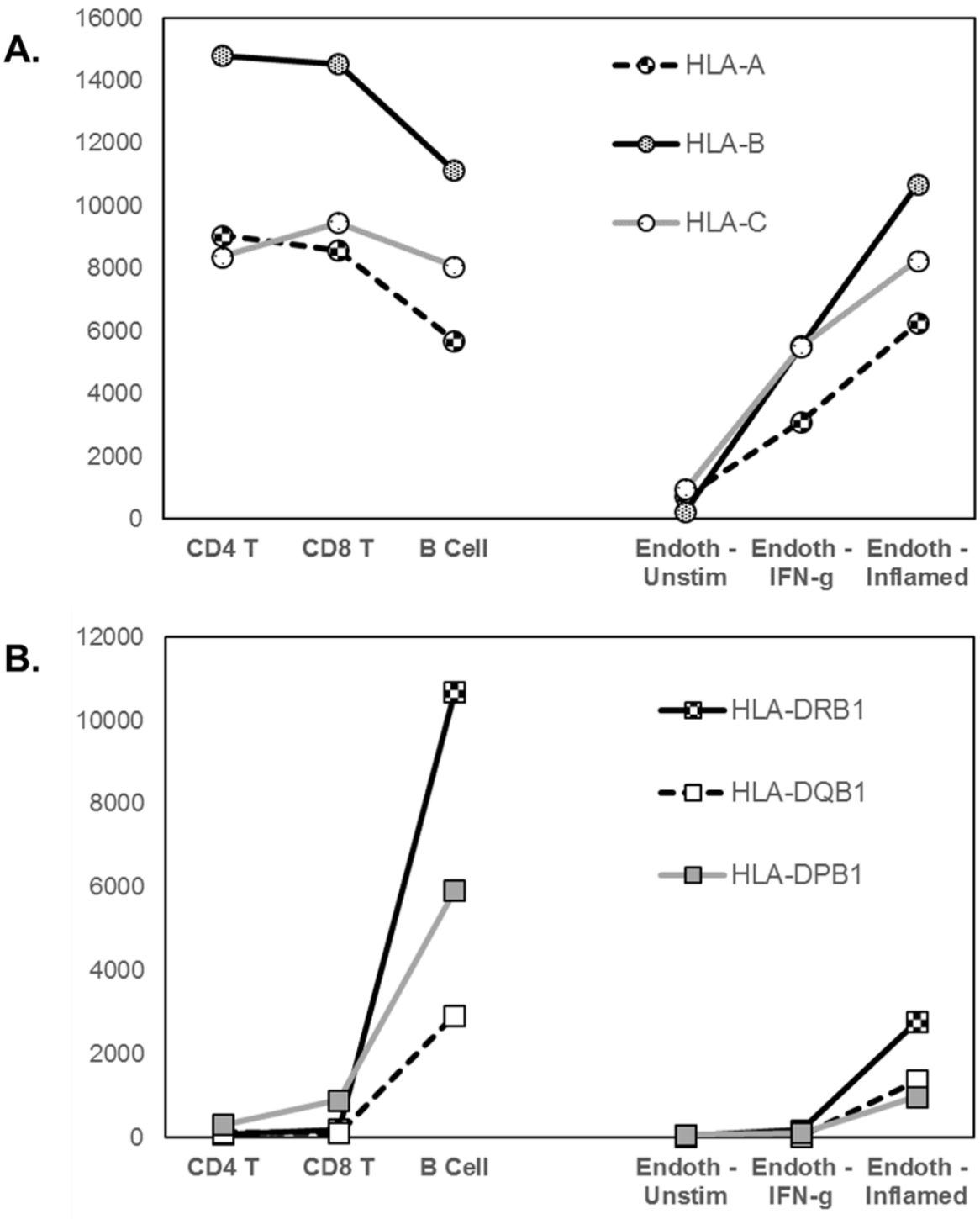
OR7

HLA EXPRESSION ON T AND B CELLS - COMPARISONS TO INFLAMED ENDOTHELIUM

Luis Hidalgo, University Alberta Hospitals, Edmonton, AB, CANADA.

Aim: Crossmatches outcome is often interpreted as absolute proof of donor-specific reactivity that directly correlates to the risk of antibody-mediated rejection (ABMR); an immune attack on donor microvascular endothelium. However, direct comparisons of endothelial cell HLA expression to that of T and B cells have not been performed. We assessed HLA expression using global gene expression to compare HLA Class I and II expression in T cells, B cells, and human umbilical vein endothelial cells (HUVEC) under conditions that simulate immune attack. **Methods:** CD4 and CD8 T cells and B cells were isolated from healthy volunteers. HUVECs were cultured under recommended culture conditions and remained unstimulated, or IFN-g treated (24 hours), or IFN-g treated followed by the addition of polyclonal HLA antibodies and NK cells/monocytes to simulate inflamed ABMR immune attack.

Expression was calculated as the geometric mean across each set of samples (≥3 for each condition). **Results:** HLA-A, -B, and -C levels were similar between CD4 and CD8 T cells but were higher than levels on B cells [Fig. 1A]. Unstimulated endothelium showed low Class I expression which was upregulated by IFN-g treatment, and most highly increased under inflamed conditions. HLA Class I levels on endothelium under inflamed conditions mirrored those in B cells but were >25% less than those on T cells for HLA-A and -B. B cells expressed high levels of HLA-DRB1, -DQB1, and -DPB1 [Fig. 1B]. However, even under intense immune attack, endothelial cells only expressed a fraction of B cell levels; 47% for -DQB1, 26% for -DRB1, and 17% for -DPB1. **Conclusions:** Our data suggest that crossmatch interpretation derived from T cell reactivity may closely resemble what is predicted to be maximal endothelial HLA Class I expression. In contrast, reactivity observed against HLA Class II on B cells likely exaggerates the maximum levels observed on endothelium under immune attack.



OR8

A NOVEL REACTIVITY PATTERN WITH LABSCREEN™CLASS I SINGLE ANTIGEN BEADS AND ITS PREVALENCE AMONG SLE PATIENTS

Christina L. Dean[1], Robert A. Bray[2], Howard M. Gebel[2], Harold C. Sullivan[1], [1]Emory University, Atlanta, GA, [2]Emory University Hospital, Atlanta, GA.

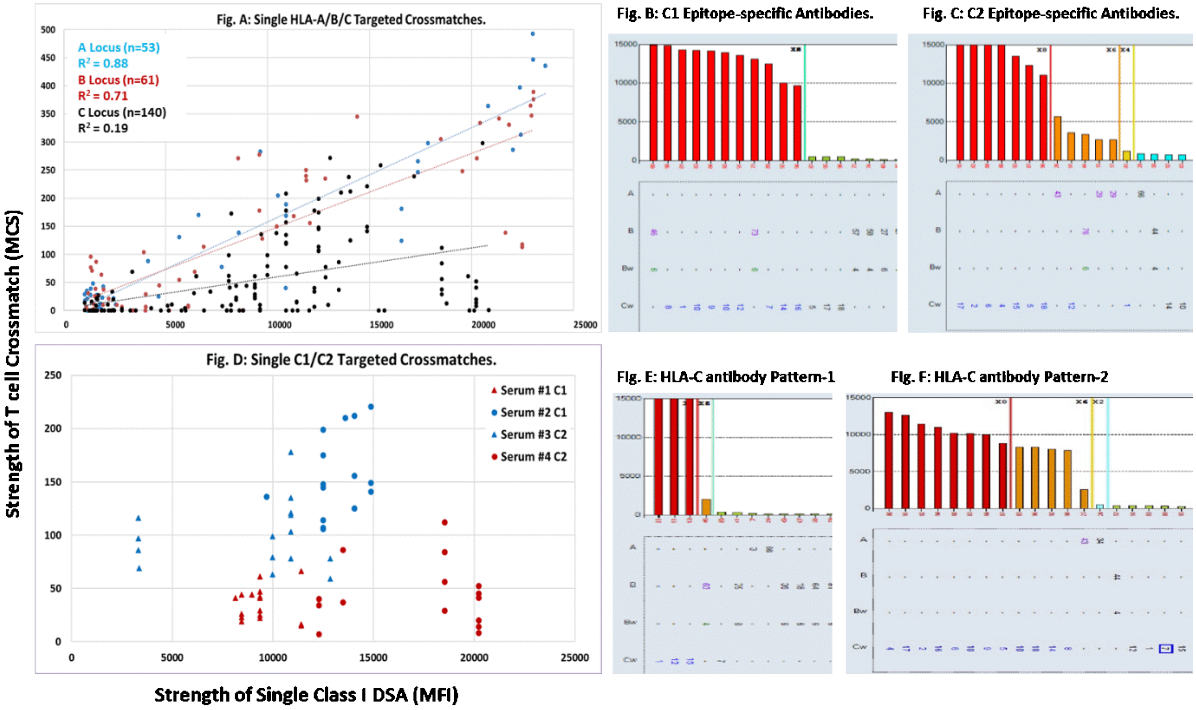
Aim: Previously, a distinct class II Luminex-single antigen bead (SAB) pattern was described among patients with systemic lupus erythematous (SLE) and attributed to antibodies targeting denatured antigens. In this study, we identify and describe a novel class I reactivity pattern shared among SLE patients. **Methods:** Routine review of serum samples from transplant patients revealed a recurring pattern of reactivity on class I single antigen bead assays (One Lambda, Inc, lot 10). The pattern included the following beads: HLA-A*33:03, -A*36:01, -A*80:01, -B*54:01, -B*53:01, -C*06:02, -C*07:02, -C*18:02, -C*14:02, -C*03:03, -C*03:04, and -C*15:02. Further investigation was performed to determine the prevalence of this pattern and the specific characteristics of patients in which it occurred. Towards this end, all class I results performed in 2017 on renal transplant candidates/recipients were reviewed retrospectively for the presence of the above pattern. Patient diagnosis and demographic data was obtained from patient laboratory and hospital records. **Results:** In total 5,992 samples from 3,027 patients were reviewed. We observed 105 (1.8%) samples from 58 patients displaying the class I pattern. Twenty-nine percent (n=17) of these patients had no history of sensitization (e.g. blood transfusion, pregnancy, transplant) and only 29% of samples had distinct antibodies as identified by FlowPRA testing. Of the positive samples, 83% had reactivity against self-antigen involving 1 or more of the alleles in question, with HLA-C*07:02 being the most common target (45%). Notably, 62% of these patients had a diagnosis of SLE. **Conclusions:** To our knowledge, this is the first description of a distinct class I Luminex-SAB pattern with a prevalence in patients with SLE. This pattern was found in patients with no sensitization history, negative FlowPRA, and/or antibody to self-antigen(s). Epitope analysis failed to reveal a common determinant(s) to explain this pattern of reactivity. Given these findings, this pattern likely represents antibody targeting denatured antigen/unique peptide, molecular mimicry, autoimmunity, or a combination of some/all these factors.

OR9

HLA-C TARGETED CROSSMATCHES ARE SERUM-DEPENDENT, AND TRANSPLANTS WITH STRONG PRE-FORMED DONOR-SPECIFIC HLA-C ANTIBODIES BUT NEGATIVE CROSSMATCHES DO NOT EXPERIENCE ANTIBODY-MEDIATED REJECTION

Kelly Cunniffe, Ahrles Cajulao, Jack Lai, Raja Rajalingam, Immunogenetics and Transplantation Laboratory, Department of Surgery, University of California San Francisco, San Francisco, CA.

Aim: To analyze the impact of donor-specific HLA-C antibodies (DSA) on crossmatch (XM) and transplant (tx) outcome. **Methods:** HLA antibodies (Ab) were analyzed using One Lambda (OL) single antigen beads (SAB). 334 T-cell flow XMs targeting a single HLA-A, B or C allotype were performed. Relationships between the strengths of XM (MCS) and DSA (MFI) were assessed by Pearson's correlations. Outcomes of 3 organ txs performed with strong (MFI>10K) pre-formed HLA-C DSAs were assessed. **Results:** Strong and linear positive-correlations were observed between MCS and MFI of single HLA-A (R^2=0.88) or HLA-B (R^2=0.71) targeted XMs [Fig.A]. Conversely, a poor correlation (R^2=0.19) was obtained with HLA-C targeted XMs. To avoid multi-epitope targeting, we focused our analyses on 66 T cell XMs that were performed using sera with strong Abs (MFI>10K) targeting only a single C1 (Cw1,7,8,9,10,12,14,16, B46 and B73) or C2 (Cw2,4,5,6,15,17 and 18), the dimorphic epitopes of HLA-C that bind different isoforms of KIR2D receptors [Fig.B & C]. Two sera with anti-C1 reactivity showed distinct T cell-binding patterns - 1 caused +ve XMs and another caused -ve XMs [Fig.D] when targets express C1-containing HLA-C allotypes. Another 2 sera with anti-C2 reactivity showed distinct bindings - 1 caused +ve XMs and another caused -ve XMs when targets express C2-containing HLA-C allotypes. One of the patients displaying anti-C2 reactivity received a kidney tx with pre-formed Cw6 DSA (MFI=20K) but -ve XM, and no rejection was observed in 1 year post-tx. Some patients display strong (MFI>10K) reactivity to subsets of Cw-beads: Cw1,12,15 (Fig.E) or Cw2,4,5,6,8,9,10,14,16,17,18 (Fig.F). These Abs neither cause +ve XM nor harm the allograft, and are self-decayed post-tx. **Conclusions:** Not all HLA-C Abs detected by OL SAB assay bind to cells. Despite high MFI, the no-binding HLA-C DSAs do not harm the allograft. The clinical impact of HLA-C Abs must be judged by their cell binding.



OR10

DATA-DRIVEN MODELING OF FLOW CYTOMETRIC CROSSMATCH: ENHANCED VIRTUAL CROSSMATCHING

Eric T. Weimer, Katherine A. Newhall, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Aim: Increasingly HLA laboratories are using virtual crossmatching to predict recipient and donor compatibility using HLA antibody data and donor HLA type. However, virtual crossmatch interpretation is based on HLA experience and expertise of individual transplant centers. The purpose of this study is to develop data-driven algorithms (DDA) that predict flow cytometric crossmatch (FXM) outcomes using HLA antibody mean fluorescent intensity (MFI) data and donor HLA typing. These methods are independent of human intuition or experience and may provide insights that are otherwise imperceptible. **Methods:** Two data sets consisting of 222 and 109 FXM with single antigen bead data for both HLA class I and II antibodies were used. Single antigen bead data was compiled against donor HLA antigens using the OneLambda assay and Luminex. The first DDA found the optimal MFI threshold using summation of mean fluorescent intensity data for class I and/or II that predicted either T cell or B cell mean channel shifts (MCS) above FXM cutoff. The second DDA applied a least-squares regression model to the HLA locus-specific data (second data set) to predict the actual T or B cell MCS. **Results:** The threshold method yielded between 84.9% and 91.1% accuracy when using class I donor specific antibody (DSA) data to predict T cell outcome, and class I and II DSA data to predict B cell outcome. Optimal MFI thresholds of 3450 and 7560 were found for T and B cell prediction. For quality assurance, prediction of T cell MCS was attempted using class II data, resulting in 61.9% accuracy. The least-squares model increased accuracy to 93.6% and 97.2% for T and B cell MCS, respectively. Class I DSA influenced T and B cell MCS more than class II. The relative importance of an individual HLA locus on T cell prediction was found to be HLA-B>-A>-C. In the least-squares fitting, B64 had a large positive effect while A34 had a large negative effect on T cell MCS. **Conclusions:** Utilizing DDA can expand accuracy of biologic systems beyond human experience and expertise. We showed how DDA can improve HLA crossmatch by generating high-level accuracy T and B cell FXM outcomes. Further improvements to the algorithm will incorporate HLA antigen expression and HLA antibody avidity.

OR11

CHARACTERISTICS OF TRANSPLANT CANDIDATES WITH CPRA≥99.95% LIKELY TO BE ALLOCATED ORGANS FROM DECEASED DONORS.

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Aim: Since the new KAS, candidates with cPRA=100% were prioritized for deceased donor (DD) kidneys, their transplant rates increased from 2.7% to as high as 19.1%. A recent simulation (CJSAN 11:505-511, 2016), predicted that not all cPRA=100% are equally advantaged. While ~75% cPRA =100% candidates were compatible with an average of 17 donors (total donors=6141), ~25% were incompatible with every donor and related to cPRA values of 99.45-> 99.99% being "rounded up" to 100%. Indeed, 91% of candidates without a compatible donor had cPRA values >99.9%. Subsequent data with actual transplants (AJT 16:1834-1847, 2016) supported those predictions. In our program, 61 cPRA=100% candidates were transplanted from 12/04/14-01/12/18. Surprisingly, 15 recipients had cPRA values ≥99.95%. In this study, we identified their characteristics. **Methods:** HLA profiles and demographic information from 15 cPRA ≥99.95% recipients were compared to 30 non-transplanted case controls with cPRA ≥99.95%. The HLA typing data of each subject was entered into Haplostats (haplostat.org) and the top ranked phased haplotypes for each were recorded. **Results:** Among cPRA≥99.95% recipients, 11/15 (73%) had one or both of their HLA haplotypes ranked among the top 125 CAU haplotypes. CAU haplotypes were the focus as CAU represent ~67% of kidney donors. Notably, 7/15 recipients had one or both of their HLA haplotypes among the top four ranked CAU haplotypes and 6/15 received transplants from homozygous donors. Among recipients, eight were AA (53%), six were CAU and one was HIS. In contrast, among 30 case controls, 4 (13.3%) had one haplotype ranked in the top 125 and 17 had one haplotype ranked in the top 8000. For 9 candidates, neither HLA haplotype was ranked among the top 10,000 CAU haplotypes. Importantly, 25/30 (83.3%) controls were African American. **Conclusions:** Kidney transplant candidates with cPRA≥99.95% were more likely to be transplanted when at least one of their HLA haplotypes was frequent (rank ≤125). This novel observation offers an opportunity to medically optimize a subset of highly sensitized candidates before deceased donor transplantation. It also suggests amendments may be necessary to minimize racial disparity in the KAS. Additionally, alternative approaches should be considered for candidates not likely to receive a DD offer.

H.M. Gebel: *2. Consultant; Company/Organization; Astellas, Thermofisher.*

Tuesday, October 2, 2018
4:30 PM - 6:00 PM
Abstract Session II: Immunotherapy/Rejection & Post Transplant Testing

OR12

MONITORING CD8$^+$ DONOR LYMPHOCYTE CHIMERISM IS A CRITICAL INDICATOR FOR DIAGNOSING AND TREATING ACUTE GRAFT VS. HOST DISEASE FOLLOWING LIVER TRANSPLANTATION

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Aim: Mortality of Acute Graft vs. Host Diseases (aGvHD) after Liver Transplantation (LT) can be as high as 70-100%, due to delays in diagnosis and severity of complications. Based on a large LT cohort with long term follow up, we investigated the role of donor chimerism (D%) in supporting expeditious aGvHD diagnosis and monitoring disease control. **Methods:** 145 of 1478 recipients with LT during 9/2007-12/2017 were clinically suspected to have aGvHD and tested for D% with peripheral blood (PB), CD3$^+$ (T), CD8$^+$ T, NK, and B lymphocytes by short tandem repeat (sensitivity 5%). D% at initial testing and peak stage, POD of aGvHD resolution, and aGvHD related mortality were retrospectively analyzed. Clinical suspicions with CD8$^+$ T D% >25% were initially treated with methylprednisolone and/or reduced immunosuppression. Thymoglobulin was added to cases with no improvement after initial therapy. **Results:** The D% in PB was uninformative. The highest D% was seen in CD8$^+$ T cells followed by T cells and NK cells. Of 145 recipients with D% test, 27 had D% > 5, these patients were then graded into

clinical severity as C0-3. Nine patients in C0 had no clinical evidence of aGvHD, whereas 18 patients were graded as C1-3. C2 and C3 (n=9) had average $CD8^+$ T D% ≥ 75±23% at initial testing and ≥ 86±24% at peak D% stages, which were significantly higher than that of C1 (n=9) with $p<0.05$ and $p<0.001$, respectively. All recipients in C3 (n=5) died of aGvHD, which gave 28% aGvHD related mortality. Individuals in C2 (n=4) survived aGvHD, but showed a concomitant longer duration of treatment than C1 ($p<0.05$). All D% of C0 was <10% and did not show the picture of aGvHD (Table 1). **Conclusions:** The D% of $CD8^+$ T cell is a sensitive and correlative indicator for timely diagnosis and effective monitoring of aGvHD after LT. High D% of $CD8^+$ T from initial testing and peak phase was associated with aGvHD related mortality or prolonged resolution of this disease. Early detection of D% of $CD8^+$ T before the donor immune system fully engrafted is critical for recovering from the damage of aGvHD following LT.

Table 1. Comparasion of CD8+ T cell Donor Chimerism Percentage among the Recipients Diagnosing for Acute Graft vs. Host Diseases after Liver Transplantation

Group	Case#	*aGvHD	aGvHD-Related Motality	Days of aGvHD Duration	**POD of ***D% Initial Testing (Day)	Initial Testing D% (%)	POD Peak D% (Day)	Peak D% (%)
C3	3	yes	yes	on	33	99	33	99
	14	yes	yes	na	51	91	134	100
	15	yes	yes	na	32	88	62	99
	16	yes	yes	na	30	28	32	39
	21	yes	yes	na	66	92	66	92
Average					42±14	80±26	65±37	86±24
C2	2	yes	no	60	30	NT	34	82
	5	yes	no	291	26	84	26	84
	6	yes	no	60	30	98	30	98
	9	yes	no	65	30	43	41	93
Average				119±99	29±2	75±23	33±6	89±7
Average of G1+G2						78±25		87±18
C1	1	yes	no	21	30	15	51	16
	8	yes	no	41	30	6	34	6
	10	yes	no	54	39	49	41	52
	11	yes	no	40	36	27	46	69
	12	yes	no	47	22	14	22	14
	17	yes	no	43	32	66	32	66
	18	yes	no	15	39	13	42	28
	23	yes	no	34	26	7	27	21
	30	yes	no	20	30	58	38	73
Average				37±12	32±5	28±21	37±9	38±25
p values				<0.05 (G2 vs G3)		<0.05 (G1+G2 vs G3)		<0.001 (G1+G2 vs G3)
C0	4	no	na	na	26	7	30	8
	7	no	na	na	27	7	27	7
	13	no	na	na	73	10	73	10
	19	no	na	na	64	7	64	7
	20	no	na	na	20	9	2	9
	22	no	na	na	4	na	4	na
	24	no	na	na	na	6	na	6
	25	no	na	na	9	5	9	5
	26	no	na	na	30	6	30	6
Average						7±2		7±2

*aGvHD: Acute Graft vs. Host Diseases

*POD: Postoperative day

**D%: Donor Chimerism Percentage

OR13

IDENTIFICATION AND CHARACTERIZATION OF A PANEL OF NON-HLA ANTIBODIES IN KIDNEY ALLOGRAFT REJECTION

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Immunogenetics Center, Los Angeles, CA, [3]Univ of California Los Angeles, Los Angeles, CA, [4]Immucor, Waukesha, WI.

Aim: The goal of this study was to identify novel anti-endothelial non-HLA antibodies (Ab) associated with kidney allograft rejection and assess a panel of novel and previously discovered non-HLA Ab in a pediatric kidney allograft recipient cohort. **Methods:** Endothelial cell cross matches (ECXM) were used to screen patients for non-HLA Ab associated with rejection. 16 of 279 kidney allograft recipients tested were positive for both ECXM and rejection. Post-transplant neat and EC eluted sera from these 16 ECXM+ Rejection+ patients, as well as available pre-transplant sera from these patients were analyzed by protein microarrays (ProtoArray, Thermo Fisher Scientific) to screen 9,000 human proteins and identify antigens bound by patient sera (Prospector software, z-score ≥3 and CV<0.05). 12 novel non-HLA Ab identified by protein microarray and 67 previously published non-Ab targets identified in kidney (n=27), heart (n=31), lung (n=8), and liver (n=1) transplant were conjugated to multiplex bead arrays. This panel of non-HLA targets was validated in a longitudinal single center pediatric cohort (n=65). **Results:** Protein microarray analysis of the ECXM+ Rejection+ sera identified 391 proteins in the pre-transplant sera, 1252 proteins in the neat sera, and 388 proteins in the EC eluted sera. 135/388 eluted sera of ECXM+ Rejection+ EC specific proteins were newly detected in post-transplant sera. 12/135 novel Ab were prioritized to select for ligands with endothelial membrane localization, kidney expression and an increased Ab binding frequency. Rank sum analysis of a single-center pediatric kidney allograft recipient cohort (n=65) identified peroxisomal-trans-2-enoyl-coA-reductase (p=0.03) and a novel non-HLA Ab (p=0.05) associated with rejection at the patient level. **Conclusions:** Non-HLA antibodies are associated with an increased risk of allograft rejection emphasizing the importance of further identification and validation of non-HLA Ab in transplantation. Assessment of a panel of non-HLA Ab appears to be useful to elucidate the incidence and specificity of non-HLA Ab associated with kidney allograft rejection.

B.L. Ray: *5. Employee; Company/Organization; Immucor, Inc.* ***E.F. Reed:*** *1. Grant/Research Support; Company/Organization; Immucor, Inc.*

OR14

RELEVANCE OF MICA-129 DIMORPHISM IN LIVE DONOR RENAL TRANSPLANTATION

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Aim: Strong or weak NK/T cell activation by NKG2D-MICA-129 met or val allele respectively can lead to different allograft outcomes by influencing the degree of NK cell activation. The aim was to understand the role of MICA-129 dimorphism in allograft rejection in live donor renal transplantation. **Methods:** A total of 176 live donor renal transplant patients were genotyped for MICA-129 dimorphism using TaqMan technique and were followed up for a minimum period of 1.5 years post-transplant. Serum samples of all recipients were evaluated at the pre and post-transplant periods, 1-2 week, 3 months, 6 months and at time of rejection (if applicable) for the presence of HLA specific antibodies using Luminex technology. The biopsy proven rejection episodes were recorded and correlated with MICA-129 dimorphism for the presence of met or val alleles and the respective genotypes *met/met, met/val* and *val/val* . **Results:** MICA-129 met allelic as well as *met/met* genotypic frequencies dominated in recipients experiencing rejection episodes, particularly those with antibody mediated rejection (AMR) as compared with the no rejection episode group (NRE) (61% vs 32%; p>0.0006 and 43% vs 13%; p>0.01 respectively). Five of the six *met/met* AMR cases developed *denovo* high titer (Mean MFI >5000) HLA-DSA for HLA class II molecules, 3-6 months post transplantation. This is in contrast to the *met/val* and *val/val* groups who maintained low titer antibodies till the time of biopsy. Presence of met allele also correlated with serum creatinine levels and C4d positivity and the data revealed an allele dose dependent increase in intensity towards AMR progression. **Conclusions:** These results point towards an important role of MICA-129 dimorphism in renal allograft rejection and suggest that recipients with MICA-129 met allele may warrant special post-transplant intervention/management.

OR15

PROGRESSIVE CHRONIC TISSUE INJURY WITHOUT ACTIVE PATHOLOGICAL CHANGES IS THE HISTOLOGICAL HALMARK OF DE NOVO CLASS II DSA

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Aim: There is no doubt that de novo anti-HLA Donor Specific Antibodies(dn-DSA) have deleterious effect on the kidney allograft. However, there is considerable controversy regarding the pattern of tissue injury generated by these antibodies. We set out to study the pattern of tissue injury of kidney biopsies procured in the early post-transplantation period (the first 5 years) in the for-the-cause biopsy practice setting at our institution. **Methods:** All the cases with dn-DSA who had at least one kidney biopsy done in the first 5 years after kidney transplantation were included in the study. The type of dn-DSA (class I, class II or combined), presence/ type of rejection and the pattern of tissue injury based on Banff score and other histological findings were evaluated. **Results:** Of the total of 36 cases fulfilling the criteria, 6(17%) had only class I, 21(58%) had only class II and 9 (25%) had combined dn-DSA. There was no difference in clinical and laboratory parameters between the groups. Chronic histological changes either in tubulointerstitial compartment (ct and ci scores), vessels (cv score) or glomeruli (cg score) were significantly higher in cases with class II or mixed DSA than class I DSA. Mean±SD for ct/ci, cv and cg in patients with class II or combined dn-DSA vs class I were 1.2±1.0 vs 0.6±0.5, 1.4±1.1 vs 0.6±0.8, 0.5±0.8 vs nil, respectively. Interestingly, the recurrence of the original glomerulonephritis was only seen in association with class I DSA. Venulitis, not part of Banff score, was seen significantly more prevalent in class I DSA group (50%) compared to class II only or mixed group (10%). **Conclusions:** Our study is limited by the low number of cases. However, some trends are readily appreciated. The tissue injury caused by class II dn-DSA is insidious and may be detected at late stages of rejection, even in early post-transplantation period. The clinical and laboratory parameters of rejection can be misleading in such cases and therefore, protocol biopsy practice is advisable.

OR16

HLA EPITOPE-MISMATCH MORE PRECISELY PREDICTS THE DEVELOPMENT OF DE NOVO DONOR SPECIFIC ANTIBODY AND ACUTE CELLULAR REJECTION AFTER LUNG TRANSPLANT

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Aim: Development of de novo HLA donor-specific antibody (dnDSA) after lung transplant (LuTx) is prevalent and associated with poorer transplant outcomes. Understanding pre-LuTx determinants helps to minimize dnDSA. The aim of this study was to investigate the impact of HLA-Epitope Mismatch Load (EpiMML) in the development of dnDSA in LuTx recipients. **Methods:** 572 recipients received LuTx during 10/2012-10/2017 and were retrospectively analyzed for the formation of dnDSA (Mean Fluorescence Intensity ≥ 1000). Antigenic mismatches were recorded among 422 recipients. EpiMMLs were determined by HLA-Matchmaker (Version 02) among 168 recipients who had four-digit high resolution typing. There were 18 recipients who were typed by both high and low resolution. Acute Cellular Rejection (ACR) and Antibody Mediated Rejection (AMR) were assessed for LuTx outcomes. **Results:** Of the 572 patients, 55% developed dnDSA; 25% Class I, 39% Class II, and 36% both Class I and II. In Class II, the most prevalent dnDSA was DQ (216/235; 92%). EpiMMLs for Class I, Class II, as well as single loci of HLA-A, B, C, DR, and DQ were all significantly higher from patients with positive dnDSA than from patient with negative dnDSA (Table 1a). However, for antigen-level mismatches, only HLA-A and DQ correlated with the development of dnDSA. As shown in Table 1b, certain mismatched epitopes were more frequent among patients who developed dnDSA than those who did not. Among a subgroup of 120 recipients, EpiMMLs of Class II, especially DQ, were significantly associated with the occurrence of ACR (p=0.02 and 0.005, respectively). There was no remarkable relevance between EpiMMLs and AMR. **Conclusions:** Compared to HLA antigen mismatching, EpiMML is a better predictor of dnDSA development in LuTx recipients. Increasing EpiMMLs, particularly for HLA-DQ, identify patients at the highest risk to develop dnDSA and ACR. Collectively, these data support the use of EpiMML assessment especially for optimizing post-transplantation immunosuppression in LuTx recipients.

Table 1a. The Associations of Epitope/Eplet Mismatched and Antigen Mistach to the Development of De Novo Donor Specific Antibody

n=168	HLA-Class I						HLA-Class II			
	*DSA		No DSA				DSA		No DSA	
**EpiMML ($\bar{x}$ ± SD)	20 ± 7		16 ± 6				35 ± 10		26 ± 12	
p value	<0.001						<0.001			
EpiMML Cutoff	>19.5						>20.5			
n=168	HLA-A		HLA-B		HLA-C		DR		DQ	
	DSA	No DSA	DSA	No DSA	DSA	No DSA	DSA	No DSA	DSA	No DSA
EpiMML ($\bar{x}$ ± SD)	10 ± 3	8 ± 3	7 ± 3	5 ± 2	6 ± 2	4 ± 3	14 ± 6	10 ± 5	23 ± 8	16 ± 9
p value	<0.001		<0.001		<0.05		<0.05		<0.001	
EpiMML Cutoff	>7.5		>7.5		>5.5		>16.5		>14.5	
n=422	HLA-A		HLA-B		HLA-C		DR		DQ	
	DSA	No DSA	DSA	No DSA	DSA	No DSA	DSA	No DSA	DSA	No DSA
*** AgMM Total n (n of 0/1/2 MM)	85 (0/5/80)	327 (9/94/224)	69 (0/5/64)	350 (1/50/294)	38 (1/3/34)	380 (6/103/271)	44 (1/2/41)	376 (7/67/302)	155 (2/6/147)	263 (21/103/139)
p value	<0.001		>0.05		>0.05		>0.05		<0.001	

* = DSA: Donor Specific Antibody

** = EpiMML: Epitope Mismatch Loads

*** = Ag MM: Antigen Mismatch

OR17

EPITOPE LOAD DOES NOT PREDICT DEVELOPMENT OF DE NOVO ANTIBODIES TO HLA IN PATIENTS WITH BK VIRUS NEPHROPATHY

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Aim: Emerging data suggest that DSA development is a frequent complication post BKV replication, and most DSA are directed against donor HLA-DQ. In the current investigation, we quantified DQ epitope mismatches between the recipient and donor in individuals with BKVN diagnosis and investigated the association between DQ epitope load and development of DSA. **Methods:** In this pilot study, using the most probable 4-digit HLA typings, we investigated epitope mismatches in individuals who developed DSA post BKVN diagnosis compared to those who did not and correlated them to DSA development and graft dysfunction defined as an increase in serum creatinine by >0.5mg/dL at 12 months post-BKVN diagnosis. We had SSOP based intermediate HLA typing available for recipients and donors in 13 recipients who developed BKVN confirmed by renal allograft biopsy. Of the 13 recipients, 2 were excluded: 1 for early graft loss and 1 who had zero DQ mismatches. None of the 11 recipients with BKVN diagnosis had a positive XM or DSA at time of transplantation. **Results:** Time from kidney transplantation to BKVN diagnosis was 8.5±5.1 months. Serum creatinine at time of diagnosis was 1.78±0.56. Of the 11 with BKVN, 36% developed de novo DSA. All DSAs were directed against DQ mismatches. Time from BKVN diagnosis to DSA development was 5.5±2.3 months. Table below lists the epitope mismatch number at each locus. Of the 4 patients with de novo DSA, 75% experienced graft dysfunction during the 12 months post BKVN and of the 7 patients without de novo DSA, 29% experienced graft dysfunction by 12 months. Our data demonstrated that the most frequent DQ epitope mismatches in those with de novo DSA were 52PL3, 45EV, 52PQ2, and 52PR. **Conclusions:** In our pilot study, the epitope load was not different between BKVN patients who developed or did not develop de novo DSA. The most frequent target of de novo antibodies were DQ locus mismatched epitopes. Knowledge of epitope targets of de novo DSA in patients with BKVN may facilitate targeted therapies and improve kidney allograft outcomes.

		Total number epitope mismatches (mm)								
Patient ID	DSA	Total #	Total Class I	HLA-A	HLA-B	HLA-C	Total Class II	HLA-DRB1	HLA-DRB345	HLA-DQB1
31	Yes (DQ6)	82	51	29	17	8	31	12	7	13
29	Yes (DQ7)	79	48	15	15	23	31	14	12	8
36	Yes (DQ7)	73	43	16	27	3	30	20	16	4
41	Yes (DQ7)	83	44	25	13	7	39	15	8	20
Mean Epitope mm DSA+ Patients		**79**	**47**	**21**	**18**	**10**	**33**	**15**	**11**	**11**
30	None	91	52	27	18	14	39	20	11	12
33	None	63	41	23	15	10	22	7	8	9
35	None	68	35	19	17	0	33	5	17	11
40	None	122	91	43	40	10	31	10	20	8
42	None	78	45	23	15	8	43	16	15	10
46	None	74	49	25	21	13	25	13	15	4
47	None	80	49	20	28	2	31	7	10	16
Mean Epitope mm DSA- Patients		**82**	**52**	**26**	**22**	**8**	**32**	**11**	**14**	**10**

OR18

COMPOSITE IMPACT OF CO-LIGATION OF HLA-I AND HLA-II ANTIBODY-MEDIATED ACTIVATION OF HUMAN AORTIC ENDOTHELIAL CELLS

Yi-Ping Jin, Elaine F. Reed, Department of Pathology and Laboratory Medicine, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA.

Aim: Solid organ transplant recipients developing donor specific HLA antibodies (DSA) are at risk for antibody-mediated rejection (AMR). It has been reported that as many as 30% of transplanted recipients develop HLA class I (HLA-I) and/or class II DSA during the first year posttransplant. We previously reported that ligation of HLA I or HLA II on the surface of endothelial cells (EC) with antibody (Ab) triggered intracellular signal networks leading to EC proliferation. However, the impact of combined HLA-I and II Ab ligation on endothelial function remains unknown. We hypothesized that combined HLA-I and II ligation cooperate to potentiate EC activation and proliferation signal networks. **Methods:** HLA-II expression in human aortic EC was induced by adenoviral vector expression of CIITA or by pretreatment with TNFα/IFNγ and confirmed by flow cytometry. Ad-CIITA infected or cytokine-treated EC were stimulated with combined HLA-I and II Ab, protein expression and phosphorylation were detected by Western Blot, EC proliferation was measured by BrdU incorporation and analyzed by flow cytometry. **Results:** Ligation of both HLA-I and II on EC with Ab stimulated a significant increase in phosphorylation of Src, FAK, PI3K, Akt, mTOR, S6K, S6RP, and ERK that was accompanied by EC proliferation. Pharmacological inhibitors targeting Src, PI3K/Akt, and MEK/ERK blocked HLA-II stimulated EC proliferation. Inhibition of PI3K with LY294002 caused ERK overactivation, but no effects on Src, FAK, nor c-Raf. Similarly, suppression of mTORC2 enhanced combined Ab-stimulated ERK activation. Interestingly, 24 hours treatment with rapamycin caused hyperphosphorylation of Akt at Thr308, which is different from either HLA-I or HLA-II Ab-mediated signal events. Furthermore, combined treatment of EC with MEK inhibitor UO126 and rapamycin abrogated combined Ab-stimulated activation of Akt and ERK, and further blocked EC proliferation. **Conclusions:** Treatment with dual PI3K/mTOR inhibitors suppresses a novel negative feedback loop mediated by mTORC2, leading to enhanced PI3K/Akt and MEK/ERK pathway activity in combined HLA-I and II Ab-activated signal networks in EC. This data suggests that combined ERK and dual PI3K/mTORC2 inhibitors will be required to achieve optimal efficacy in controlling combined HLA I and II Ab-mediated AMR.

Wednesday, October 3, 2018
2:30 PM - 4:00 PM
Abstract Session III: Genetic Polymorphism & Anthropology/Disease

OR19

INTEGRATE CRISPR/CAS9 FOR PRECISE PROTEIN EXPRESSION OF *HLA-B*38:68Q*

Yuxin Yin, Elaine F. Reed, Qiuheng Zhang, UCLA Immunogenetics Center, Los Angeles, CA.

Aim: The determination of null- or low-expressed HLA alleles is clinically relevant in both hematopoietic stem cell transplantation and solid organ transplantation. Here we investigate a questionable (Q) allele *HLA-B*38:68Q,* which carries a 9-nucleotide (nt) deletion at codon 230-232 on exon 4 (Fig1A), using CRISPR/Cas9 gene editing to study the effect of this mutation on *HLA-B*38:68Q* expression. **Methods:** The CRISPR/Cas9, targeting gRNA and a non-PAM (Protospacer Adjacent Motif) ssDNA oligo were co-transfected to EBV-B cell line TEM665 (Fig1B) by Neon (Thermo Fisher Scientific) to generate homozygous deletions at codon 230-232 of *HLA-B*38:01:01*. After single cell seeding and expanding, Sanger sequencing and next generation sequencing were performed to validate gene editing in single cell derived clones. Cell surface expression was determined by flow cytometric analysis using a FITC-conjugated Bw4 IgG antibody (One Lambda). **Results:** The gene editing of *HLA-B*38:01:01* homozygous EBV-B cell line by CRISPR/Cas9 resulted in 25 single cell derived clones. The data showed that 21/25 (84%) clones were successfully edited. Among the edited alleles, 5 alleles were through homology directed repair (HDR) pathway and 36 alleles were through non homologous end joining (NHEJ) pathway (Fig1C). We acquired two precise edited clones: one was *HLA-B*38:68Q/B*38:01:01* heterozygous and the other one was *HLA-B*38:68Q* homozygous (Fig1D). Based on the flow cytometric analysis (Fig1E), the expression of *HLA-B*38:01:01* homozygous was 2.2 fold higher than *HLA-B*38:68Q/B*38:01:01* heterozygous cells, and the *HLA-B*38:68Q/B*38:01:01* heterozygous cells was over 2.0 fold higher than *HLA-B*38:68Q* homozygous. We demonstrated that *HLA-B*38:68Q* is a low-expressed allele using CRISPR/Cas9 gene editing. **Conclusions:** CRISPR/Cas9 can induce precise gene editing in HLA genes. It provides a useful tool to study the functions of insertion, deletion and intron sequences of HLA genes.

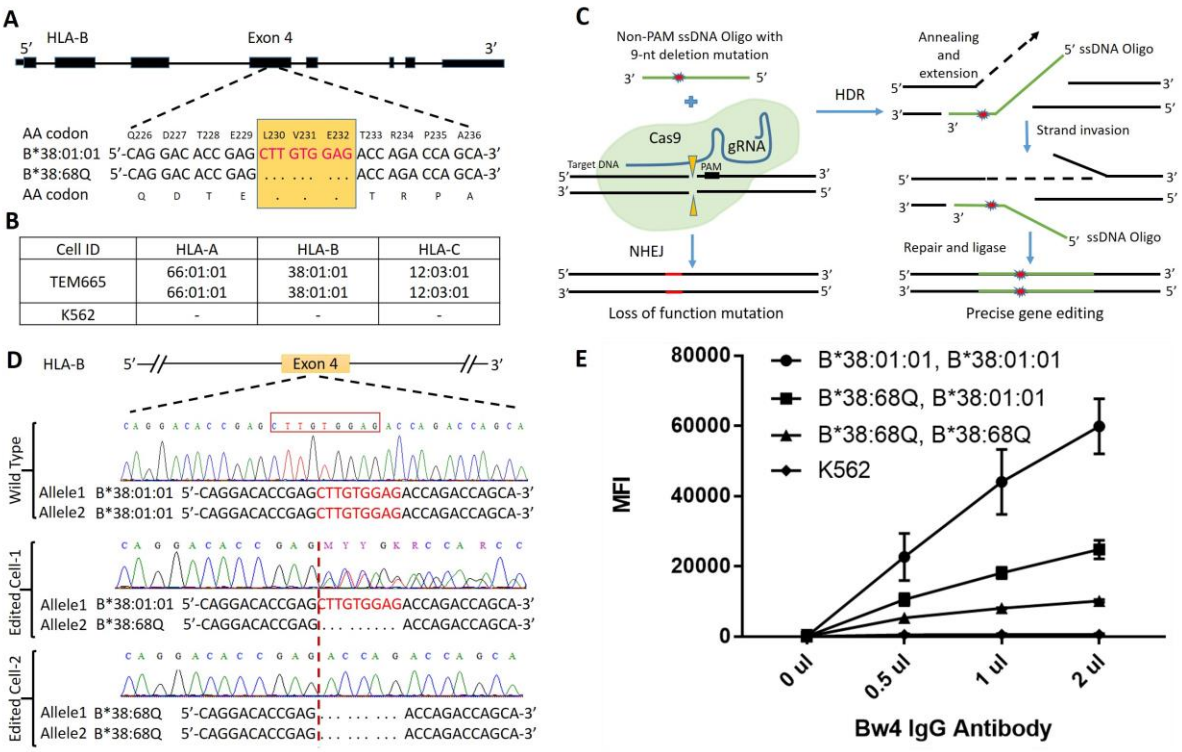

Figure 1. Overview of the precise gene editing using CRISPR/Cas9. (A) Nucleotide and protein sequence of *HLA-B*38:68Q*. Yellow box highlights the 9-nt mutation and "." Indicates the deletion. Numbers above sequence correspond to amino acid codon positions. **(B)** HLA class I typing of TEM665 and negative control K562. **(C)** Gene editing pathways for non-PAM ssDNA oligo. The non-PAM ssDNA oligo is defined as the strand complementary to the PAM strand. **(D)** Allelic analysis by sequencing. For each panel, upper part is Sanger sequencing, and lower part is consensus sequence by next generation sequencing. Red box indicates the position of the 9-nt in wild type *HLA-B*38:01:01*. Red dash indicates the precise gene editing location. **(E)** Flow cytometric analysis of *HLA-B*38* expression on gene edited cell lines and control cell lines. Cells were in triplicate incubated with increasing amount of Bw4 IgG antibody. Graph depicts mean fluorescence intensity (MFI)±SD.

OR20

INVESTIGATING THE MICRORNA PROFILE OF B CELL LINES FOR THEIR DIFFERENTIAL EFFECT ON HAPLOTYPE SPECIFIC DPB1 GENE EXPRESSION

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Aim: SNP rs9277534 in the 3'-UTR region of the HLA-DPB1 gene is associated with differential expression of DP molecule on the cell surface of lymphocytes. It appears that this differential expression of DP influences risk of GVHD in unrelated donor hematopoietic cell transplantation and course of hepatitis B infection. We aim to uncover mechanisms for such alterations in protein expression by identifying haplotype-specific microRNAs (miRNAs) and their differential *in silico* targeting patterns that occur at polymorphic sites, with eventual experimental confirmation. **Methods:** Novel and miRBase-annotated miRNAs expressed in COX (high DP expression) and PGF (low DP expression) B-lymphoblastoid cell lines (BLCL) were characterized using miRDeep2. Higher-confidence miRNAs were obtained by requiring >80% estimated true positive probability, RANDFOLD $p \leq 0.05$, and removal of entries overlapping non-miRNA Rfam elements. A miRNA target prediction scheme utilizing complementary TargetScan v7 (TS) and RNA22v2 (R22) was employed, using the following relatively stringent parameter settings to identify the highest-precision targets: context++ score <-1 for TS; no mismatches/wobbles in seed, heteroduplex (HD) energy <-20 kcal/mol, ≥14 base pairs in HD, and "Sensitivity of 21%, Specificity of 92%" for R22. **Results:** COX and PGF BLCLs demonstrated differential miRNA expression and predicted targeting at polymorphic sites of their respective DPB1 genes. Differential predicted targets were identified in exon 2, introns 1, 2, and 3 and the 3'-UTR of the two DPB1 sequences. These identified miRNAs have the potential to explain altered protein expression levels via putative cytoplasmic or nuclear miRNA effects. rs9277534 is not included among the high-confidence targeted sites; however, if thresholds are relaxed, then that region is differentially targeted as well. **Conclusions:** We have identified miRNAs with differential expression in COX and PGF BLCLs, which demonstrate unique predicted

targeting patterns at polymorphic sites of their respective HLA-DPB1 alleles. This catalog of miRNAs can now be tested in further experiments to identify true mediators of HLA-DPB1 expression and help to identify actual causative polymorphisms in such regions that involve extensive and confounding linkage disequilibrium.

OR21

HLA-C DOWNREGULATION BY HIV ADAPTS TO HOST HLA GENOTYPE

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Aim: It was recently found that HIV can downregulate HLA-C on infected cells. This is mediated by the viral protein Vpu, and is in addition to the downregulation of HLA-A/B by Nef. The magnitude of HLA-C downregulation varies widely between primary HIV viruses, but the selection pressures resulting in either HLA-C downregulation or preservation are not clear. **Methods:** To better understand viral immune evasion targeting HLA-C, we have characterized HLA-C downregulation by a range of primary HIV-1 viruses. **Results:** 100 replication competent viral isolates, from limiting dilution outgrowth assays of 20 individuals with effective anti-retroviral therapy, show a substantial minority of individuals harbor reservoir virus which strongly downregulates HLA-C. 20 longitudinal samples from 4 untreated individuals demonstrate no changes in HLA-C downregulation during acute infection, but show variation between viral quasispecies that can persist in chronic infection. 200 Vpu molecules cloned from plasma of treatment naïve individuals in chronic infection, show that downregulation of HLA-C adapts to host HLA genotype. HLA-C alleles differ in the pressure they exert for viral downregulation of HLA-C, and individuals with higher levels of HLA-C expression favour greater viral downregulation of HLA-C. Studies of primary and mutant molecules show the transmembrane regions of both Vpu and HLA are responsible for their interaction when removing HLA-C from the cell surface. **Conclusions:** Finding that reservoir virus can downregulate HLA-C could have implications for HIV cure therapy approaches in some individuals. The adaptation of HLA-C downregulation to host HLA genotype which is observed, indicates a subset of HLA-C alleles restrict HIV-specific CTL responses which are subverted by viral downregulation of HLA-C. Differential viral modulation of HLA-C, compared to HLA-A/B, can be used to understand differences in the biological roles of these HLA molecules in HIV infection.

OR22

HLA-G REGULATORY AND CODING REGION HAPLOTYPES IN PAPILLARY THYROID CARCINOMA

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Aim: To evaluate *HLA-G* coding and regulatory (promoter and 3' untranslated region-3'UTR) haplotypes in papillary thyroid carcinoma (PTC) patients and their associations with clinical and histopathological features. **Methods:** We studied 185 PTC patients and polymorphic sites distributed along the three different *HLA-G* gene regions were characterized by Sanger sequencing. *HLA-G* haplotype associations were analyzed using the Fisher exact test, calculating odds ratio (OR), confidence interval (CI) and *P*-values. **Results:** More than 90 variation sites were observed along the whole gene. Considering the **promoter region**, i) 010101d haplotype was less frequent in patients presenting classical histological variant of PTC (OR=0.2789, CI 95%=0.0755-1.0304, *P*=0.0499), ii) 0104a haplotype was less frequent in patients presenting tumor multicentricity (OR=0.3360, CI 95%=0.1446-0.7810,

P=0.0089), and iii) 0103a haplotype was more frequent in patients presenting advanced stage of PTC at diagnosis (TNM staging III and IV) (OR=0.3541, CI 95%=0.1360-0.9219, *P*=0.0370). Regarding the **coding region**, the *G**01:01:12$^{(+324G)}$ allele was associated with the presence of tumor multicentricity (OR=11.2857, CI 95%=1.3438-94.7784, *P*=0.0094) and Hashimoto's thyroiditis (OR=6.4851, CI 95%=1.2383-33.9649, *P*=0.0224). At **3'UTR**, the UTR-02 haplotype was overrepresented (OR=1.6759, CI 95%=1.0616-2.6456, *P*=0.0328) and UTR-03 haplotype was underrepresented (OR=0.4106, CI 95%=0.1912-0.8815, *P*=0.0200) in patients presenting tumor multicentricity. No association regarding tumor size, local invasion, metastasis at diagnosis and extrathyroidal extension was observed. **Conclusions:** Although HLA-G is expressed in more than 80% of PTC specimens, *HLA-G* alleles were primarily associated with tumor morbidity, indicating that local factors may transcriptional and posttranscriptionally modulate *HLA-G* expression.

OR23

HLA CLASS II GENES CORRELATE WITH PROTECTIVE NEUTRALIZING ANTIBODY TITERS IN A DENGUE VACCINE EFFICACY TRIAL

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Aim: A tetravalent, live attenuated dengue vaccine demonstrated efficacy, safety and immunogenicity in several clinical trials in Asia and Latin America. Efficacy differed based on infecting serotypes, presence of pre-existing dengue neutralizing antibody (NAb) titers and age. HLA class II molecules expressed on antigen presenting cells mediate CD4+ T cell stimulation of antibody production by B cells involved in vaccine-induced responses. We hypothesized that the differences in observed vaccine efficacy could be due to variation in NAb immune responses in conjunction with host HLA class II genes. **Methods:** Samples were available from a subset of subjects that took part in the first tetravalent dengue vaccine efficacy trial conducted in Thailand. DNA was extracted from 335 saliva samples and HLA genotyping was performed using next-generation sequencing (NGS) of full-length genes. A panel of ancestry informative markers (AIMs) was genotyped to assess population stratification. Serotype-specific NAb titers were measured by plaque-reduction neutralization test 28 days after last injection. The association of NAb titers and HLA class II on dengue infection was tested by logistic regression. Linear regression was used to test association of HLA class II alleles with NAb levels after accounting for sex, age, and serotype as covariates. A minimal false discovery rate to account for multiple comparisons, with a two-sided p-value<0.05 and q-value<0.20 was considered statistically significant. **Results:** NGS identified 197 HLA class I and II alleles in the Thai Dengue vaccine trial. AIMs analysis did not identify population stratification comparing cases and controls. Magnitude of NAb levels post vaccination was significantly higher in the presence of HLA-DRB1*11 (p=0.002,q=0.08). HLA-DPB1*03:01 and *05:01 presence correlated with pre-existing NAb titers in the placebos (p=0.005, q=0.09; p=0.001, q=0.04). We did not observe a direct effect of HLA on dengue infection in either the placebo or treatment arm. **Conclusions:** These findings suggest that specific HLA class II alleles modulate protective NAb titers in dengue infection. This is an exploratory study to identify signals to replicate in other dengue vaccine clinical studies. Understanding this HLA class II mechanism will enable improved vaccine design.

OR24

HLA ALLELE AND HAPLOTYPE FREQUENCIES CHARACTERIZED USING NEXT-GENERATION SEQUENCING METHODS IN UNRELATED WORLD-WIDE POPULATIONS: SUMMARY FROM THE 17th INTERNATIONAL HLA AND IMMUNOGENETICS WORKSHOP

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Aim: The primary goal of the 17th IHIWS was to advance the field of Histocompatibility and Immunogenetics research as well as clinical practices through the application of NGS technologies. Analyses of very-high resolution

NGS HLA profiles sampled from different populations will be useful for reconstructing human history and may improve match predictions for unrelated donor selection algorithms. **Methods:** In the unrelated population HLA diversity project, 15 laboratories from 10 different countries contributed HLA genotype data generated by various NGS methods. HLA population data included well-defined large datasets from the USA and East Asia and smaller samples from Europe, Australia, and Western Asia. Participants uploaded data in HML/XML format into the 17th IHIW database, data was systematically reviewed, and statistical analysis was performed using the genetic software PyPop v.0.7.0 to estimate; (i) allele frequencies, (ii) heterozygosity, (iii) conformity to HWE, (iv) selective neutrality, (v) 2-locus haplotypes and (vi) LD. The software BIGDAWG was used to estimate extended haplotype frequencies by an EM algorithm. Inter-population variance was determined using Nei's genetic distances which were then used to construct phylogenetic dendrograms. **Results:** Amongst each population, test of selective neutrality often revealed an excess of heterozygous compared with neutral expectations. A comparison of the HLA genetic diversity estimated at the 4 and 2 fields revealed that diversity at the majority of loci, particularly for populations of European descent, was lower at the 2-field resolution. African descent populations had a lower number of distinct HLA alleles at class I and II loci compared to other groups but exhibited higher levels of heterozygosity. Two-locus haplotype analyses revealed distinct HLA associations at the 4-field. Analyses of extended haplotype frequencies, estimated both in the presence and absence of DP loci, showed unique haplotypes that were common to each ethnic group although some haplotypes were shared amongst different ethnic groups. **Conclusions:** This workshop project provided a vast amount of new information about HLA genetic diversity at the intronic level and will be a useful resource for anthropological studies, disease association studies and transplantation.

OR25

THE HIGHLY POLYMORPHIC HLA-DRA GENE: CONSERVED AND DIVERSE REGIONS WITHIN THE HLA-DRA GENE IMPLY HAPLOTYPES OF DRA~DRB3/4/5~DRB1~DQB1

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Aim: HLA-DRA is highly conserved compared to the other HLA genes. The gene is represented in IPD-IMGT/HLA by 7 alleles encoding two distinct proteins, and we observed that the known DRA alleles have two deletions in intron 4, forming 3 characteristic patterns. We are interested in elucidating the polymorphism of DRA in relation to DR-DQ haplotypes using full-length genomic sequencing. **Methods:** Our laboratory performed full-length DRA sequencing on 70 samples, using MinION and confirming novel SNPs with Sanger SBT. Full-length reads were aligned against reference sequences from IPD-IMGT/HLA to identify sequence polymorphisms and deletion patterns. These polymorphisms were analyzed in the context of HLA-DRB1, -DRB3,4,5, and -DQB1 alleles. **Results:** HLA-DRA alleles represented in IPD-IMGT/HLA are a underestimation of the diversity in the sequence polymorphism. More than 40 novel DRA alleles were identified based on intronic polymorphisms, but exon 2 remains remarkably conserved. Polymorphisms in exons 3 and 4 were confirmed, but no new exonic polymorphism was identified. 19 previously unrepresented SNP positions were identified within the 5711bp region represented in IPD-IMGT/HLA, and the remaining SNPs are combinations of the 61 known polymorphisms. HLA-DRB1 alleles were correlated with the observed intronic deletion patterns. Specifically, DRA alleles corresponding to DRB1*04, DRB1*07, and DRB1*11 groups are correlated with one deletion pattern. DRA deletion patterns that are linked with the DRB1*03 and DRB1*13 were correlated with specific DRB3 and DQB1 alleles. Similarly, DRA deletion patterns correlated with DRB1*15 alleles were also correlated with DRB5 and DQB1. **Conclusions:** HLA-DRA is highly polymorphic, but has conserved exons, suggesting the gene plays a crucial role in immune responses. The observed polymorphism patterns suggest the existence of recombination site(s) within DRA, and the intron deletions give clues about the DRA~DRB3/4/5~DRB1~DQB1 haplotypes. Patterns in polymorphisms may represent different evolutionary lineages, suggesting different origins of the DRA genes in the ancestral haplotypes. Extending the samples for further analysis by computational haplotyping will elucidate the origin of the DRA genes in this haplotype.

Wednesday, October 3, 2018
2:30 PM - 4:00 PM
Abstract Session IV: Bone Marrow, HSCT, Chimerism

OR26

INVESTIGATING THE IMPACT OF PATIENT'S NON-SHARED HLA-C ALLOTYPE EXPRESSION LEVELS IN A 9/10 SINGLE HLA-C MISMATCHED HEMATOPOIETICSTEM CELL TRANSPLANTATIONSETTING USING TWO DIFFERENT HLA-C EXPRESSION PROXY MODELS

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Aim: Petersdorf et al. reported in 2014 an association between patient high expressed HLA-C (C) allotypes and inferior HSCT outcome using an imputed C-expression model (Apps et al. 2013). This study aims at: a) examining the validity of Apps et al. model in Caucasians by using the same methodology in a sample of 400 healthy German blood donors. b) specifically investigating the effect of patient's non-shared (PNS) C expression levels on outcome by applying C expression imputed data in a 9/10 HLA C-mismatched HSCT setting. **Methods:** Buffy coats from 400 healthy German blood donors were tested by flow cytometry as previously described (Apps et al. 2013) in order to determine C expression on lymphocytes. With reference to the mean fluorescence intensity (MFI) values measured, C antigens were categorized as high- or low- expressed. In a cohort of 324 single C-mismatched transplant pairs both C expression models were used in order to investigate the impact of high- and low-expressed PNS-C on HSCT outcome. Overall survival (OS), disease free survival (DFS), relapse incidence (RI) and non-relapse mortality (NRM) were set as outcome endpoints, while statistical significance was set at $p<0.05$. **Results:** With the exception of HLA-C*15, the two expression models were fully concordant as to the definition of low- and high-expressed C antigens. Analysis of PNS-C expression's effect on HSCT outcome revealed an unexpected correlation between high expression and better OS (49% vs 33% p=0.04; HR=0.43, p=0.002) due to lower NRM (37% vs 47%, p=0.02; HR=0.29, p<0.001). Although similar trends were observed, statistical significance was only met with the Apps et al. model. Death cause analysis revealed a tendency for lower infection-related mortality in the high expressed PNS-C (10% vs 14%), while no differences were observed for GvHD-associated death. Interestingly, overall patient's C expression had no influence on any outcome endpoint. **Conclusions:** a) HLA-C antigens exhibit similar expression patterns regardless of race. b) The lower OS observed in lower expressed PNS C mismatches may be attributed to inferior infection control and thus higher NRM. Albeit noteworthy, these findings must be confirmed by larger independent cohorts, before definitive conclusions can be drawn.

OR27

NOVEL NEXT GENERATION SEQUENCING BASED CHIMERISM ASSAY FOR ENGRAFTMENT MONITORING IN HEMATOPOIETIC CELL TRANSPLANTATION

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Aim: Most laboratories providing chimerism testing use electrophoretic analysis of STR loci or qPCR of SNP polymorphisms. STR assays are limited to 1-5% sensitivity and qPCR requires substantial amounts of DNA which may not be feasible in patients with low blood cell counts or for analysis of rare cell subsets. We developed a chimerism assay based on NGS of SNP and InDel polymorphisms and validated it in a clinical transplant

immunology lab supporting a large HCT program. **Methods:** Multiple polymorphic loci were evaluated for specificity, sensitivity and accuracy using artificial admixtures as well as direct comparison to qPCR results from patient specimens post HCT. An initial panel comprised 37 SNPs on 18 chromosomes and 12 InDels on 10 chromosomes. Target generation is performed in 2 multiplexed PCRs with 5 ng sample DNA per reaction. All NGS benchwork is accomplished within 6 hours, followed by a 7 hour MiSeq run, so that results are available for reporting early on day 2. **Results:** Initial NGS testing used artificial admixtures of unrelated individuals, with mixtures from 10% to 0.1%, see Table 1. On average, 30 informative markers were identified between unrelated individuals and NGS achieved 0.5% sensitivity among all 4 admixture sets. Table 2 shows the results for NGS versus qPCR for 7 patient post HCT specimens.

Table 1. NGS analysis of artificial admixtures of 2 unrelated individuals.

		Set 1		**Set 2**		**Set 3**		**Set 4**	
Titration	**Admixture ratio (%)**	**Ave (%)**	**SD**	**Ave (%)**	**SD**	**Ave (%)**	**SD**	**Ave (%)**	**SD**
1/10	10	10.47	1.20	9.95	1.40	11.73	3.72	11.43	1.06
1/50	2	2.13	0.22	2.15	0.70	2.48	1.14	2.15	0.43
1/100	1	1.17	0.21	1.28	0.50	1.13	0.33	1.13	0.33
1/200	0.5	0.68	0.31	0.63	0.21	0.59	0.26	0.59	0.26
1/500	0.2	0.42	0.23	0.33	0.23	0.31	0.13	0.31	0.13
1/1000	0.1	0.29	0.19	0.17	0.08	0.28	0.20	0.28	0.20

Table 2. qPCR of patient specimens post HCT.

	NGS		**qPCR**	
Case	**Patient %**	**SD**	**Patient %**	**SD**
1	99.41	5.28	100	0
2	2.63	4.7	1.56	2.49
3	2.67	4.04	1.09	0.13
4	1.36	4.39	1.06	0
5	3.97	0.72	5.27	1.09
6	12.84	2.48	11.21	0.79
7	4.1	2.44	3.44	1.27

Conclusions: These preliminary results show that NGS technology can provide accurate, informative, and sensitive detection of a minority species with minimal DNA quantity requirements, rapid turnaround and minimal hands on time. As laboratories implement NGS technology for high resolution genotyping of HLA and other genetic loci, extension to engraftment monitoring might also offer workplace efficiencies.

M.Z. Askar: *3. Speaker's Bureau; Company/Organization; Immucor. 4. Scientific/Medical Advisor; Company/Organization; Illumina.* ***S.E. Pereira:*** *4. Scientific/Medical Advisor; Company/Organization; Scisco Genetics Inc.* ***A.G. Smith:*** *2. Consultant; Company/Organization; Scisco Genetics Inc.* ***C. Pyo:*** *4. Scientific/Medical Advisor; Company/Organization; Scisco Genetics Inc.* ***W. Nelson:*** *5. Employee; Company/Organization; Scisco Genetics Inc.* ***D.E. Geraghty:*** *6. Stock Shareholder; Company/Organization; Scisco Genetics Inc.*

OR28

HAPLOIDENTICAL DONORS FOR HEMATOPOIETIC CELL TRANSPLANTATION (HCT): CHANGING DEMOGRAPHICS AND THE ECONOMICS OF TYPING THIS POPULATION

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Aim: HLA-mismatched allogeneic donors allow HCT of patients lacking an HLA identical sibling or matched unrelated donor. Most patients find a haploidentical or a partially-HLA mismatched donor among their first- and second-degree relatives or unrelated donors. We examined process improvements to reduce donor search time and cost in the new era of alternative donors and the less costly Next Generation Sequencing (NGS) technology for high resolution typing. **Methods:** HLA class I typing was performed using reverse sequence specific oligonucleotide probe (rSSO) hybridization (One Lambda, Inc) to identify potential haploidentical donors. Select donors were typed at high resolution using Sanger sequence base typing during 2015 and 2016 (local and commercial kits) or *NGS* in 2017 (lllumina). Specimen (blood) collection kits were sent to donors using a third party vendor (Path-Tec). **Results:** We performed a retrospective cost analysis of 2017 data. Currently, rSSO class I typing is performed on non-parent or child family members to rule out HLA disparate donors. Of 444 individuals, 188 appeared to be haploidentical and were reflexed to HRT. Only 3.9% of donors reflexed to NGS were disparate. If all 444 individuals had been initially typed by NGS, the cost for the program would have been 63.2% higher. As show in Table 1, the number of 2nd degree relatives has increased. As the donor pool expands beyond the patient's nuclear family, the incidence of incorrectly reported donor relationships has increased, causing inappropriate testing and delays in reporting results. Furthermore, the donors' geographical locations have expanded coast to coast, and even globally with increase of shipping costs. Of 413 kits sent in 2017, 20.1% were not returned.

Conclusions: With the steady increase in the use of alternative HCT donors, the staggered class I typing schema remains cost effective even with the advent of NGS. Areas for cost reduction include obtaining correct relationships prior to testing and improving the return rate of collection kits.

A.M. Jackson: *3. Speaker's Bureau; Company/Organization; One Lambda / Thermo Fisher Scientific.* ***M. Bettinotti:*** *3. Speaker's Bureau; Company/Organization; One Lambda / Thermo Fisher Scientific.*

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HIGHER PERCENT RECIPIENT CHIMERISM IN BONE MARROW THAN PERIPHERAL BLOOD IS RISK FOR RELAPSE

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Aim: In patients after allogeneic stem cell transplant, chimerism is monitored on peripheral blood (PB). Occasionally, bone marrow (BM) samples are tested for chimerism and results are often similar. We investigated the significance of results that differ between PB and BM. **Methods:** Retrospective review of post-allogeneic stem cell transplant patients, who were tested for chimerism on both PB and BM (total cells, unfractionated) within 7 days interval. The % recipient difference between PB and BM was compared with relapse/remission status (BM biopsy results, cytogenetics, molecular testing, CBC, and clinical follow-up documents. For patients with multiple time points of concurrent BM and PB results, only the first point was included in this evaluation. **Results:** Concurrent BM and PB chimerism results were compared in 74 patients. Median age at transplant was 54 years (range: 3-76 years). Date of PB and BM sample post-transplant was median 207 days (range: 27-3102). Test methods were XY FISH (N=9), short tandem repeat (N=55) and quantitative PCR (N=10). Diagnoses were myeloproliferative /dysplastic disease (N=61), aplastic anemia (N=2), lymphoma/lymphocytic leukemia (N=11). % Recipient was higher in BM than PB (difference: $\Delta \geq 2\%$) in 11 patients, and similar ($\Delta < 2\%$) in 63 patients. Of the 11 patients with

Δ ≥2%, disease relapse was detected in 5 patients at BM collection and two more patients within the following 2 months (total N=7/11). Of the 63 patients with Δ <2%, disease relapse was detected in 2 patients at BM collection and five more patients in the following 2 months (total N=7/63). In the patients with Δ ≥2% compared to the patients with Δ <2%, relapse was higher at BM collection (p=0.0005 by Fisher exact) and cumulatively in the following 2 months (p=0.000004, chi-square). Mixed chimerism status (recipient ≥5%) in PB was observed in 4/11 patients with Δ ≥2% and 9/63 patients with Δ <2%, not statistically different (p=0.76). Mixed chimerism was associated with relapse in the patients with Δ <2% (p=0.022, chi-square), but not in patients with Δ ≥2% (p=0.55, Fisher exact). **Conclusions:** Higher % recipient in BM than in PB indicates risk of concurrent/imminent relapse, regardless of PB mixed/complete chimerism status. This status may reflect increasing mixed chimerism.

OR30

HLA EXPRESSION ON CD34+ STEM CELLS: IS A FLOW CYTOMETRIC CROSSMATCH A GOOD GATEKEEPER FOR MISMATCHED HEMATOPOIETIC CELL TRANSPLANTION?

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Aim: Primary graft failure associated with donor specific antibody (DSA) is influenced by the level of circulating DSA at time of infusion and by the density of HLA antigens expressed on cell populations needed for engraftment. It is not yet known what HLA antigens are expressed on CD34+ stem and progenitor cells nor what level of DSA, if any, is permissible. We compared surface expression levels of class I (HLA-A, B, C) and class II (HLA-DR, DQ, DP) molecules on CD34+, B, and T cells to determine whether a lymphocyte flow cytometric crossmatch is an adequate method to assess risk of DSA mediated graft failure. **Methods:** Samples consisted of peripheral blood (PB) from PB stem cell donors or an aliquot of bone marrow (BM) aspirate from BM donors. CD34+, CD19+ (B cells) and CD3+ (T cells) were simultaneously analyzed using monoclonal antibodies specific for each HLA target and flow cytometry. Selected samples were analyzed by flow cytometry using dilutions of DSA containing sera and staining with anti-human IgG. **Results:** Despite individual variations, higher levels of HLA-A and B and lower levels of HLA class II were observed on CD34+ cells compared to lymphocytes (Fig 1). Titration of sera containing antibody specific for donor's HLA-A antigens revealed higher reactivity of CD34+ cells than of B or T cells (Fig 2-representative result). **Conclusions:** Antibodies specific for all class I and II HLA targets may be deleterious given their expression on CD34+ cells. HLA-A and B specific antibodies may be especially harmful given the high expression of these loci. Use of peripheral T and B donor lymphocytes flow cytometric crossmatch may underestimate the risk of graft failure due to the relative lower expression of HLA-A and B loci in these cell populations.

M.P. Bettinotti: *3. Speaker's Bureau; Company/Organization; One Lambda Thermo Fisher.* ***A.M. Jackson:*** *3. Speaker's Bureau; Company/Organization; One Lambda Thermo Fisher.*

OR31

CHARACTERIZATION OF THE CCR5 DELTA 32 MUTATION BY RACE IN UNRELATED DONORS LISTED ON THE NATIONAL MARROW DONOR PROGRAM'S BE THE MATCH REGISTRY

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Aim: Donors homozygous for the CCR5 delta 32 deletion are of interest in hematopoietic stem cell transplantation (HCT) for patients infected by both HIV-1 and a hematologic malignancy. The mutation has been published to confer natural HIV resistance to individuals carrying two copies, while heterozygous individuals show increased resistance and lower viral loads compared to wild type. The aim of this study was to characterize the CCR5 delta 32 deletion frequency in the diverse unrelated donor populations listed on the National Marrow Donor Program's Be The Match Registry. **Methods:** Next Generation Sequencing by the Illumina MiSeq was used to target the 32 base pair deletion in the CCR5 gene for 495,530 URDs between 2016-2018. The frequency of donors identified as homozygous, heterozygous and wild type for the mutation was calculated for each of broad race/ethnic groups; Black, Asian/Pacific Islander, White, Hawaiian, Hispanic and Native American Indian. **Results:** Overall, .73% URDs were identified as CCR5 delta 32 homozygous in our cohort. The frequency of homozygotes found in unrelated registry donors self-identified as race group of White was similar (.95%) to previously published data on European individuals (1%). The frequency of homozygotes in the remaining populations was low with .04% observed in Black, .01% in Asian/Pacific Islander, .21% in Hispanic and .24% in Native American. Within donors carrying the homozygous genotype, the population was highly homogeneous, with 98.9% self-identifying as White, given predominate donor recruitment numbers and higher frequency. **Conclusions:** The frequency of self-identified White U.S. registry donors homozygous for the CCR5 delta 32 deletion is consistent with previous studies on individuals of European descent. Given the low frequency of homozygous CCR5 delta32 genotypes in the unrelated donor population, additional treatment methods using multiply mismatched donors can extend the feasibility of this use case for concurrent HCT and infection therapy in HIV patients.

Thursday, October 4, 2018
2:30 PM - 4:00 PM
Abstract Session V: New Assays

OR32

DIFFERENTIAL DETECTION OF GLYCOSYLATION OF HLA DONOR SPECIFIC ANTIBODIES (DSA) USING SINGLE ANTIGEN MICROBEADS

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Aim: Differential glycosylation of Immunoglobulin G (IgG) is known to affect the functions of IgG by modulating the interactions with its binding proteins, such as C1q and Fc receptors. Our research aim was to develop an assay to analyze glycosylation of donor specific antibodies (DSA) using conventional single antigen microbead technology, and subsequently to analyze glycosylation profiles of kidney transplant patients with DSA and biopsy data for graft function assessment. **Methods:** Conventional Class I and II LABScreen® single antigen beads were pretreated to eliminate glycans on the Class I and II antigens. Patient serum was incubated with the pretreated beads according to the standard assay. Glycans of the DSA bound to the beads were detected with biotinylated lectins known to specifically detect sialic acid, galactose, or fucose residues, along with neutravidin-PE (phycoerithrin). Data acquisition was performed using a LABScan 3D™. **Results:** Pretreated Class I and II single antigen beads maintained DSA binding capabilities comparable to the untreated conventional beads when PAN IgG-PE was used as the detection agent. Using pretreated beads, glycans on the bound DSA were specifically detectable with lectin/neutravidin based reporters with slight modification of conventional LABScreen® single antigen protocol (Figure: Detection of Class I DSA with lectin specific to galactose vs. PAN IgG). **Conclusions:** We have demonstrated a simple and robust assay for detection of glycans on DSA using modifications of conventional LABScreen® single antigen technology. We used lectins as specific glycan probes and neutravidin-PE as a reporter. Further research is in progress to assess the clinical validity of the assay and to assess the predictive value of glycosylation profiles of Class I and II DSA on the outcome of kidney transplant recipients.

OR33

A NOVEL METHOD FOR HLA ANTIGEN SPECIFIC B CELL DETECTION

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Aim: HLA-Ag specific B cells play an important role in the allograft humoral immunity through producing donor specific HLA-Abs and presenting donor HLA-Ag to regulate allograft cellular immunity. The aim of the present study was to develop a HLA antigen specific B cell multiplex detection system through B cell receptor capture

approach by flow cytometry beads array platform(Flow-BCR) . **Methods:** LabScreen Single Antigen Beads (LSAB; LMX-IgG): One Lambda protocol; Flow-BCR: LSAB were co-incubated with $1.0X10^6$ patient PBMC for 1 hour, the cells were lysed and the beads were washed 3 times. The beads were resuspended in stain buffer containing PE-anti-hIgG and incubated for another 30 mins. After washes. the final beads were acquired in a Canto-II flow cytometer. The PE positive and negative beads of each population were gated and counted. **Results:** A hybridoma cell line with HLA-A2 membrane BCR was used to evaluate the method sensitivity. Flow-BCR was able to detect one A2-BCR hybridoma cell spiked into 1000,000 PBMC cells (Fig 1). A total of 55 patient PBMCs including 20 circulating HLA-Ab negative and 35 circulating HLA-Ab positive patient samples were tested by multiplex Flow-BCR. Compared to Non-sensitized group, significantly higher frequencies of HLA-Ag specific BCR cells were identified in HLA antigen class I and class II (Fig 2a,b) sensitized patient samples. **Conclusions:** The novel Flow-BCR is able to detect HLA-Ag specific memory B cells in sensitized patients and opens a new window of HLA antigen specific allograft immunity. The procedure of Fow-BCR is simple, specific, and very sensitive (1/1000,000 cells). It may provide a new tool to assess HLA sensitization before transplantation and monitor graft rejection after transplantation.

OR34

MODERNIZING ABO ANTIBODY DETECTION: DEVELOPMENT OF A BEAD-BASED ABO ANTIBODY DETECTION ASSAY

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Aim: Accurate, clinically relevant characterization of ABO antibodies (ABO-Ab) is critical to assess their impact in ABO incompatible transplantation. The current ABO-Ab detection method using erythrocyte agglutination is limited by lack of ABO subtype specificity, difficulty in ABO-Ab isotype differentiation, and poor reproducibility. We previously developed a slide micro-array method for ABO-Ab analysis to address these limitations. Our aim was to create a similar bead solid phase assay. **Methods:** ABO A subtype antigens (I,II,III,IV,V,VI) were coupled to Luminex beads and quantified using monoclonal ABO-Ab. Bovine serum albumin and alpha-Gal antigen were coupled as negative/positive control beads, respectively. Optimal plasma and anti-human IgG and IgM labelling-antibody dilutions were determined. IgG and IgM isotypes with specificities for ABO A-subtypes were measured (n=40 healthy donors) by mean fluorescent intensity (MFI). Levels of IgG *vs* IgM ABO-A-Ab were compared (Wilcoxon signed-rank test). Preliminary positive MFI thresholds for each ABO A-subtype bead were calculated based on ABO-A-Ab in ABO A control plasma. **Results:** Variation in ABO-Ab levels between ABO A-subtypes was detected. There was no significant difference between IgG and IgM ABO-Ab levels in paired MFI for all ABO types. IgG and IgM ABO-Ab are clearly detectable in all non-ABO A controls. Calculated MFI thresholds

accurately interpreted positive ABO-A-Ab in ABO O samples for the IgG assay and most beads in the IgM assay. ABO B controls had similar IgM ABO-A-Ab levels to ABO O controls but lower levels of IgG. Reactivity to alpha-Gal was present but inconsistent. **Conclusions:** This bead-based method successfully measures ABO-A-Ab and shows promise for clinical laboratory implementation. The specificity of these solid-phase assays will facilitate accurate assessment of ABO-Ab to subtypes, which are known to be expressed differently in endothelium than on erythrocytes. The ability to accurately measure both IgM and IgG ABO antibodies makes it possible to evaluate of the role of each ABO-Ab isotype in transplantation; this may be particularly relevant in the setting of plasmapheresis, which more efficiently removes IgM antibodies. Further optimization of this assay, controls, and ABO B and ABO H panel development are underway.

OR35

A NOVEL MIXED LYMPHOCYTE CULTURE METHOD TO EVALUATE DONOR HLA SPECIFIC IMMUNITY IN DIFFERENT HLA TYPE MISMATCHED DONORS AND RECIPIENTS

Hong Liu[1], Dolly B. Tyan[2], Ge Chen[1], [1]Stanford Blood Center HIDPL, Palo Alto, CA, [2]Stanford University, Pathology, School of Medicine, Palo Alto, CA.

Aim: A novel flow cytometry based mixed lymphocyte culture assay (Flow Cellular Crossmatch: Flow-CXM) was developed for detecting antigen or donor specific T cell immunity. The preliminary study demonstrated that the donor specific T cell response in recipient was correlated with HLA mismatch (MM) numbers between donors & recipients. The aim of this study is to further evaluate the significance of Flow-CXM in different HLA type MM donors and recipients. **Methods: CXM** (Fig 1): $0.1X10^6$ irradiated donor PBMCs were incubated with $0.1X10^6$ recipient's PBMCs and Th1 (IFNɣ & IL-2) or Th2 (IL-4, 5, 10) cytokine capture beads for 16 hrs. The cells were lysed and the captured cytokines were detected by corresponding PE-labeled 2nd antibodies. The beads with different fluorescence were gated and PE stained beads (Flowspot %) were counted to represent the relative number of donor specific responding T cells. **HLA Typing:** High-resolution HLA typing of all donor and recipient samples were performed by NGS or SSO. **Results:** A total 109 paired bone marrow donor and recipient samples were tested by Flow-CXM. Compared to the HLA identical group, IL-2 but not IFNɣ response significantly increased in HLA-class I (CI) ($P<0.05$) and HLA-class II ($P<0.0001$) MM groups. Furthermore, all single or multi-antigen or allele MM groups (A, B, DP, DR, A+C, DR+DP and DR+DP+DQ MM) have significant increased IL-2 responses as compared to HLA identical group (Fig 2). There are no significant differences on Th2 cytokine secretion between HLA identical and MM groups. **Conclusions:** The newly developed Flow-CXM is able to detect earlier T cell activation of donor HLA specific immunity in recipient within 24 hrs. The multiple T cell activation markers (Th1/Th2 cytokines) can be measured in a single reaction. The methodology of Flow-CXM is simple, sensitive, and HLA specific. It hold a potential to be used in clinical transplantation for predicting and monitoring GVHD/GVL in BMT and cellular rejection in solid organ transplantation.

OR36

NGS ALLELE-LEVEL TYPING STRATEGY FOR NINE KIR GENES

Loes A. van de Pasch, Karin van Ham, Sanne Vendelbosch, Maarten T. Penning, Erik H. Rozemuller, GenDx, Utrecht, NETHERLANDS.

Aim: Killer-cell Immunoglobulin-like Receptors (KIRs) are encoded by 17 genes in the Leukocyte Receptor Complex (LRC). These genes are highly polymorphic, on the level of structural variants, and copy-number variation. These characteristics make it challenging to design specific amplifications, and to perform reliable analysis of each KIR allele present in a sample. To enable allele-level resolution KIR genotyping, we developed an NGS-based strategy that involves whole gene amplification of KIR genes. **Methods:** We developed KIR-gene specific amplification primers, with specificity for one or more KIR genes (KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL4, KIR3DS1, KIR3DL1, KIR3DL2, KIR3DL3 and KIR3DP1). Amplicons were processed in the NGSgo® (GenDx) library preparation procedure (GenDx) and paired-end sequenced on an Illumina MiSeq platform (2x151 bp). Data was genotyped with NGSengine® software (GenDx), using KIR database IPD-KIR 2.7.1. **Results:** Multiple samples with known and unknown KIR genotypes were analyzed using this new strategy. KIR amplification, sequencing and subsequent typing was successful for the majority of samples. Many samples could be typed unambiguously, despite the fact that phasing was not fully accomplished due to the length of the genes (up to 17 kb). Remaining challenges in analysis are samples with KIR copy number variation and high degree of homology, which can hamper the data analysis. **Conclusions:** KIR genotyping at allelic level is feasible using the short read sequencing technology of Illumina. This new typing strategy may be an attractive alternative to existing KIR genotype assays that only determine KIR gene content at a limited resolution, or complex sequencing methods aiming to sequence the entire LCR.

L.A. van de Pasch: *5. Employee; Company/Organization; GenDx.* ***K. van Ham:*** *5. Employee; Company/Organization; GenDx.* ***S. Vendelbosch:*** *5. Employee; Company/Organization; GenDx.* ***M.T. Penning:*** *5. Employee; Company/Organization; GenDx.* ***E.H. Rozemuller:*** *5. Employee; Company/Organization; GenDx. 6. Stock Shareholder; Company/Organization; GenDx. 7. Other (Identify); Company/Organization; Founder of GenDx.*

OR37

A METHOD FOR ASSESSING IMMUNOGENICITY OF HLA-EPITOPES TO CIRCUMVENT POTENTIAL IMMUNOTOXICITY OF THERAPEUTIC GENE EDITING

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Aim: Targeted genome editing has tremendous potential for treating monogenic diseases, especially for those that affect the hematopoietic system. Efforts to reduce the immunogenicity of administered proteins such as Cas9 and Cas12a are critical for ensuring safe CRISPR treatment. Moreover, corrections via gene editing could result in novel HLA-epitopes for host defense. We aim to expand our Minor Histocompatibility Antigen Identification Pipeline (MiHAIP) to automatically assess the immunogenicity of HLA-epitopes for optimization of therapeutic gene editing. **Methods:** Using ClinVar as a source of pathogenic target mutations, MiHAIP simulates antigen processing given certain genetic variants and HLA allele types. NetMHCpan was used to predict the binding affinities of all possible peptides (8- to 11-mers) to the HLA alleles. Thirty-two HLA class I alleles with sufficient known binders as a training set were included in the experiment. The outputs from MiHAIP were used to generate an HLA-peptide-affinity matrix for downstream analyses. **Results:** From 57,484 missense variants in ClinVar, 932,052 binders were returned out of 40,993,568 HLA-peptide combinations. Approximately 31.8% are considered to be the strong binders. Examination of the variants of typical monogenic diseases uncovered alterations in amino acids that commonly affect the immunogenicity of HLA-epitopes. This indicates that editing these variants may turn a non-binder into a binder. In sickle cell anemia, the homozygous allele of rs334(T) encodes valine[V] that affects the disease. However, carriers of rs334(T) and rs334(A/G/C), which encode glutamic acid [E], glycine [G] and alanine [A] respectively, are disease free. Our results show that peptides of TP[E/A/V]EKSAVTAL are strong binders of HLA-B*07:02, and that editing on both rs334(T>C) and rs334(T>A) could introduce high immunogenicity epitopes. In contrast, editing rs334(T>G) could be a better option if patients have HLA-B*07:02 allele. This approach enables us to assess the immunogenicity of HLA-epitopes from any variants. **Conclusions:** This is a preliminary strategy paired with an efficient analytical pipeline to evaluate immunogenicity for therapeutic gene editing. It could be extensively applied to screen appropriate editable targets to circumvent immunotoxicity.

OR38

NOVEL METHOD USING REVERSE ENGINEERING TO DEFINE HLA EPITOPE ANTIBODY TARGETS

Fumiko F. Yamamoto[1], Lin Wang[2], Chia-Jung Chang[3], Dolly B. Tyan[4], [1]Stanford Hospital, Stanford Blood Center, Palo Alto, CA, [2]Stanford Histocompatibility, Immunogenetics & Disease Profiling Laboratory, Palo Alto, CA, [3]Stanford Genome Technology Center, Palo Alto, CA, [4]Stanford University, Palo Alto, CA.

Aim: Donor specific antibodies (DSA) lead to increased risk of antibody mediated rejection (AMR), but not all mismatched HLA induce an antibody response. Using full length genomic DNA next generation sequencing (NGS) for actual recipient and donor pairs, and sera known to contain DSA by single antigen beads (SAB), we developed a novel computer algorithm to define the exact regions being recognized, unbiased with respect to locus or position in the transcribed sequence including the leader sequence. **Methods:** We analyzed 241 kidney and cardiac transplant pairs for DSA using SAB (One Lambda) and 17th IHIWS standardized procedures for Luminex® IgG and Bio-C1q. Donor and recipient were high resolution typed at all loci using TruSight HLA v2 Sequencing Panel and analyzed by Assign 2.1 (Illumina). We compared triplet amino-acids (AA) between donor and recipient starting at the first AA in exon 1 and stepping down the entire transcribed sequence of every allele one AA at a time, identifying every triplet present in the donor but not the recipient, irrespective of locus. We developed an in-house "triplet scanning program" to perform these analyses instantaneously. We grouped pairs by DSA, starting with those with one or a limited set of DSA(s), e.g., A2 by IgG (N=6) or C1q (N=5), and mapped the residues on 3-D images using iCn3D Structure Viewer. **Results:** Two A2 DSA triplet clusters were common to IgG and C1q, 2 more were observed only for C1q, and one more was observed more often for IgG. For DQ2, 5 common DQA1*05:01 clusters and, separately, 5 common DQB1*02:01 clusters were found by IgG (N=12) and/or C1q (N=8). Failure to see an expected triplet in the cluster was explained by a shared recipient triplet at another locus. **Conclusions:** SAB and NGS provide comprehensive data to map serologically reactive epitopes. Using an unbiased computer algorithm, we have found clear serologic epitopes shared by multiple disparate donor/recipient pairs increasing confidence in the definition. Differences between IgG+ and C1q+ epitopes provide an opportunity to assess clinical relevance of the

two. Clear and unbiased epitope definition will allow the creation of a robust database and an opportunity to develop a standardized nomenclature.

Thursday, October 4, 2018
2:30 PM - 4:00 PM
Abstract Session VI: Solid Organ Pre/Post - Transplant Testing II

OR39

POTENTIAL CLINICAL RELEVANCE OF ANTI-HLA-DP ANTIBODIES TARGETING THE 96K AND 96R EPITOPES

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Aim: HLA-DP is conserved across the human population with the majority of polymorphism occurring at six hypervariable regions (HVR) within the β-chain. Anti-DP antibodies commonly react to specific epitopes in close proximity to AA 55, AA 84, and to a lesser extent the AA 33 region. Reactivity to these polymorphic HLA-DP epitopes can be associated with a positive flow crossmatch when single antigen bead (SAB) reactivity is high. Here we confirm another variable region of HLA-DP located at AA 96, and characterized by either a lysine (96K) or arginine (96R) residue. **Methods:** We identified two paradoxical, robust, HLA-DP reactivity patterns by SAB, and MFI data was analyzed with HLA Matchmaker to investigate potential epitope patterns—which suggested variability at AA 96. Using patient serum samples obtained before and after the appearance of AA 96 reactivity, we performed surrogate flow crossmatch to determine the validity of antibodies targeting AA 96 epitopes. **Results:** Using HLA Matchmaker, we have verified a variable region at AA 96, and have observed that differences at this position are capable of eliciting 96K (DP: 1, 3, 5, 6, 9, 10, 11, 13, 14, 15, 18, 19, 20) or 96R (DP: 0201, 0401, 0402, 17, 23, 28) antibody response. We identified a patient that was homozygous for 96R, received a lung from a heterozygous 96K/96R donor, and quickly developed strong anti-96K DSA determined by SAB. Flow crossmatch of this 96K reactive serum versus a surrogate donor homozygous for the 96K epitope (DP5, --) yielded a strongly positive B-cell crossmatch, while crossmatch versus a surrogate donor that was heterozygous at AA 96 (K/R: DP9, DP0201) only elicited a weakly positive B-cell crossmatch. In comparison, serum samples targeting the 96R epitope were also identified and additional surrogate testing is ongoing. **Conclusions:** We have confirmed a HLA-DP epitope located at AA 96 with potential clinical relevance. Antibodies targeting the 96K epitope were capable of eliciting a positive B-cell crossmatch. Additional surrogate testing is ongoing to better characterize HLA-DP 96K and 96R antibodies.

OR40

INTEGRATIVE ANALYSIS OF HLA NGS GENOTYPING DATA AND SINGLE ANTIGEN BEAD ASSAYS FOR MAPPING SEROLOGIC EPITOPES

Chia-Jung Chang[1], Dolly B. Tyan[2], [1]Stanford Genome Technology Center, Palo Alto, CA, [2]Stanford University, Palo Alto, CA.

Aim: Defining HLA serologic epitopes contributing to antibody-mediated rejection (AMR) is a key to untangle the molecular mechanisms of epitope recognition. We proposed a reverse engineering strategy to define new epitopes from the protein sequences of transplant recipients sharing the same donor specific antibody, and developed a set of tools implementing the idea. **Methods:** The hypothesis is that amino acid (AA) polymorphisms shared between the recipient and the donor will not be immunogenic and therefore cannot lead to the generation of antibodies. Without other assumptions, we developed a program which: 1. retrieves all AA triplets of the recipient by sliding a window of size three with a step of size one starting from the first AA on the full AA sequences of all the alleles, 2. reads the AA sequences of the donor with a sliding window in the same manner and outputs the allele, the position, and the triplet itself whenever an AA triplet is not in the triplet set of the recipient. We call the results unbiased donor specific AA triplets because we do not restrict the comparison within the same class nor to just the mature protein. We also developed web-based functions in the 17th IHIWS database to collect the unbiased triplets so that we could group the transplant pairs with the same DSAs and find shared unbiased triplets within the groups as potential epitopes. Please see the figure for an illustration.

Results: To verify the strategy, we collected the NGS HLA typing combined with the donor specific antibodies identified by Luminex® IgG and Bio-C1q SAB for 206 renal and 133 cardiac transplant recipients with AMR from nine labs. We ran the program and imported the results into the 17th IHIWS database, further mapping the epitopes onto the 3D protein structure for reference. **Conclusions:** We proposed and verified an unbiased reverse engineering strategy to define new serologic epitopes.

OR41

POTENTIAL ROLE OF NON-HLA ANTIBODIES IN C4D NEGATIVE AND C4D POSITIVE ANTIBODY MEDIATED REJECTIONS [AMR] FOLLOWING LIVING/DECEASED DONOR RENAL TRANSPLANTATION

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Aim: To investigate the prevalence and potential immunological significance of antibodies reactive against non-HLA antigens in acute/chronic antibody mediated rejection/chronic rejection evidenced by histopathological findings. **Methods:** 19 Patients with biopsy proven C4d+ AMR; 37 with C4d- AMR, and 8 patients with T-cell mediated rejection [TCR] were tested for a panel of 39 non-HLA antibodies using a commercial Kit. The ability of these non-HLA antibodies to bind C1q was also done. The patients were scored positive for non-HLA antibodies based on a 95% cut off above the normal limits for each antibody in the general population. **Results:** All patients had AMR with varying degrees of Interstitial Fibrosis and Tubular Necrosis. Majority of patients with C4d+ / C4d- AMR had more than 3 non-HLA antibodies [39 total targets] above 95% cut off. Most also had donor-specific HLA antibodies [DSA]. No specific pattern in terms of antibodies against structural proteins affecting cellular transformation, migration and fibrosis were observed. Notably, high levels of Glutathione S-Transferase tehta-1[GSTT1] antibodies with Acute Tubular Necrosis [ATN] was predominant in C4d- AMR cases [60%] while only 8% with C4d+ AMR patients with GSTT1 had ATN. Only 25% patients with TCR had Non-HLA antibodies and only towards two targets while almost 85-90% of AMR patients had multiple non-HLA antibodies. **Conclusions:** While non-HLA antigens like GSTT1 could be polymorphic and may induce allogenic response, many others could be against cryptic antigens exposed by injuries due to DSAs, or against altered self-antigens. Distinguishing immunological rejection from non-immunological injuries due to potential concurrent ATN and immunological injuries is challenging. The GSST1 antibodies detected were mostly C1q binding, but the mechanistic details of this finding is not clear now. The significance of this non-HLA Ab assay depends on more research into the genesis and predictive value of these pre-existing antibodies towards immunological rejection. Our current data tend to correlate with the concept of alloimmune mediated injuries leading to autoimmune responses based on concurrent HLA DSA

and Non HLA antibodies. However AMR with no HA DSA and with non-HLA ab will be required for the de novo induction of non-HLA antibodies.

OR42

REACTIVITY OF MONOCLONAL ANTIBODIES FOR THE DETECTION OF HLA CLASS II SURFACE EXPRESSION: MIXING APPLES WITH ORANGES?

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Aim: The flow cytometry crossmatch (FCXM) is a widely used method to assess pre-transplant immunologic risk. One variable that may affect FCXM reactivity is variability in lymphocyte HLA surface expression. Therefore, assessing HLA density on donor cells in FCXM can help guide FCXM interpretation, particularly HLA Class II, due to the inherent high background reactivity of B cells. Using single antigen beads (SAB), we defined the reactivity of two commercially available monoclonal antibodies (moAb) that claim pan-reactivity to all HLA class II. **Methods:** Mouse anti-human HLA class II clones WR18 and Tu39 were tested by Luminex SAB from two vendors. PE-labeled goat anti-mouse IgG was used as a detection antibody for both clones. Multiple dilutions of the moAb were tested to ensure bead saturation. Patterns of reactivity across antigens of different HLA loci were compared. **Results:** Whereas WR18 bound to all HLA-DR antigens, Tu39 showed lower reactivity to DR7, DR12, and specific alleles of DR9 (DRB1*09:01), DR14 (DRB1*14:01, 14:04 and 14:54) and DR52 (DRB3*01:01 and 03:01) [Fig. 1A, B]. This pattern aligned with a lack of reactivity to a specific epitope 60Y on these HLA alleles. Although reactivity varied, WR18 detected all HLA-DQ antigens. In contrast, Tu39 only bound to DQ2, DQ4 and a specific allele of DQ6 (DQB1*06:01) [Fig. 1C, D]. HLA-DP was detected by both clones with similar broad reactivity to all antigens. **Conclusions:** In contrast to WR18, Tu39 lacks reactivities to specific HLA-DR and -DQ antigens. This limited reactivity would affect assessment of HLA expression in FCXM when donor HLA types include antigens to which Tu39 is weakly or not reactive. Detailed knowledge of the specific reactivities of commercially available moAb is imperative for their usage in FCXM when analysis of HLA expression is part of the assessment. This information may also be valuable to the broader scientific community that may not have the technology readily available to investigate HLA-specific reactivity of moAb.

OR43

RACIAL INEQUITY AMONG 0-ABDR MISMATCHED KIDNEY RECIPIENTS

Robert A. Bray, Howard M. Gebel, Emory University Hospital, Atlanta, GA.

Aim: The implementation of the new Kidney Allocation Schema (KAS) in Dec. 2014 brought about important changes to deceased donor (DD) kidney allocation. Most notable has been for Highly Sensitized candidates who receive national, regional and local priority based on CPRA. However, the second ranked allocation category, 0-ABDR mismatch (MM), has actually experienced a drop in transplant rates from ~8.2% pre-KAS to ~4.7%, 1yr post-KAS(AJT 16:1834; 2016). As both equity and utility are part of UNOS mission, we sought to assess the racial makeup of 0-ABDR MM recipients and donors. **Methods:** A data request was made via the OPTN web site (optn.transplant.hrsa.gov/data/request-data/) and the returned data-set contained 1,517 0-ABDR mismatched kidney recipients transplanted between 12/04/2014 and 6/30/2017. Demographics collected included: CPRA, Race, Gender and HLA type. Analysis focused on the racial distribution of recipients by the following CPRA groups: 100%, $\geq$50<100%, >0<50 and 0%. **Results:** Among 0-ABDR MM recipients, 47% were females while 60% of the donors were male. The overall racial distribution of DDs for 0-MM recipients was: White: 78.4%, Black: 4.2%, Hispanic: 14.2%, Am Indian-Alaska Native: 0.7%, Hawaiian-Pacific Islander: 0.06% and Multiracial: 0.7% which differs from all DDs. The racial distribution of 0-MM recipients, by CPRA group, is shown in Figure 1. **Conclusions:** Across all CPRA groups, African Americas (AA) and Asians receive disproportionally fewer 0-ABDR mismatch allografts than Whites or Hispanics when compared to their proportion of the wait list. Among the 100% CPRA group AAs receive more transplants than Hispanics likely due to KAS national allocation policy. However, in all other CPRA groups, Whites and Hispanics receive more allografts than any other group likely due to the racial composition of DDs. Given that the frequency of 0-ABDR MM transplants has dropped significantly since KAS, and the clear racial inequity in allocation, it may be time to reassess the priority/utility of this category.

OR44

HLA EXPRESSION FOLLOWING LYMPHOCYTE ENRICHMENT: TISSUE SOURCE STILL MATTERS FOR DECEASED DONOR CROSSMATCHING

Elizabeth M. Moore, Donna P. Lucas, Karl Schillinger, Melissa Jeresano, Annette M. Jackson, Johns Hopkins University, Baltimore, MD.

Aim: We previously reported differences in HLA expression on lymphocytes isolated from different tissues and the impact tissue source can have on reactivity. In the current study, we re-examined HLA expression and the impact of lymphocyte enrichment using multiple class I and II specific monoclonal antibodies and different fluorochromes. **Methods:** Deceased donor lymphocytes were isolated from spleen, nodes, and peripheral blood. Following ficoll hypaque (FH) isolation, the EasySep™ Total Lymphocyte Enrichment Kit was used to increase purity of lymphocytes by negative selection. Cells were stained with four sets of monoclonal antibody-fluorochrome combinations. Data were acquired on either the BD FACSCanto™ II or the BD FACSAria™ using FACSDiva™ software. **Results:** Lymphocytes isolated from deceased donors were assessed for variability of HLA expression among different tissues and mean values are presented in Table 1. Lymphocytes isolated from spleen had two-fold greater class I expression as PBLs and two-fold greater class II expression as nodes. Results using different combinations of monoclonal antibodies for class I and II show similar findings. HLA expression measurements were higher for lymphocytes isolated with bead-enrichment compared to FH isolation alone (not shown). Paired analysis using class I specific FITC labeled antibodies revealed lower than expected class I expression on B cells indicative of a possible autoquenching artifact due to similar excitation and emission spectra (not shown). **Conclusions:** Similar to our previously published results, the spleen derived lymphocytes consistently yielded the highest HLA expression across T and B cells. We observed similar results across antibody clones but identified a possible FITC autoquenching artifact. Given increased transplant rates for highly sensitized candidates and the role of crossmatch tests to confirm compatibility, these HLA expression data should be corroborated in a larger dataset and used to guide deceased donor procurement protocols.

A.M. Jackson: *3. Speaker's Bureau; Company/Organization; ThermoFisher.*

OR45

IS IT SAFE TO TRANSPLANT A KIDNEY FROM DECEASED DONORS IN THE PRESENCE OF DSAs BUT WITH A NEGATIVE FLOW CROSS MATCH?

Jorge Neumann, Juliana Montagner, Tiago Schiavo, Elizete Keitel, Valter Garcia, Santa Casa De Porto Alegre, Porto Alegre, BRAZIL.

Aim: We observed a strong association between MFI values > 5k (SAB) on anti-A, B and DR DSAs and a positive flow cross. Since 2011 we do not transplant in the presence of one or more DSAs over this value. Our hospital offers a deceased donor kidney to every patient, provided that the flow cross is negative, regardless of the presence of DSAs below 5k MFI. The purpose of this paper is to observe if this policy is justifiable, comparing the results of our transplants performed with and without DSAs. **Methods:** 360 deceased kidney Tx performed between March 2015 and December 2017 with minimum 3 months follow up were included. The graft survival, number of rejections and last serum creatinine was compared in 306 non-DSA and 54 DSAs. **Results:** The overall graft survival was 94.4% for the DSA group and 92.5% for the non-DSA group (P=0.611). The mean creatinine was 1.65 (SD 0.92) in the

DSA group and 1.61 (SD 1.19) for the non-DSA group (P=0.822). The proportion of patients with rejection was 19.73% in the DSA group and 15.09% on the non-DSA group (P=0.568). Mean PRA was 37% (Class I) and 41% (Class II) for the DSA group, and 9% (I) and 5% (II) for the non-DSA group. Five patients on the DSA group had PRA Class I >90%. Four (80%) are free of dialysis, and 6 patients had PRA Class II >90%. Five (83%) are free of dialysis. Of interest is the finding that 42% of the DSAs were directed to cryptic epitopes. **Conclusions:** Our results showed no difference on survival, function and number of rejections between the patients transplanted with or without DSAs, provided that the DSA MFI is < 5k and the flow cross is negative. We believe that our policy to offer a deceased kidney donor to our patients even in the presence of low (>1,000 to 5k MFI) DSA levels is justifiable.

Thursday, October 4, 2018
2:30 PM - 4:00 PM
Abstract Session VII: Case Studies

OR46

DONOR-DERIVED ANTI-HLA ANTIBODIES IN A HAPLOIDENTICAL HEMATOPOIETIC CELL TRANSPLANT RECIPIENT SHORTLY AFTER TRANSPLANT

Paula Y. Arnold, Shane Cross, Brandon Triplett, St. Jude Children's Research Hospital, Memphis, TN.

A 15-year-old Caucasian male with high risk AML was referred to our institution for investigational therapy after relapsing from an 11/12 HLA-A mismatched (graft vs. host direction only) unrelated donor hematopoietic cell transplant (HCT). Prior to second HCT, the patient's serum was negative for antibodies to class I and class II HLA by microarray single antigen bead assay. The patient received two progenitor cell infusions from his haploidentical mother; the first was depleted of TCRαβ+ cells and the second was depleted of CD45RA+ cells. These procedures remove naïve T cells, the major alloreactive component of donor T cells, while preserving TCRγδ+ and CD45RO+ memory T cells respectively, which may provide infection control and graft vs. leukemia effects. Eight days post-transplant and prior to neutrophil engraftment, the patient became refractory to platelets and was found to be highly sensitized to multiple HLA-A and B antigens, although none were donor-specific. Because the patient received rabbit anti-thymocyte globulin (rATG) during the preparative regimen, there was a concern that antibody reactivity could be due to residual rATG. However, dilutions of the rATG preparations from the same lots used in the patient revealed no specific activity in the single antigen bead assay. Serum from the patient's mother/donor was tested and found to be positive for many of the same antibodies present in the patient, suggesting the patient's antibodies originated in the donor. Antibody testing of patient serum at five and seven weeks post-transplant showed magnified and expanded sensitization, despite undetectable circulating B cells secondary to administration of rituximab in the preparative regimen for EBV prophylaxis. Escalating sensitization is unlikely to reflect passive transfer of antibody from donor to recipient. Plasma cells are long-lasting, antibody-secreting B cells that do not express CD20 and therefore would not be targets for destruction by rituximab. Taken together, the data indicate active transfer of rituximab-resistant antibody-producing passenger lymphocytes (likely plasma B cells) from a haploidentical donor to a transplant recipient at the time of progenitor cell infusion.

B. Triplett: *7. Other (Identify); Company/Organization; Travel support (flight, hotel and registration) from Miltenyi to present previously published data at EBMT.*

OR47

THE MYSTERY OF THE MISSING DRB1 ALLELE - SOLVED BY NGS!!!

Anajane G. Smith[1], Shalini E. Pereira[2], Amanda Willis[3], Chulwoo Pyo[1], Wyatt Nelson[1], Medhat Z. Askar[3], Daniel E. Geraghty[1], [1]Fred Hutchinson Cancer Research Center, Seattle, WA, [2]University of Washington, Seattle, WA, [3]Baylor University Medical Center, Dallas, TX.

The mystery began when HLA typing, of two apparently unrelated individuals at two different institutions, identified the presence of a DRB5*01 gene without any evidence of an expected linked DRB1*15, DRB1*16, or, in rare cases, DRB1*01 allele. Historic data from Sanger sequence-based typing included DRB1*14, DRB3*02:02, DRB5*01:01, DQB1*05:03, 06:02 for Individual 1 and DRB1*04:01; DRB4*01; DRB5*01:01; DQB1*03:01, 06:02 for Individual 2. Since those analyses only assessed exon 2, the apparent absence of a DRB1 allele associated with the DRB5 gene raised the possibility of an entire linked DRB1 gene having gone missing. Both specimens were recently re-examined using next generation sequencing technology. HLA-A, B, C genes were assessed for exons 1-7; DRB1, DRB3/B4/B5, DQA1, DQB1, and DPB1 were assessed for exons 1-4; and DPA1 for exons 2-4. In

Individual 1, NGS identified DRB1*14:54:01 and a DRB1*15 exon 1 sequence, but without any evidence of DRB1*15 sequences for exons 2 and 3. Since the exon 4 sequences of DRB1*14:54:01 and DRB1*15 are identical, it could not be determined whether the DRB1*15 exon 4 sequence was present. Individual 2, typed as DRB1*04:01:01 and, again, a DRB1*15 exon 1. In the latter case, it was clear that no exon 2, 3, or 4 sequences were present for the DRB1*15 gene, since exon 4 sequences differ between DRB1*04:01:01 and DRB1*15. The table shows the full NGS typing data. The HLA alleles shared by both individuals suggest a putative extended haplotype: A*25:01:01-C*12:03:01-B*18:01:01-DRB1*15new-DRB5*01:01:01-DQA1*01:02:01-DQB1*06:02:01-DPA1*01:03:01-DPB1*23:01:01. NGS typing solved the missing allele mystery through identification of a truncated DRB1*15 allele comprising an exon 1 sequence but without exons 2, 3, or 4. Similarly, there is DRB4*03:01N which has an exon 3 sequence of a DRB4*01:01, but without an exon 2 sequence. These unusual alleles appear to be the results of historic multi-exon deletion events and add to the astounding diversity of the human major histocompatibility complex.

Proposed Likely Haplotypes

	A	B	C	DRB1	DRB345	DQA1	DQB1	DPA1	DPB1
Baylor-105	*01:01:01	*18:01:01	*07:01:01	*14:54:01	B3*02:02:01	*01:04:01	*05:03:01	*01:03:01	*81:01
	***25:01:01**	***18:01:01**	***12:03:01**	***15 variant**	**B5*01:01:01**	***01:02:01**	***06:02:01**	***01:03:01**	***23:01:01**
HIP01149	*02:01:01	*44:02:01	*05:01:01	*04:01:01	B4*01:03:01	*03:03:01	*03:01:01	*01:03:01	*04:01:01
	***25:01:01**	***18:01:01**	***12:03:01**	***15 variant**	**B5*01:01:01**	***01:02:01**	***06:02:01**	***01:03:01**	***23:01:01**

A.G. Smith: *2. Consultant; Company/Organization; Scisco Genetics Inc.* ***S.E. Pereira:*** *4. Scientific/Medical Advisor; Company/Organization; Scisco Genetics Inc.* ***C. Pyo:*** *4. Scientific/Medical Advisor; Company/Organization; Scisco Genetics Inc.* ***W. Nelson:*** *5. Employee; Company/Organization; Scisco Genetics Inc.* ***M.Z. Askar:*** *3. Speaker's Bureau; Company/Organization; Immucor. 4. Scientific/Medical Advisor; Company/Organization; Illumina.* ***D.E. Geraghty:*** *6. Stock Shareholder; Company/Organization; Scisco Genetics Inc.*

OR48

HAPLOIDENTICAL HEMATOPOIETIC CELL TRANSPLANTATION IN A HIGHLY SENSITIZED PATIENT WITH DONOR-SPECIFIC ANTIBODIES- A SUCCESS STORY

Po-Chien (Elaine) Chou[1], Ewelina Mamcarz[1], Sallyanne C. Fossey[2], Paula Y. Arnold[1], [1]St. Jude Children's Research Hospital, Memphis, TN, [2]DCI Transplant Immunology Laboratory, Nashville, TN.

A nine-year-old female with refractory Acute Myelomonocytic Leukemia (AML) was referred to our institute for Hematopoietic Cell Transplant (HCT) in 2015. While preparing for transplant, this patient became severely refractory to platelet transfusion. HLA antibody identification using single antigen bead technology revealed that she was highly sensitized to both HLA class I and class II antigens and had donor specific antibodies (DSA) to both parents (antibodies against HLA-B35 specific for her father; antibodies against A36 and DQ2 specific for her mother). Flow cytometric T cell allo-crossmatch of patient serum was positive with both parents. Unfortunately, this patient had no full siblings and an unfavorable matched unrelated donor search. The patient had few options other than haploidentical HCT from one of her parents and was therefore given a desensitization regimen of rituximab and plasmapheresis. Following two cycles of desensitization, overall class I PRA remained high (91%), but the patient's DSA against her father (B35) became negative. The patient's maternal DSA to DQ2 persisted. Patient/father T cell crossmatch after the desensitization treatment was diminished to borderline. The patient proceeded to HCT using her father as the donor, and was supported with HLA compatible platelets in the post-transplant period. She had neutrophil and platelet engraftment by days +13 and +19 respectively, following HCT. HLA antibody testing on the patient's serum nine months post-transplant revealed greatly reduced antibody positivity with only two positive class I specificities and no antibodies to class II. This patient continues to remain in remission nearly three years post-transplant. This case study highlights the value of desensitization regimens for patients with donor specific antibodies and few donor options for HCT.

OR49

A PITFALL OF VIRTUAL CROSSMATCHING IN PEDIATRICS: MULTIPLE SOLID PHASE REAGENTS CAN SHOW STRONG HLA ANTIBODIES THAT ARE NOT DETECTABLE IN FLOW CYTOMETRY CROSSMATCHES

Molly Weisert, Jondavid Menteer, Nicholas Fotiadis, William Lyle, Lee Ann Baxter-Lowe, Childrens Hospital of Los Angeles, Los Angeles, CA.

The usefulness of virtual crossmatching (VXM) is limited by the accuracy of current assays for HLA antibodies (Ab). We describe two cases of a specific false-positive pattern of class II Ab in toddler-age heart transplant (HTx) candidates. Subject 1 was a 13 month old female awaiting HTx for dilated cardiomyopathy, with no history of sensitizing events other than routine immunizations. Ab binding to DR4, DR7, and DR9 molecules in single antigen reagents from One Lambda (lot 11, 5,000 - 8,000 MFI) and Immucor (11,000 - 15,000 MFI) was suspect because it included a self-antigen (DRB1*07:01). These specificities were also detectable in sera pretreated with DTT or fetal calf serum as well as with phenotype reagents (One Lambda and Immucor). Surrogate flow cytometry crossmatches (SFCXM) with DR4+ or DR7+ B cells treated with pronase were negative (positive without pronase). DR4, DR7, and DR9 specificities persisted during the 3 mo transplant waiting period. The patient underwent successful HTx with a DR4+ donor. The FCXM with donor B cells was negative (positive without pronase). Soon after transplant the strong DR4, DR7, DR9-signature was not detected. Nearly 2 years after transplant, no clinical or pathologic Ab-mediated or cellular rejection has occurred. Subject 2 (DR1,13) was listed for HTx due to dilated cardiomyopathy as an 18 month old male, without history of sensitizing events other than routine immunizations. His HLA Ab profile with single antigen reagents (One Lambda lot 12) showed a similar DR4, DR7, DR9 pattern (9,000 - 14,000 MFI) which was supported by data from phenotype reagents. SFCXM with DR4+ and DR7+ B cells were negative. This patient is still awaiting heart transplantation, and donors with these HLA types will not be excluded by VXM. DR4, DR7 and DR9 avoidance would exclude 50% of the American population, which may tragically slow the HTx waiting process for this patient population. We are skeptical of this particular pattern of antibodies, particularly in toddlers without previous HLA presensitizing events. These observations suggest that exclusive use of VXM without cell-based crossmatches to support clinical significance of the antibodies may unnecessarily limit donors. Investigation is underway to determine the cause for this discrepancy between the solid phase assays and cell-based crossmatches.

OR50

DONOR SPECIFIC HLA ANTIBODY FORMATION FOLLOWING DISTAL TIBIAL ALLOGRAFT AND SUBSEQUENT GRAFT RESORPTION: A CASE REPORT

Christopher Liwski, Daryl Dillman, Michael Gross, Robert Liwski, Ivan Wong, Dalhousie University, Halifax, NS, CANADA.

Background*:* The association between donor specific Human Leukocyte Antigen (HLA) antibody formation and small bone allograft resorption has not been studied. We present the case of a patient treated for glenoid bone loss with a distal tibial allograft who formed donor specific HLA antibodies against the allograft and had subsequent graft resorption. ***Methods:*** The patient received a right glenoid reconstruction using distal tibia allograft with Bankart repair in June 2015. X-Ray and CT-Scans were performed pre-and post-surgery at standard checkpoints. Patient blood and serum samples were collected pre-and-post-surgery for HLA typing and HLA antibody testing. ***Results:*** Pre-operative CT scans revealed large Hill-Sachs and bony Bankart lesions, and pre-operative HLA antibody testing revealed no HLA antibodies. The 2-week post-operative radiograph revealed proper graft fixation, and the patient had good range of motion 6-weeks post-surgery. However, 6-week post-operative HLA antibody testing revealed development of donor specific HLA antibodies directed at HLA-A2 antigen as well as several antigens belonging to the A2 crossreactive group (A24, A68, A69, B57 and B58), cPRA of 69%. Eplet analysis showed reactivity to 62GE and 151AHV eplets. At 3-months post-surgery, the patient was weak in internal rotation, and the HLA-A2 antibodies continued to be detected at 5-months post-surgery. Shortly after the antibody peak, the 6-month post-operative CT arthrogram revealed significant graft resorption. Interestingly, the donor specific HLA-A2 antibodies were undetectable 6 months after resorption was complete. ***Discussion:*** This case report shows that small bone allografts can lead to HLA sensitization. The temporal correlation between donor specific HLA antibody formation and clinical findings strongly suggest an association between HLA antibody formation and graft resorption. It is unclear what impact HLA antibody formation may have on future regrafting in this patient. This case shows a clear association between the formation of donor specific HLA antibodies and graft resorption, which

suggests a link between the two events. Our future studies will address the frequency of HLA antibody formation, and investigate whether tissue processing techniques could be improved to reduce bone allograft antigenicity.

OR51

TO TREAT OR NOT TO TREAT: LONG TERM STABLE KIDNEY ALLOGRAFT FUNCTION IN PRESENCE OF STRONG COMPLEMENT-FIXING DONOR-SPECIFIC ANTIBODY (DSA) TO HLA-DQ ALPHA CHAIN

Sam Ho[1,2], Nicholas Dyson[2], Christine Peiter[1], Bradford West[2], [1]Gift of Life Michigan, Ann Arbor, MI, [2]Memorial Medical Center, Springfield, IL.

The clinical significance of HLA antibody specific to the DQ alpha chain is controversial. Here we present a case of long term stable kidney allograft function in presence of strong *de novo* complement-fixing DQA DSA. A 27-year-old Caucasian male (non-sensitized; 0% CPRA) with ESRD to IgA nephropathy received a living donor kidney transplant (TX) 10+ years ago with a negative pre-TX CDC crossmatch (XM). Mismatched donor HLA were A1, A11, B8, DR13, DR17, DR52, DQ2, DQ6. Beginning 6 years post-TX, routine antibody screening by single antigen beads (SAB) revealed the emergence of *de novo* DQ antibody, with the reactivity to donor DQ2 and DQ6 initially detected at 700-10400 and 100-2400 MFI, respectively (Table 1). Subsequent SAB profiles indicated that the DSA had progressively converted to be primarily directed against DQA1*04/05/06, likely a result of sensitization from donor DQA1*05:01. Epitope analysis suggested that the DSA was directed against a well-defined eplet 40GR3 shared by all DQA1*04/05/06 alleles and may represent three distinct eplets 40G, 47C, 50V51L53Q, all located in the alpha-1 domain accessible to antibody binding. Testing by phenotype beads confirmed this strong DQA reactivity (5900-14100 MFI) and flow XM with surrogate donors expressing DQA1*05 were strongly B-cell positive (210-420 MCS), suggesting the DSA was directed against native DQA chain. Functionally, C1q testing demonstrated the DQA DSA's considerable ability to fix complement (5300-9500 MFI), while CDC XM with DQA1*05+ surrogate donors were all negative. Despite the prolonged presence of this DQA DSA, other than an episode of acute cellular rejection (Banff II C4d-) diagnosed shortly after transplant which was promptly resolved, the patient's 10+ years of post-TX course has been unremarkable with a stable kidney function (creatinine 1.5±0.2 mg/dL; BUN 13.4±2.7 mg/dL; GFR 55.0±6.8 mL/min). This case highlights the fact that not all HLA DSA are created equal in terms of their clinical significance, and may also suggest a protective effect of a subset of DSA when directed against only to the DQ alpha chain.

OR52

IDES DESENSITIZATION THERAPY: BREAKING DOWN ANTIBODY BARRIERS TO TRANSPLANTATION

Gail Larkin[1], Melissa Jeresano[1], Karl Schillinger[1], Julie A. Houp[1], Kristin Gay[1], James Rager[1], Niraj Desai[2], Annette M. Jackson[1], [1]Johns Hopkins University Department of Medicine, Baltimore, MD, [2]Johns Hopkins University Department of Surgery, Baltimore, MD.

Despite increased transplant rates for highly sensitized candidates, inequities for those with CPRA >99.7% remain. This case highlights the efficacy of an endopeptidase (IdeS) to cleave IgG HLA-specific antibodies and complications for donor-specific HLA antibody (DSA) monitoring in the presence of therapeutic agents. DSA was evaluated using flow cytometric crossmatch tests (Flow-XM) and Luminex immunoassays (Lifecodes classes I and II single antigen; Immucor-Lifecodes, Stamford, CT and Single Antigen Beads; One Lambda, Canoga Park, CA). Sera were pretreated using Melon Columns™. A 43 year old male with a CPRA of 99.99% was entered into a clinical trial investigating the efficacy of IdeS to cleave IgG molecules and render them ineffective in eliciting complement activation or antibody-dependent cellular cytotoxicity. A deceased donor was identified to whom the patient had HLA-DP DSA yielding a positive prospective Flow-XM. Within 2 hours of IdeS administration, immunoassays and a Flow-XM revealed no detectable DSA (Figure 1). Due to concern for DSA rebound, alternate day DSA monitoring and intense post-transplant immunosuppression were employed. Two single antigen panels confirmed DSA reappearance on POD7 prior to high dose immunoglobulin (IVIG) dosing. Administration of IVIG produced an increase in the negative and positive control beads and reactivity with self HLA-antigens (3400 mean fluorescent intensity, MFI). Utilization of multiple bead assays, Flow-XM tests, and HLA pattern analysis rather than MFI was needed to monitor further increases in DSA that would prompt therapeutic plasmapheresis (TPE). Cumulative DSA test analysis revealed a stabilization of DSA accompanied by declining serum creatinine values with no initiation of TPE. New therapeutics hold promise for removing DSA barriers; however, post transplant

monitoring can be challenging due to test interference that accompany these biologics. This case illustrates the use of multiple assays and HLA pattern analysis to guide post transplant care.

A.M. Jackson: *3. Speaker's Bureau; Company/Organization; Thermo Fisher Scientific.*

OR53

POST TRANSPLANT MONITORING TO INCLUDE DONOR DERIVED CELL FREE DNA (%ddcfDNA): STEPS TOWARD PERSONALIZED MEDICINE

Kristin L. Gay[1], Annette M. Jackson[1], Pali Shah[1], Sean Agbor-Enoh[1,2], Hannah A. Valantine[2], [1]Johns Hopkins University, Baltimore, MD, [2]National Heart, Lung and Blood Institute, Bethesda, MD.

Aim: Elevated %ddcfDNA levels have been shown to correlate with allograft injury and acute rejection in lung transplantation. This case highlights how conventional post-transplant monitoring may underestimate the complex interactions between infection, injury, and de novo donor specific antibody (DSA) development. **Methods:** Quantitation of %ddcfDNA via next generation sequencing was performed as part of the Genomic Research Alliance for Transplantation (GRAfT) consortium. DSA monitoring utilized Luminex phenotype and single bead immunoassays. Spirometry was used to assess pulmonary function and transbronchial biopsies to assess rejection. Bronchoalveolar lavage, blood samples and sputum cultures were used to diagnose infections. **Results:** A 60 year old male with interstitial lung disease received a single lobe lung transplant. This patient was entered into GRAfT, a blinded prospective trial to investigate the use of %ddcfDNA to monitor allograft injury and predict long term outcomes. Immediately after transplant, high %ddcfDNA was detected in the recipient's plasma which declined over several weeks. A biopsy on post-operative day (POD) 192 indicated mild acute cellular rejection concomitant with an infection and a spike in %ddcfDNA. The elevated %ddcfDNA (>1% threshold) suggested substantial injury to the allograft despite stable lung function. The patient experienced several infections prior to the detection of HLA-DQB1 de novo DSA. The second %ddcfDNA spike was concurrent with an increase in DSA and loss in lung function. Therapeutic plasma exchange reduced DSA with corresponding %ddcfDNA regression and improved lung function. An infection on POD 338 correlated with a third %ddcfDNA spike (last available measurement). The patient experienced further infections with a diminishing trend in lung function. **Conclusion:** %ddcfDNA monitoring to detect cumulative clinical and subclinical injury may aid in assessing de novo DSA risk and in the development of interventions to prevent chronic allograft loss.

A.M. Jackson: *3. Speaker's Bureau; Company/Organization; Thermo Fisher Scientific.*

Tuesday, October 2, 2018
12:00 AM - 12:00 AM
Posters

P001

VALIDATION OF ALLTYPE™ NEXT-GENERATION SEQUENCING IN BRAZILIAN SAMPLES

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Aim: Validation of HLA typing by next-generation sequencing (NGS) demands evidence of the assay's accuracy compared to Sanger sequencing. We evaluated the AllType™ NGS (One Lambda) and TypeStreamVisual™ NGS analysis software (One Lambda) for accuracy on HLA genotyping. **Methods:** 48 Brazilian DNA samples selected for allelic diversity were sequenced for HLA class I and class II genes following AllType™ NGS protocol using automation of the Ion Chef and S5 sequencing system (One Lambda). Sequencing data was analyzed by TypeStreamVisual™ software 1.0.0 with IMGTHLA database 3.27.0.8. All samples were previously typed for HLA- A, B, C, DRB1, DQB1 and DPB1 by SeCore Sequence-Based Typing kits (One Lambda) using uTYPE HLA sequence analysis software (One Lambda).Evaluation was done comparing NGS results with previously assigned by Sanger's method. **Results:** AllType™ NGS and SeCore results were 100% concordant for HLA- A, B, C, DRB1, DQB1 and DPB1 loci. Following ambiguities were detected for class I and class II :B*15:01:01 B*15:03:01 /B*15:39:01 B*15:54; B*07:02:01 B*08:01:01/B*08:156 B*42:01:01; DRB1*12:01:01 DRB1*13:02:01/DRB1*12:10 DRB1*13:02:01; DQB1*06:03:01 DQB1*06:09:01/DQB1*06:41 DQB1*06:88;

DPB1*04:01:01 DPB1*04:02:01/DPB1*105:01 DPB1*126:01 Allelic imbalance cases occurred most in DQB1 locus, all involving DQB1*02 . All null alleles required identification by the National Marrow Donor Program could be ruled out as potential results. Reproducibility was validated by 3 samples tested 3 times in the same assay run and 4 samples tested in 3 different runs. 20 blind samples sequenced also by NGS were tested and approved up to 3-field result for all loci. **Conclusions:** We concluded that AllType™ NGS is reliable to be introduced in clinical routine for HLA-A, B, C, DRB1, DQB1 and DPB1 loci. Although HLA-B ambiguities needed additional tests, there was enormous reduction in the number of ambiguities detected by AllType™ providing highest cost benefit than Sanger sequencing.

P002

STANDARD DUO TYPING OF 1196 SPECIMENS REVEALED A STRATEGY TO USE NEXT GENERATION SEQUENCING AS A STANDALONE HLA TYPING METHOD IN CLINICAL LABORATORY

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Aim: Next generation sequencing (NGS) permits sequencing of the entire *HLA* genes and generate unambiguous phased-resolved *HLA* typing. NGS is increasingly used in clinical HLA laboratories for both stem cells and solid organ transplantation. Herein, we investigated if NGS can be used as a standalone *HLA* typing method in clinical laboratory. **Methods:** 1,196 samples were typed in parallel using NGS (Omixon) and high-definition rSSO (One Lambda) methods. In addition, SSP (Olerup) was used to type *HLA-DRB4* alleles. The frequency of allelic dropout by NGS was evaluated for all nine *HLA* loci (*A, B, C, DRB1, DRB3/4/5, DQA1, DQB1, DPA1*, and *DPB1*). **Results:** No allelic dropout was observed at *HLA-A, -B, -C, -DRB1, -DRB5, -DQA1, -DQB1*, and *-DPA1* loci. However, 4.6% of the samples (55/1,196) showed allelic dropouts at one of the other three loci: *HLA-DRB3, -DRB4*, and *-DPB1*. Frequent allelic dropouts were observed at *HLA-DRB4* (3.7%; 44/1,196 samples) and *HLA-DPB1* (0.8%; 10/1,196 samples) loci. *HLA-DRB3* allele dropout was noted in a single sample (0.1%). The table below lists the specific dropped out alleles and their associated haplotypes or other alleles. **Conclusions:** Our results suggest that the NGS can be used as a standalone method for *HLA-A, -B, -C, -DRB1, -DRB5, -DQA1, -DQB1*, and *-DPA1* typing. However, additional typing assays are required to confirm the homozygosity of *HLA-DRB3, -DRB4*, and *-DPB1* loci. *DRB1-DRB3/4/5* linkage can be used to identify the allelic dropouts in *HLA-DRB3/4* loci for certain cases. Concurrent typing of *HLA-DRB3/4* and *-DPB1* by rSSO would improve the NGS workflow and turnaround time.

P003

APPLYING SEQUENCING PHASING FOR THE DETECTION OF NOVEL *HLA* ALLELES USING NEXT-GENERATION SEQUENCING (NGS) DATA

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Aim: Next Generation Sequencing (NGS) assays provide unambiguous allelic determination, shortening of workbench time and reduction of technical errors. The phase-defined complete gene sequencing accounts for most of the typing improvements brought by different NGS protocols and also can be used as a powerful tool for novel polymorphism detection. In some cases, phasing is the only way by which a new variant can be detected. Here we describe a novel polymorphism at 4th exon of HLA-C locus, which would be missed and wrongly typed if not by phasing. **Methods:** DNA sample was extracted from blood hematopoietic cell transplant patient. We have obtained the HLA-C sequence lenghth (from 5 to 3'UTR) by applying the TruSight HLA v2 panel and Assign^{TM} 2.1 software from Illumina. The sample has been submitted to Sanger Sequencing, following our workflow for novel allele

confirmation, using Secore Kits from Thermo Fisher. **Results:** Without phasing information, the sample would be mistyped as HLA-C*04:01:01 + HLA-C*12:03:06, which has been confirmed by Sanger sequencing. However, the pattern of linkage disequilibrium among the following sites: 258.3, 261.3 and 267.3 (based on the reference IMGT/C 3.29.0) was in disagreement with the aforementioned genotype. A nucleotide sequence "GTG" was expected at these sites for HLA-C*04:01:01, respectively, and an "AGA" for HLA-C*12:03:06. However, based on the phasing information, we have found codon GGA, which is compatible with the HLA-C*12:03:01 and a codon "ATG" belonging to a novel allele differing from HLA-C*04:01:01 by a "G" to "A" at the 258.3 site. **Conclusions:** It becomes clear that the phasing information is essential for the correct typing. In this case, the new allele information would be completely loss, although, it would not lead to any clinical harm to the patient, because is is a new silent mutation. Nevertheless, this result has shown that only by taking the phasing information, laboratories will be able to accurately identify the HLA allelic variation which is crucial for clinical applications.

P005

RETROSPECTIVE STUDY ON THE EFFECT OF NON INHERITED MATERNAL ANTIGEN TRANSPLANTATION RELATED MORTALITY IN HLA MISMATCHED CORD BLOOD TRANSPLANTATION: HONG KONG'S EXPERIENCE

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Aim: Transplantation-related mortality (TRM) is well-recognized complication of HLA-mismatched cord blood transplantation (CBT). In utero, non-inherited maternal antigen (NIMA) is exposed and recognized by the fetus, which induces T regulator cells to the antigens. It is conceivable that the matching of donor NIMAs in CBTs may alleviate some of the excess mortality associated with this treatment in the recipients. We aim to review if the same concept is observed in the CBTs performed in two paediatric haematopoietic stem cell transplantation centers in Hong Kong. **Methods:** A retrospective analysis was performed and a review of the total of 41 paediatric CBTs performed for haematological malignancies from January 2008 to December 2017 in Hong Kong when the maternal blood samples could be retrieved for HLA typing. **Results:** Only 4 NIMA-matched CBTs for haematological diseases (i.e., the NIMA of the donor cord blood units (CBUs) matched to the patient) were identified and the remaining 37 were non-NIMA-matched CBTs. Due to the small sample size, cases and controls were unable to match on age, disease, disease status, transplantation-conditioning regimen, HLA matching status, and infused cell dose. Two out of 4 (50%) recipients NIMA-matched CBTs died of engraftment failure and disease relapse while 25 out of 37 (68%) non-NIMA-matched died. TRM was similar after NIMA-matched UCBTs compared with NIMA-mismatched CBTs. Overall survival was not higher after NIMA-matched CBT. The 3-year probability of overall survival rates were 50% after NIMA-matched CBTs and 32% after NIMA-mismatched CBTs. **Conclusions:** This retrospective study demonstrated better outcome of NIMA-matched CBTs as compared with NIMA-mismatched CBTs however limited by small sample size and wide variation in the patients' parameters. When facing the choice of multiple HLA-mismatched CBUs containing adequate cell doses, the advantage of selecting NIMA-matched CBUs on improving survival after mismatched CBT requires further collection of data and experimental evidence.

P006

NK KIR RECEPTOR GENOTYPES ARE ASSOCIATED WITH RELAPSE IN MEXICAN HLA MATCHED SIBLING PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

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Aim: The outcome of Hematopoietic Stem Cell Transplantation (HSCT) is strongly associated with the HLA identity of donor/recipient, and GVHD remains the most frequent associated factor with mortality and impact on the

quality of life. A successful HSCT depends also on T-cell mediated graft vs. leukemia (GvL) effect. Relapse is a major important challenge. NK cells, KIR and their HLA ligands are involved with an increase in overall survival, as well as better engraftment and reduced incidence of GVHD in patients with AML. The aim of the study was to determine the KIR gene content, their HLA ligands and their association on the outcome of ALL patients transplanted with HLA-identical sibling donors. **Methods:** Twenty seven Mexican patients with their HLA-identical siblings were included. Of these, 20 had ALL and 7 AML. KIR gene content was determined with the LinkSēq™ KIR kit and was run on an ABI 7500 Real-Time PCR instrument. Analysis was done with SureTyper 5. HLA intermediate resolution typing was performed by SSOP on a Luminex. HLA alleles were analyzed with the Match it DNA 1.2.0 software. KIR haplotypes and HLA ligands were assessed using https://www.ebi.ac.uk/ipd/kir/ligand.html. Allele Frequency (AF) and multiple parameter comparison analysis was performed using the SPSS17 software. **Results:** We looked if activating or inhibitory KIR genes had any effect on relapse, long term disease free survival (DFS), GVHD or mortality. An association with relapse was observed in ALL patients, when the matched sibling donor carried the *3DL1*-Bw4 ligand (50% of ALL patients were carriers); (Chi2= 5.93; p=0.03) with no differences in Bw4 80I and 80T ligand. The AA and Bx haplotypes did not have any association with mortality, GVHD, relapse or DFS. **Conclusions:** The results in ALL regarding relapse show that the presence of *3DL1*-Bw4, conveys higher susceptibility for relapse, as shown for AML. Contrary to AML, no effect was detected with 80I or 80T with intermediate relapse and mortality, implying a non benefit of education and sensitivity to inhibition as reported for AML. More cases of ALL are being analyzed to confirm if HLA and KIR allele typing in donor selection, enables the antileukemic benefits of NK alloreactivity in HSCT, in ALL, as it has been pointed out in AML, since the affected cell population in ALL, is totally different of the latter.

P007

PURITY AND YIELD OPTIMIZATION OF EASYSEP HUMAN WHOLE BLOOD CD34+ CELL ISOLATION KIT

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Aim: Relapse of the underlying hematological malignant disease after stem cell transplantation remains a major cause of mortality. Decline of CD34+ cell subset donor chimerism is an early marker of relapse before it can be detected in unfractionated peripheral blood or other cell subsets. One of the main challenges in performing CD34+ cell donor chimerism is obtaining pure CD34+ cell due to very low frequency of this cell population in peripheral blood. In this report, we describe our experience in optimizing the EasySep Human Total CD34+ isolation protocol to maximize the yield without compromising the purity of this rare cell subpopulation. **Methods:** CD34 subset is isolated from 6ml of buffy coat using EasySep Human Total CD34+ Kit, StemCell Technologies. CD15+, CD3+, CD19+, and CD34+ subsets are isolated by immunomagnetic positive selection in series from buffy coat. First, CD15+ subset is isolated using 1ml buffy coat. The remaining 5ml sample is used to obtain pure lymphocyte using Lymphocyte Enrichment Kit. CD3+ subset is isolated and followed by CD19+ subset isolation from supernatant of CD3+ subset isolation. Supernatant of CD19+ subset isolation was treated with RosetteSep cocktail provided in the kit and 10% whole blood mixture. CD34+ subset was then isolated from RosetteSep cocktail treated supernatant. Lineage-specific subsets were measured in flow cytometry. **Results:** CD34+ purity was confirmed via flow cytometry. **Conclusions:** The optimized method improved the efficiency of vendor recommended protocols by using less starting blood sample and producing higher yield and purity.

P008

ASHI VALIDATION WITH HOLOTYPE HLA FOR HIGH RESOLUTION TYPING

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Aim: Clinical validation of Next Generation sequencing(NGS)for HLA typing has been a high priority for many HLA laboratories worldwide, giving the opportunity to assess the correct allele matching of high-resolution genotypes to improve transplantation outcomes and decrease the rate of GvHD.Holotype HLA (Omixon Inc.) is an optimized, pre-configured assay and software combination product that provides comprehensive gene characterization of multiple HLA loci for sequencing on the Illumina platform. The goal of this study is to provide a validation of high-resolution HLA typing using Omixon's NGS kits. **Methods:** To test the robustness of the NGS protocol for HLA typing by Omixon Holotype HLA Assay and validate its use in our lab, we will type 96 DNA samples from peripheral blood using an 11-locus kit configuration to genotype HLA class I and Class II genes. All DNA samples have been previously typed by Sanger sequencing. HLA typing will be assigned using Omixon's HLA Twin software that combines two independent computational algorithms to ensure high confidence in allele calling. Inter-laboratory reproducibility will be assessed with 20 samples from Children's Hospital of Philadelphia, Immunogenetics Laboratory, as a courtesy from Dr. Dimitri Monos. The validation will follow the Minimum Guidelines for Validation of NGS for HLA standards from American Society for Histocompatibility and Immunogenetics (ASHI). **Results:** Sequence concordance will be assessed, where allele calls (1st and 2nd fields)

from each sample will be compared with those obtained by Sanger sequencing. Reproducibility between technologists will be also assessed. We expect to demonstrate the advantages of the NGS-based HLA genotyping over SSO and SBT techniques, as it may provide credible and more informative HLA genotyping with the first passage without any reflexive testing. **Conclusions:** It's expected fully concordance of sequencing results for allele call for first and second field. Concluding this validation will provide the confidence that HLA typing by NGS is ready to be introduced in our laboratory. According to our best knowledge, this will be the first validation using Omixon Holotype Assay in Brazil, and the second laboratory in Brazil to validate NGS following the ASHI standards.

P009

CONCORDANCE OF VIRTUAL AND PHYSICAL CROSSMATCH RESULTS WITH WEAK DONOR SPECIFIC ANTIBODY.

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Aim: Virtual crossmatch is becoming more routine in some transplant programs. We interested in to evaluate the concordance of crossmatch results and weak donor specific antibodies (DSA) using proficiency testing survey results. **Methods:** Crossmatch results with weak DSA (1K-2K median fluorescence intensity (MFI)) were analyzed using ASHI AC and CAP MX1 & MX2 PT reports conducted between 2015-2017. Multi-antigen and Single antigen bead assays by Luminex were used for antibody (Ab) screening and Ab identification tests, respectively. Positive cut-off for Ab testing was 1000 MFI. Only T cell Flow cytometry (TFXM) results are presented. **Results:** In ASHI survey, 8 out of 9 challenges with class I DSA ranged from 1K to 2K were resulted Negative TFXM. Four of them were not graded due to insufficient consensus. In CAP MX1 survey, 4 out of 9 challenges with weak class I DSA up to 2K had Negative TFXM and rest of them resulted Positive TFXM. Five out of 9 challenges were not graded due to insufficient consensus. In CAP survey, the majority of labs (64%) reported Positive TFXM in one challenge with below threshold (MFI: 942-990) DSA to HLA-B7 (virtually negative). In contrary, the consensus was reached (>90% of labs) with Negative TFXM in another challenge with 2K DSA to HLA-B27 (virtually positive). Of note, 2 sera presenting anti-HLA-B7 DSA with MFI-1093 and MFI-1407 resulted Negative (graded) and Positive (68% of labs) TFXM, respectively in 2 separate challenges. The similar cases were also observed in ASHI PT surveys. **Conclusions:** Nearly 50% of crossmatches with weak DSA in PT surveys were not graded due to insufficient consensus. In addition, less concordant crossmatch results were observed with weak DSA. Our findings suggest that virtual crossmatches with weak DSA should be confirmed by physical crossmatches as possible.

P010

HETEROGENEOUS FLOW CYTOMETRY CROSSMATCH REACTIONS IN THE PRESENCE OF PATIENT SERA WITH PAN-DR ANTIBODIES

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Aim: During routine clinical evaluations of antibody profiles using single antigen bead (SAB) assays, several instances of widespread reactivity exclusively to DR antigens ('pan-DR' antibodies) were observed. Additional assays were performed to investigate reactivity of these pan-DR positive sera against different antigenic conformations present on bead products and donor cells. **Methods:** Four serum samples taken two months apart from two hematopoietic cell transplant candidates were evaluated. Mixed bead and C1q assays (One Lambda, Inc) were performed to verify that the antibodies being detected were indeed against DR antigens and not cryptic epitopes that are present on SAB. Flow cytometry crossmatch (FCXM) was performed against two different donor cells. ELISA was performed to assess the involvement of AT1R antibodies in positive FCXM reactions based on reports of the capacity of anti- AT_1R to cause positive FCXM. **Results:** All sera were negative for Class II antibodies using mixed beads and C1q screening assays, contradicting the Pan-DR reactions with SAB. From patient#2, FCXM showed that serum #3 was T+/B+ crossmatched against cell #1 but became T-/B- crossmatched against cell #2. From patient #1, serum #1 was borderline T+/B+ when crossmatched against cell #1 but became T+/B- when crossmatched against cell #2. FCXM with all serum samples from both patients against cell#2 were negative. Additionally, auto-crossmatches for both patients were negative despite the presence of auto-antibodies. There was no association between AT_1R antibody level and pan-DR observation. **Conclusions:** The presence of

antibodies against both naïve and denatured antigens was indicated in pan-DR positive SAB/negative mixed beads, and in the positive T-cell FCXM and/or B-cell FCXM reactions. It is plausible that pan-DR antibodies described by the SAB data are not HLA antibodies at all but rather non-HLA antibodies with cross reactivity between cryptic HLA-DR antigens and yet unidentified non-HLA epitopes on lymphocytes.

P011

HLA-B13 DONOR-SPECIFIC ANTIBODIES YIELD ATYPICAL FLOW CYTOMETRY CROSSMATCH OUTCOMES

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Aim: To investigate observations of high rates of discrepancies between virtual crossmatch (VXM) positive and flow cytometry crossmatch (FCXM) negative cases in the presence of HLA-B13 DSA. **Methods:** Sera with antibodies against HLA-B*13:01 and/or B*13:02 falling within a MFI ranging from 3286 - 11793, as determined by LABScreen Single Antigen beads (SAB) (One Lambda), were used in FCXM against cells expressing HLA-B*13:01, B*13:02 (sera n=8, surrogate cells n=5). Each serum sample was obtained from a unique donor. FCXM with non-B13 HLA class I DSA of a similar MFI were also conducted for comparison to B13 FCXM data. A linear regression analysis was conducted to determine correlation between MFI and MCS for both B13 and non-B13 DSA. **Results:** Sera containing non-B13 DSA had a significant correlation between MCS and MFI values for both T ($p < 0.001$) and B-cells ($p < 0.001$), whereas sera containing B13 DSA did not. This lack of correlation translated to discrepant crossmatch evaluation with sera containing B13 antibodies of MFI strength > 5000 producing positive reactions in only 66.7% of cases, compared to non-B13 DSA producing all positive FCXM reactions for DSA MFI > 5000. Additionally, of the five B13 sera tested on more than one cell, only two sera (#4 & #8) gave the same result (Fig1). Sera #1, 5 and 7 each resulted both negative and positive reactions. **Conclusions:** High incidences of discrepancies in B13 FCXM indicate that the unpredictable nature of antigen availability at the cell surface occurs extensively in HLA-B13. One factor in these weak antigen-antibody interactions may be a lower salt bridge formation deduced from decreased protein-ligand interaction between B13 and KIR3DL1, which is unlike the other Bw4+alleles. Another possibility, is that affinity level of anti-B13 reactions may be susceptible to certain eplets accessible only in naïve antigen conformation. For certain specificities, including B13, it will be vital to evaluate FCXM reactions to avoid unnecessarily eliminating potential donors.

P012

HLA-DP TYPING IS NEEDED TO DISTINGUISH HLA MATCHED SIBLINGS FROM HAPLOIDENTICAL OR NON-HLA MATCHED SIBLINGS

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Aim: HLA typing by Next Generation Sequencing (NGS) enables three to four field typing of the HLA-A,-B,-C,-DRB1345,-DQA1,-DQB1,-DPA1 and -DPB1 loci in one step, making it easier to identify potential haematopoietic cell family donors who are HLA matched. This study presents three cases where HLA-DP typing, as part of the standard NGS protocol, distinguished HLA matched siblings from haploidentical or non-haplotype matched siblings. **Methods:** HLA-A,-B,-C,-DRB1345,-DQA1,-DQB1,-DPA1 and -DPB1 typing by NGS was carried out using the Illumina TruSight HLA v2 Sequencing Panel on the Illumina MiSeq instrument, with Assign 2.1 TruSight HLA Analysis software (Illumina, San Diego, CA, USA). The study includes 58 adult patients and 124 siblings; and 10 paediatric patients, both parents and 19 siblings. **Results:** NGS typing showed 24 (41%) of the adult patients had at least one HLA-A,-B,-C,-DRB1345,-DQA1,-DQB1,-DPA1 and -DPB1 matched sibling, while 43 (74%) had a presumed haploidentical sibling, (no parents were available to confirm haplotypes). In one case, the adult patient and all five siblings were fully matched at HLA-A,-B,-C,-DRB1345,-DQA1 and -DQB1. However, three siblings had different DPB1 alleles to the patient, indicating they were haploidentical, rather than being HLA matched. In another case, two siblings were HLA-A,-B,-C,-DRB1345,-DQA1, and -DQB1 identical to each other, but differed at DPB1. The DP difference meant only one sibling was haploidentical to the adult patient. With the paediatric cases, there was one family where a crossover appeared to have occurred between HLA-DQ and HLA-DP in one of the two healthy siblings, although neither was a haplotype match to the patient. **Conclusions:** With the implementation of NGS typing, HLA-DPA1 and -DPB1 typing is now routine in our laboratory for all haematopoietic cell transplant workups. This has shown some siblings, who previously would have been thought to be HLA matched (based on HLA-A,-B,-C,-DRB1345,-DQA1 and -DQB1), were only haplotype matched, while other siblings thought to be haplotype matched were not, once HLA-DP typing was considered. Therefore it is beneficial to include HLA-DP typing for all haematopoietic cell transplant workups.

P013

STEPS LEADING TO DISCOVERY OF A RARE ALLELE

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Sanger sequencing-based typing (SBT, Life Technologies), and LABType Sequence specific oligonucleotide (SSO) Typing (One Lambda) are performed on all bone marrow/stem cell transplant recipients and prospective donors at Mayo Clinic, Rochester. SSO performed on an 11 yr-old African American male with Hemophagocytic Lymphohistiocytosis needing a stem cell transplant and his full-sibling donor, resulted in C*04/C*07 with group 1 results showing common allele possibilities. SBT of exons 2, 3, and 4 resulted in either a rare C*04 allele, a rare C*07 allele, or both C*04 and C*07 being rare alleles. (Fig. 1a). At base pair 862 in exon 4, a heterozygous A/G result was seen. At this base the G result was consistent with a C*07:18 while the A suggested C*04:165. (Fig. 1b). This nucleotide exchange from G → A in codon 264 resulted in an amino acid change from Glutamic Acid to Lysine GAG → AAG. Ambisolv Sequence Specific Primers (SSP, One Lambda) were performed to help differentiate the common alleles C*04:09N, C*04:82, C*07:01, C*07:06, and C*07:18. Except for the C*07:18, all were negative and led to an initial interpretation of C*04:165 and C*07:18. Olerup C*04 and C*07 SSP trays were set up with C*04:01/C*07:18 results obtained. Included in the C*04 SSP tray was C*04:165 with negative results. To further confirm the presence of C*07:18, exons 5 and 6 were sequenced by SBT where results at position 1043 showed a T/C. (Fig. 1b). After SSO typing a maternal half-sibling showing a haplotype match at class I, SBT was performed showing a C*04:01 homozygous result. (Fig. 1c). To help resolve the typing, Next Generation Sequencing (NGS) using Omixon Holotype HLA was performed. This led to a conclusion of C*04:01/C*07:18 novel allele (Fig. 1d). The allele has been newly discovered and named C*07:607 in the IPD-IMGT/HLA Database. It was originally found in a Black East African female. NGS phasing proved extremely valuable in identifying the nucleotide change from G→A at position 862 (codon 264) belonging to the C*07 and not the C*04.

P014

NEXT GENERATION SEQUENCING IS CAPABLE OF DETECTING CONTAMINATING ALLELES FROM MALIGNANCY FOR A BONE MARROW TRANSPLANT RECIPIENT

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HLA typing using Next Generation Sequencing (NGS) for bone marrow transplant (BMT) patients has been recognized to outperform traditional molecular typing methodologies in detecting contaminating alleles from malignancy. Here we report a case with ambiguous HLA typing that was clearly resolved by NGS. A 61 year old female with atypical chronic myeloid leukemia was evaluated for allogeneic BMT. Initial HLA typing by NGS on peripheral blood (PB) of the patient and her 2 siblings showed that one sibling was most likely HLA identical, and the other was haplo-identical to the patient, except an extra HLA-DRB1* allele was detected in the patient. NGS presented a mixture of DRB1*03:01 and 03:16 for the patient and DRB1*03:01 only for both siblings (Table 1a). The difference between DRB1*03:01 and 03:16 is located in Exon 2 at NT 229 as C and T, respectively. The number of NGS reads (R#345) from the patients' PB at this position showed 10 reads mapping to DRB1*03:01 and 335 to DRB1*03:16, indicating 2.9% DRB1*03:01 and 97.1% DRB1*03:16. To investigate the possible involvement of malignant cells, DNA from the patients' buccal swab (BS) was typed by NGS and also revealed a mixture of DRB1*03:01 and 03:16. The number of NGS reads (R#543) showed 420 and 123 reads (77.3% and 22.7%) mapped to the DRB1*03:01 and 03:16, respectively (Table 1b). Confirmatory typing of the patients' PB and BS verified the initial typing result, thus concluding the DRB1*03:16 allele originated in the malignancy of the patient. BMT was performed with the HLA identical sibling. Parallel testing by NGS typing and engraftment monitoring by short tandem repeat (STR) was performed with post-BMT PB. Interestingly, NGS analysis indicated 63.1% of DRB1*03:01 and 36.9% of DRB1*03:16, which presented a close correlation with 61% and 39% donor

and recipient chimerism, respectively, as detected by STR (Table 1c). This case study highlights the great potential of NGS in detecting minor component alleles from malignancy in a semi-quantitative manner.

P015

BONE MARROW DONOR SELECTION IN THE PRESENCE OF BOTH ALLOGENEIC AND SYNGENEIC DONORS

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Syngeneic transplants provide an interesting dilemma with respect to donor selection. The risk of relapse is higher for patients with AML undergoing syngeneic transplant vs. HLA-matched sibling transplant, however, rates of graft versus host disease and transplant related mortality have historically been lower with syngeneic transplants. Thus, HLA -matched sibling donors are preferred to identical twin donors for patients undergoing transplant for malignant diseases (ie. AML) to optimize the impact of graft vs. leukemia (GvL) whereas identical twin donors are ideal for non-malignant disorders. We present a patient with AML who had both an HLA matched sibling donor as well as an identical twin. He was initially transplanted with the HLA matched sibling donor successfully however, at 6 months post transplant he developed severe neutropenia (ANC = 0). Chimerism studies revealed 95% DNA from the related donor T cells at this time. The cause of his neutropenia was autoimmune in nature and refractory to multiple immunosuppressive medications and therefore he underwent a second transplant this time using his identical twin as the donor. The patient's neutrophils engrafted and he has undergone a complete recovery post transplant. Chimerism

studies after the second transplant reveal that 85% of the DNA is from the second donor's T cells. Typically, chimerism studies with syngeneic transplant are not helpful as the patient and donor are identical. However in this case, chimerism studies were possible to determine the engraftment of the initial related donor to the patient/twin. This case illustrates that optimal donor selection depends on disease when an identical twin and an HLA matched sibling donor are available. It also showcases the interesting results of chimerism testing in a patient who has received both an allogeneic and a syngeneic transplant.

P016

CHROMOSOMAL ABNORMALITY IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES MAY RESULTS IN FALSE HOMOZYGOSITY RESULTS

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We and few other centers have reported the false homozygosity of one or two HLA antigens in several leukemia patients. However, only one case of leukemia with false homozygous typing of one haplotype has been reported. In all these reports, HLA typing were performed by luminex based sequence specific oligonucleotide (SSO), sequence based typing (SBT) or sequence specific primers (SSP) methods and only SSP could identify heterozygosity. We report three cases of leukemia patients, two with AML and one with ALL diagnosis, with chromosomal abnormalities who failed the HLA genotyping of one chromosome. As a results, false homozygous results were obtained. All testing were performed when patients were initially diagnosed. All three patients were typed by Next Generation Sequencing (NGS), SSO or one by Sequence Based Typing (SBT). Repeat HLA genotyping by both NGS and SSO using buccal swab or blood after induction therapy and patient's remission revealed heterozygosity. The above cases indicates for the first time, that not only SSO or SBT, but also the revolutionized NGS HLA genotyping may reveal false homozygosity in leukemia patients who have many tumor cells in their blood with chromosomal abnormality. The fact that SSP can detect the second haplotype is more likely due to the use of much higher DNA in testing which results in higher amount of normal cells DNA from and therefore, amplified DNA may be visualized. We conclude that in order to avoid false HLA homozygosity, everyone dealing with patients with hematologic malignancies, must be aware of this problem and take proper precautions to either repeat testing with buccal swab when homozygous HLA typing has been detected or blood when patients is in remission. Laboratories utilizing only NGS must also be aware of this problem and take precautions in reporting homozygous results. Confirmation of HLA testing must be performed prior to selection of related donors or searching for an unrelated donors.

P017

THE FIRST CASE OF NEONATAL ALLOIMMUNE THROMBOCYTOPENIA DUE TO HLA ANTIBODIES IN HONG KONG

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We report the first case of neonatal alloimmune thrombocytopenia (NAIT) secondary to anti-HLA antibody in Hong Kong. The baby girl was born at 39 weeks gestation by spontaneous delivery, with a birth weight at 4.165 kg. She was kept in hospital for investigations of large for gestational age. A full blood count incidentally showed: moderate thrombocytopenia (Platelets, 64×10^9/L), but normal leukocyte count and haemoglobin. No red cell fragments or spherocytes nor platelet clumps were noted. Over the next two days, her platelet count dropped to 8.0×10^9/L on Day 2, but there were no major bleeding. Her and her mother's blood groups were both B+ and direct antiglobulin test was negative. Antibody screen was negative on the maternal serum. Her mother did not have thrombocytopenia. In view of her marked thrombocytopenia, she was transfused one unit of random donor group B+ platelet concentrate and IVIG 4.2g, and the platelet count rose to 98×10^9/L on Day 3. The mother had no history of blood transfusion. No anti-HPA antibodies were detected by antigen-captured ELISA. Maternal serum was crossmatch incompatible with father's platelets by PIFT. Luminex SAB test demonstrated broad spectrum anti-HLA IgG

antibodies with 2,000-5,000 MFI against several HLA-A, -B antigens in the maternal serum, and NAIT caused by HLA antibodies was suspected. Anti-A203 and anti-B55 IgG antibodies were found against the paternal inherited HLA antigens. These specific antibodies with 1,500-2,000 MFI were also detected in her pre-treatment serum. Although the initial platelet response was satisfactory, the platelet count gradually dropped again from 82×10^9/L on Day 4 to 49×10^9/L on Day 7. She was then given further IVIG 4.2g with the platelet count rose to 79×10^9/L on Day 8. The platelet count then stabilized up to Day 10. Although the mother was a primipara, anti-HLA antibodies could still occur since even non-transfused men may have 'naturally occurring' anti-HLA antibodies. Based on the anti-HLA antibodies in the maternal serum, the mother's and baby's HLA typing results and the PIFT results, we believe that there is evidence this is a rare and unique case of NAIT secondary to anti-HLA antibodies.

P018

UNEXPECTED SINGLE MISMATCH CAUSED BY PATERNAL CROSSOVER IN HLA CLASS I IN A PATIENT WITH SICKLE-CELL DISEASE

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We report 23-Year-old male patient, known case of sickle-cell disease, with frequent pain attacks and multiple time admission in the hospital, no history of stroke, priapism and acute chest syndrome was noticed. The patient was given multiple transfusions during the last few weeks. His blood film showed microcytic hypochromic picture with notable presence of sickle cells. His hemoglobin electrophoresis showed Low A1-Hb (17%), F-Hb (8.6%) and S-Hb (69%). The patient was offered related allogeneic BMT as a possible treatment. Patient's HLA typing for class I and II was performed by using One Lambda sequence specific oligonucleotide reverse and sequence specific primers (SSOr/SSP). Also, 6 family members underwent HLA Typing for Class I and II, including his parents which were noted as possible donors. The parents are cousins. The result is based on haplotype illustration; Patient (A*24,24, B*48,50, C*08,06, DRB1*07:01,07:01, DQB1*03:03,02:02), Father (A*01, 24, B* 50,48, C*06,08, DRB1*07:01,07:01, DQB1*02:02,03:03), Mother (A*24,68, B*51,50, C*15,06, DRB1*04:03,07:01, DQB1*03:02,02:02), Brother (A*24,68, B*48,50, C*08,06, DRB1*07:01,07:01, DQB1*03:03,02:02), Sister-1 (A*24, 24, B*48,51, C*08,15, DRB1*07:01,04:03, DQB1*03:03,03:02), Sister-2 (A*24,68, B*48,50, C*08,06, DRB1*07:01,07:01, DQB1*03:03,02:02), Sister-3 (A*01,24, B*50,51, C*06,15, DRB1*07:01,04:03, DQB1*02:02,03:02). As we could interpret the results the patient has an unexpected unique haplotype. Therefore, all tests were repeated with new samples to exclude possible sampling error. However, the same results were confirmed, so we initiated further investigation study in the family. This showed a crossover in the region of paternal A locus, as the patient showed A*24 instead of expected A*68. The illustration of HLA haplotypes and the significant similarity of HLA typing in class I and II in the parents explained the presence of single mismatch in class I between the patient and his sister-2. Possibility of rare crossover must be considered in the interpretation of unexpected HLA results of families worked up for BMT.

P019

HLA-MATCHED PLATELET TRANSFUSION RESCUED THE LIFE OF A CHILD WITH SEVERE THROMBOCYTOPENIA

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A four-year-old female child who was initially diagnosed with myelodysplastic syndrome was admitted in the bone marrow work up. A fully matched HLA allogeneic bone marrow transplantation was performed. Post-transplant the patient received multiple platelets transfusions to manage extreme thrombocytopenia, but without any positive effects. The platelet count was zero/µl and the life of the patient was seriously in danger. At our center, we define the refractory for a patient as an increase of platelet count of less than 10k after the transfusion of at least two fully functioned platelet units containing a sufficient number of platelets ($>2x10^{11}$ per unite). As a further therapeutic attempt, the young girl received Prednisolone 4 mg/kg/day for 4 days and IVI g 1 g/kg/day for 2 days. However, the medications did not render any response and the count of platelet was still zero and the life of this child was still threatened as she developed rapidly and increasingly petechial exanthema with a massive hemorrhagic in the skin. The HLA-workup showed that the patient developed multiple HLA antibodies against HLA class I antigens (B8. B55. B27. B18. B56. B7. B47. B13. A69. B54. B41. B37. B46. B35. B75. Cw10. B67. B76. Cw1. B53. B64. A68.

Cw8. Cw9. B65. Cw14. A68. A34. Cw7. A66. A43). The screening for platelet specific antibodies against human platelet antigens (HPA) was negative. Using our in-house established platelet HLA-matched program, corresponding HLA-matched platelet units donated by 4 selected donors was given over several sessions. The child responded excellent to most of the given HLA-matched platelets. After the second transfusion, the platelets count raised to $51x10^3/\mu l$. Generally, we consider an increase in platelet count of more than 30 k per transfused unit as a sufficient transfusion. After the clinical symptoms and platelet count were stabilized the patient was discharged with a platelet count of $40x10^3/\mu l$. This case highlights the importance and the benefits of platelet matched HLA-program in rescuing the life, decreasing the mortality rate as well as the stay in the hospital and the treatment cost.

P020

IDENTIFICATION OF A NEW C*06 NULL ALLELE BY SBT TYPING IN AN ARABIC FAMILY

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A 12 y/o female from Saudi Arabia with chronic granulomatous disease was evaluated at Duke for HSCT. Patient and sibling typing were performed by SBT method (Protrans, Germany) which separates alleles by group-specific PCR before sequencing. The patient was identical to her sibling at HLA-A, B, DRB1, DRB3/4/5 and DQB1 loci. However, the analysis software could not assign alleles at HLA-C locus for both the patient and sibling due to the presence of double peaks in exon 3 electropherogram. For both the patient and the sibling, exon 2 and exon 4 sequences showed no polymorphism. Sequence with forward primer of exon 3 was good 1-224 nt in exon 3 but became double peaks after 224. Reverse primer sequence of exon 3, however, had double peaks 1-224 nt then good after 224. Analyzing the results by ignoring the double peak sequences showed a perfect match to C*06:02 in the database. This indicated there were C*06:02 and another variant of C*06:02 with a G deletion at 224 of exon 3. This corresponds to nt 568 in cDNA which changes codon 166 from GAG to .AG and is predicted to be a null allele due to shift of open reading frame. In order to investigate whether the new variant was inherited from the family, we also obtained the samples from the parents. It turned out the C*06 new null allele was carried by the father. The sequence was submitted to GenBank (accession number MH031344). The patient's sibling was identified to be the 10/10 matched donor and HSCT was performed in 2018 successfully.

Table 1: Typing results of the family.

	A	B	C	DRB1	DRB3/4/5	DQB1
Patient	02:ATRDH 03:01	50:01 -	06 new null 06:02	04:06 07:JDKZ	4*01:AUSYV	02:02 04:02
Sibling	02:ATRDH 03:01	50:01 -	06 new null 06:02	04:06 07:JDKZ	4*01:AUSYV	02:02 04:02
Mother	N/A	N/A	07:ATRDJ 06:02	N/A	N/A	N/A
Father	N/A	N/A	06 new null 06:02	N/A	N/A	N/A

P021

DESENSITIZATION IS BENEFICIAL IN HIGHLY ANTI-HLA ANTIBODY SENSITIZED PATIENTS WITH PLATELET REFRACTORINESS UNDERGOING HEMATOPOIETIC CELL TRANSPLANTATION

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Introduction: Management of platelet (Plt) refractoriness can be very challenging, expensive and time consuming. Inability to find compatible Plts for hematopoietic cell transplantation (HCT) recipients may result in delay in transplantation and post-transplantation morbidity/mortality. While desensitization protocols are often utilized in DSA-sensitized organ transplant recipients, there is little information available about utility of desensitization in the

setting of Plt refractoriness in highly-sensitized HCT recipients. We describe our successful experience with desensitization using a specified protocol. **Methods:** Desensitization protocol: 3weeks, IVIg (daily), plasmapheresis (PP) 3 days/week, and rituximab and bortezomib (once/week after each PP). Anti-HLA antibody studies were performed using LABScreen® (One Lambda®) after each PP and compared with historical antibody results of each patient. Plt-XM studies were performed using Capture-P®, Immucor® assay. Mean MFI values were determined for each HLA antibody, and in aggregate, before and after desensitization. Statistical analyses were by Student t-test, $p<0.05$ was considered significant, for each and total anti-HLA antibodies. **Results:** Slight reduction in mean MFI values were noted after desensitization (see table), however no statistical significance was noted. %cPRA did not change. None of the individual anti-HLA antibodies changed with statistical significance. All patients achieved adequate Plt incremental increases with transfusions after desensitization. All patients achieved adequate engraftment. **Conclusions:** 1) "Desensitization" is beneficial in the management of Plt refractoriness and allows for successful HCT engraftment. 2) MFI and % cPRA do not appear to be reflective of successful desensitization. (In previous studies we have found that >12-16 weeks of treatment are generally required to see a demonstrable fall in MFI values.) 3) Additional studies are needed for understanding the mechanism(s) that result in the improvement in Plt refractoriness with desensitization, since it does not appear to be due to reduction in anti-HLA antibodies.

	Mean MFI			**% cPRA**		**Adequate platelet incremental increase post-transfusion (Tx)**		**Adequate Engraftment**
	Pre	**Post**	**P value**	**Pre**	**Post**	**Pre**	**Post**	
Patient 1	4955	4401	0.18	100	100	No	Yes	Yes
Patient 2	2237	1704	0.07	92	93	No	Yes	Yes
Patient 3	5707	5414	0.71	100	100	No	Yes	Yes

Table 1: Desensitization Results

T. Harville: *4. Scientific/Medical Advisor; Company/Organization; Medical Director, HLA Lab UAMS, AURORA Advisory Board.*

P022

NGS HOMOPOLYMER DETECTION ERROR RESULTED A*03:01 MISTYPED AS A*03:21N

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Aim: Next Generation Sequencing (NGS) has enabled the characterization of complete HLA gene sequences and has become widely recognized as the preferred method for HLA typing. The importance of accurately adapting NGS into routine clinical testing has emerged. Homopolymer (HP) is a sequence of consecutive identical bases. Approximately 1.43 million HPs (also known as mononucleotide microsatellites) exist in the human exome, with the size of 4-mer and up. NGS have a relatively high error rate in determining HP due to the principles used for detection. Here we report an HLA sequencing error resulted from NGS HP detection limitation and hope to raise awareness and develop preventative steps. Methods: Routine clinical HLA allelic typing was performed using Immucor Mia Fora Flex kit. Stringent QC pass/fail criteria based on vendor's recommendation were followed. Mia Fora Smart Flagging System Genotype status was incorporated into routine analysis. Two complementary bioinformatics, mapping and phasing, were used for analysis. Heterozygous calls were made if two nucleotides were present at a position with coverage ratio >20%. Results: Two samples were typed as A*03:21N during two NGS runs, A*03:21N, A*33:01 and A*03:21N, A*68:02 respectively. A*03:21N is a well-documented allele, both assignments were acceptable by lab quality criteria. The frequency of A*03:21N assignment caused concern and both samples were reflexed to Sanger SBT and confirmed as A*03:01. The difference is in exon 4, position 628, codon 186 where A*03:01 has 7-mer C vs. 8-mer C in A*03:21N. A*03:21N was defined as 627-628insC caused

frameshift and premature stop at codon 196. Detailed NGS analyses revealed the number of 'C' call at position 628 were >20%, resulted A/C heterozygous calls and A*03:21N assignment. Calling the common A*03:01 as non-expressed A*03:21N has significant clinical impact. Even more problematic is that the assignments lacked warning sign. Conclusions: NGS has revolutionized the field of genomic sequencing and rapidly adapted by HLA lab for clinical typing. However, NGS limitations, such as GC bias, preferential amplification and HP, are less known. Better understanding of these hidden risks and vendor software improvement can provide valuable benefit for labs utilizing NGS as their primary typing method.

P023

A NOVEL FLOW CYTOMETRY BASED CELLULAR CROSSNATCH (FLOW-CXM) PREDICTS GRAFT FAILURE AND GVHD POST BONE MARROW TRANSPLANTATION

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Aim: Pre-Transplant samples of two bone marrow transplantation(BMT) cases were tested by new developed Flow-CXM assay. The aim of this study is to evaluate the Flow-CXM assay in prediction of graft failure and GVHD in BMT. **Methods:** Short Tandem Repeat; Luminex IgG/C1q SAB assay; Flow-CXM: $0.1X10^6$ irradiated donor PBMCs were incubated with $0.1X10^6$ recipient's PBMCs and Th1 (IFNɣ & IL-2) cytokine capture beads for 16 hrs. The cells were lysed and the captured cytokines on the beads were detected by PE-labeled 2nd antibodies. The positive PE stained beads were counted to represent the relative number of donor specific responding T cells. **Results: Case 1:** A 53/F patient was diagnosed with Sezary disease and has received two BMTs. No DSA to 1st and 2nd donors was detected in pre-Tx patient serum by Luminex Ab testing. STR results showed primary graft failure on day 45 after 1st BMT and 100% engraftment on day 28 after 2nd BMT. However, patient deceased 2.5 month after 2nd BMT. The retrospective Flow-CXM results showed that recipient has increased IFN-r and IL-2 response to 1st donor cells(HvG). In addition, there was weaker IFN-r and IL-2 responses in graft vs host (GvH) directions. Decreased IL-2 secretion was found in HvG direction to 2nd donor but strong positive IFN-r response in GvH directions. The patient finally was diagnosed as GI GVHD. **Case 2:** A 64/ F patient was diagnosed with Mycoses fungoides Sezary disease. The patient got one BMT and maintained a low level engraftment. LMX IgG/C1q DSA results were negative in pre-Tx serum. STR assay has been performed to monitor engraftment from day 28 to day 2264 (2011-2018) post BMT. No graft failure and rejection were reported even though the engraftment was relatively low and varies with large scope (CD3 17%-65%, CD15 6%-92%, CD19 12%-83%, CD56 17%-86% and whole blood 9%-77%). Flow-CXM results showed that no positive allo response in both HvG and GvH directions. **Conclusions:** The newly developed Flow-CXM may predict graft failure by positive IFN-r and IL-2 response in HvG direction. Positive IFN-r response in GvH direction may indicate the occurrence of GVHD.

P024

SENSITIZATION BY STEM CELL INFUSION: A CASE STUDY

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A 70 yo male with patient medical history of gastroesophageal reflux disease, colonic polyps s/p resection, insulin-dependent diabetes mellitus, hypercholesterolemia, and biopsy-proven idiopathic pulmonary, was evaluated for lung transplantation. The patient denied any sensitization events including transplantation, transfusion, prior surgery, and infection during the initial evaluation. However, both phenotype ID beads based and single antigen beads based testing showed only one weak HLA-A2 antibody (MFI= 2500, cPRA= 0% for antibodies with MFI> 5,000). 3 month later, we performed another single antigen testing and the patient became highly sensitized (cPRA= 94% for antibodies with MFI> 5,000). MFI of HLA-A2 antibody soared to 21,000 and HLA-A2 was C1q binding antibody. The patient also displayed antibodies against HLA-A23, A24, A68, A69 and other HLA-A locus antibodies. In addition, the patient had antibodies against HLA-Cw7 and Cw17. We were puzzled by the development of strong antibodies without a clear sensitization event. We decided to repeat antibody testing on a new sample one month later. During this period, the patient acknowledged that he received stem cell infusion several times for his wellbeing. The repeated single antigen testing confirmed the previous antibody findings. After explanation, the patient agreed to stop stem cell infusion. Single antigen testing 6 wks after the last stem cell infusion showed that the antibody level remained high. A decision was made to wait to see if the antibodies would reduce over time. Unfortunately, the patient passed away one month since the last antibody testing. Stem cells infusion was thought to be at low immunological risk due to the low level of HLA expression, but this case demonstrated that stem cells can stimulate antibody production effectively. No HLA class II antibody was detected during the entire course, which is in consistence with the previous finding that HLA class II antigens are not expressed on the surface of stem cells.

P025

PLATELET REFRACTORINESS IN A 5 MONTH OLD INFANT WITH HYOPLASTIC LEFT HEART ON ECMO

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Platelet transfusion refractoriness in patients ranges from 7-34% and the etiology is typically attributable to formation of HLA antibodies from pregnancy or previous transfusion. Antibody development in children <9 months old is extremely uncommon due to their immature immune system. We report platelet refractoriness in a 5 month old male with hypoplastic left heart. Status post the Glenn procedure, the patient developed pulmonary hypertension and difficulty with oxygenation and ventilation. Eventually, he required placement on extracorporeal membrane oxygenation (ECMO). During the Glenn and later chest closure, the patient required transfusion with 4 units of RBCs for bleeding. Placement on ECMO required priming with several units of RBCs and additional blood product transfusions. Several days after starting ECMO, the patient was noted to be increasingly thrombocytopenic requiring platelet transfusions. After surgery and up to this point, he had received 33 RBCs, 51 apheresis platelets, 10 plasma, and 2 packs of cryoprecipitate. The etiology of his platelet refractoriness was investigated. He was found to have numerous class 1 HLA antibodies using the Luminex Class 1 SAB with a cPRA of 92% (HLA A: 1, 3, 11, 23, 24, 29, 36, 66, and 80; B: 7, 8, 13, 27, 37, 41, 42, 44, 45, 47, 48, 54, 56, 59, 60, 61, 67, 72, 73, 76, 81, and 82). A complement-dependent cytotoxicity (CDC) assay was done and the cPRA was found to be 85%. A little over 1 week later, the class 1 SAB assay was repeated and the cPRA was found to be 63% (HLA A: 26, 32; B: 13, 35, 37, 46, 47, 49, 50, 51, 53, 55, 57, 58, 63, 64, 65, 71, 72, 75, and 78); however, the CDC assay cPRA was 93%. Most of the antibodies identified were different. During the interim period, the patient had received 11 RBCs, 13 platelets, and 1 pack of cryoprecipitate. Thus, given the immaturity of the child's immune system, his severe illness, and the dissimilarity between the testing results, the HLA antibodies were believed to have been transfused with various blood products. Per AABB guidelines, all platelet donors are tested for HLA antibodies if they have ever been pregnant or transfused before. Unfortunately, this child passed away from cardiac complications prior to further evaluation. This case serves to highlight that infants and very young children with immature immune systems who have been transfused blood products can be platelet refractory due to acquired HLA antibodies.

P026

TIMING IS EVERYTHING - EVEN FOR DP ANTIBODIES

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Introduction: Donor Specific Antibodies (DSA) can be a contraindication for kidney transplants. The clinical relevance of DSA to DP antigens and correlation with crossmatch (XM) remain unclear. We report the conversion of a negative XM to positive due to development of DSA to DP6, most likely caused by an unreported sensitizing event. **Methods:** A 38 yo female received a deceased donor (DD) kidney transplant (Tx) after negative T/B cell flow XMs using sera drawn 35 and 44 days pre Tx (day -35 and -44). Delta channel shifts (DCS) in these XMs were: T=0 for both sera; B=37 and 31 for days -35 and -44. These sera were negative for DSA. We received a serum drawn at admission for Tx (day -1). The patient received blood transfusions within the previous week (day -6). The day-1 serum had high level DSA to DP6 (MFI=13,723), compared to day -44 (1,321) and day-35 (1,653). By flow XM, the day -1 serum was T negative, B positive (DCS=228), likely due to the increase in DSA to DP6. In addition to standard immunosuppression, this recipient also received thymoglobulin and basiliximab once the program was notified of high level DSA and positive flow XM. Post Tx monitoring showed an immediate decline in DSA to DP6: post Tx day 6 (MFI=7,380), day 12 (5,669), day 21 (4,042), day 63 (485). The DSA to DP6 remained low (MFI<400) for five years. However, the recipient had BK viremia and a kidney biopsy five years post Tx showed "arteriolar hyalinosis and arteriosclerosis related to possible CNI toxicity". The recipient is re-listed in UNET for another kidney Tx, the primary reason for graft failure deemed transplant glomerulopathy. **Discussion:** This case emphasizes the importance of timely antibody testing in relation to XM testing, awareness of sensitizing events, and updated HLA typing. Our lab now employs stricter guidelines to ensure more frequent receipt of patient sera, especially following potential sensitizing events. At the time of this patient's Tx, DP typing was not performed on DD. After discovering the increase in antibodies at pre Tx day -1, but absence of DSA, we performed DP typing. Finding DP6 as a DD antigen helped explain the change to positive XM, as the level of DSA to DP6 significantly

increased following the sensitizing event pre-Tx. This early peri and post Tx antibody response may have contributed to reduced graft survival and transplant glomerulopathy.

P027

POTENTIAL INFLUENCE OF BACTERIAL INFECTIONS IN AUGMENTED HLA-C ANTIBODY RESPONSES WITH CONSISTENT CROSS MATCH POSITIVITY

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A patient listed for renal transplant had consistent positive cross matches with several sequential donors over a period of time when there are no DSAs that were listed as Unacceptable Antigens [UAs]. The listed patient had several Class I antibodies especially towards HLA-C antigens during the entire monitoring period. But none of them matched the criteria of ≥ 5000 MFI which we use for all patients as a cut off value unacceptable C antigens [UAs]. From September 2017 the patient got several offer till 01/22/2018 and all B cell Flow cross matches were decisively positive. The DSAs were all directed towards HLA-C antigens except on two occasions where HLA-B 8 and or A33 DSAs were also seen along with one or more HLA-C DSAs. Upon investigation of the antibody specificity trend, the following pattern for HLA C antibodies indicating a significant spike around between 05/04/2017 and 01/09/2018 was observed. The arrow where there is a dip shows that none of them were C1q binding.

The patient complained about a potential infection of chest catheter on 09/26/2017 through telephonic encounter with nurse practioner with no fever but noted redness around the site. According to the patient, this is how his prior infections started indicating previous infections as well. Later the patient developed foot ulcer which was not notified but found out during the investigation of spike in HLA antibodies after repeated positive cross matches with several deceased donors. Bacteriological investigation of the foot ulcer revealed presence of two species of *Staphylococcus* by MALDI-TOF method which was eventually treated with appropriate antibiotics and got resolved. It is a long recognized fact that several bacterial antigens shares amino acid sequences with various HLA-Loci. It is known that infections and immunizations can induce polyclonal B cell activation resulting in increased HLA antibody responses in previously sensitized patients. This case signifies the importance of non-conventional ways of HLA antibody boosting and importance of antibody profiling following any infections. Had we known about the infections we could have periodically monitored the antibody profile of the patient and avoided some of the crossmatches by entering unacceptable C antigens.

P028

IMPORTANCE OF EVALUATING ALLELE-LEVEL HLA TYPING OF THE DONOR TO ASSESS THE IMMUNOLOGICAL LOGISTICS OF IMPORTING A RENAL ALLOGRAFT FOR HIGHLY SENSITIZED RECIPIENT AND FACILITATING A SUCCESSFUL TRANSPLANT.

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Renal transplant candidates with more than 99% CPRA are at greater disadvantage for a match run. Even with a match-run, some of the allelic DSAs not used for Unacceptable Antigens [UA] entry criteria, could make the decision to import an organ from a distant location difficult without knowing the donor's allele level typing. We describe the case of a patient where the first potential importable organ had to be rejected and the second one could be accepted leading to a successful transplant based on inferred 2 field HLA typing information of the donor. **Case:** Patient JG, a 24 year old female with a hx of ESRD secondary to pauci-immune glomerulonephritis with a kidney txp in 2001 that failed in 2012 with > 99% CPRA got an import kidney offer in Aug 2017 for a donor with an HLA Genotype of A68/-; B35**, 39**; C1, 7; and **DRB1 *0401/04/02/03 at both HLA-DRB1 Loci**. The Recipient had strong antibody to **DRB1*04:02 [MFI 7000 consistently]** and B39 **MFI=3117**. Since the donor had a potential DRB1*04:02 on both DR4 loci and a B39, the offer was rejected. HLA-DR4 could not be entered as UA despite a high MFI value since listed patient was DRB1*04:01/*04:07. Another offer came through and the donor had a DRB1*04 but the most possible alleles were depicted as **DRB1*04: 03 /*04:07** and **DRB1*04:02** was not an option. So the only HLA DSA the patient had towards this donor was B39 [MFI=3117]; Accepted the organ and the real cross match was negative, the transplant was done and the Recipient is doing well as of now. Allele-specific antibodies and their impact on real cross matches have been highlighted by failed NKR Chains not knowing allele specific DSAs. Also, when a patient is listed as DRB1*04, we cannot list any UAs as DR4. And as we propagate our ability to assign antibody specificity based on the single antigen bead based assays, we are still at large with allele-specific antibodies that could be crucial. We have an example of a case where the Recipient had antibodies against B*35:01 and DRB1*14:01/02/54; and had a donor with B*35:12 and DRB1*14:07 -And No beads to assess antibodies against B*35:12 or DRB1*14:07. **Conclusion**: Virtual crossmatch can be an excellent tool as long as we have reliable data on DSAs in terms of the allele-specific antibodies where applicable, and it is extremely important when one make decision to import an organ based on potential allele-specific DSAs.

P029

A CASE OF ACUTE TUBULAR NECROSIS, ANTIBODY MEDIATED REJECTION, OR BOTH

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We investigated the cause of impaired allograft function in the case of biopsy-proven Acute Tubular Necrosis (ATN) with C4d deposits, in the absence of other Antibody Mediated Rejection (AMR) features. ATN, an acute injury of renal tubular epithelial cells, occurs more often in deceased donor transplants (Tx) due to ischemia and nephrotoxic drugs.[1] Distinguishing ATN from an acute immunological rejection [AMR or cellular] immediately after the Tx is crucial yet challenging, due to the possibility of both occurring simultaneously. The delay in anti-rejection treatment could result in an irreversible damage to the allograft. A 42-year-old male received a kidney from an ABO compatible, HLA mismatched, deceased donor. Although, HLA DSAs of low intensity were present prior to the Tx, flow XM was negative. Two weeks post-Tx, patient experienced acute shutdown of kidney function (eGFR <15, CR 8.3mg/dl and BUN/CR ratio 7.1). Biopsy indicated ATN, with nonspecific tubular vacuolization likely associated with calcineurin inhibitory toxicity, strong diffuse C4d deposition in peritubular capillaries and no typical histologic features of AMR. Several strong non-complement binding HLA DSAs were detected. Patient was treated for AMR with plasmapheresis/IVIG and responded well (eGFR and BUN/CR ratio returned to normal, CR was reduced to 1.51 mg/dl along with significant decrease in HLA DSA levels). The intriguing fact was the cause of such strong C4d deposition. Allograft injury, ischemia and calcineurin toxicity are known to cause induction of auto antibodies (Ab) capable of causing additional damage. To further explore the potential pathological factors in this suspected ATN and AMR, a non-HLA auto Ab assay was performed to compare patient's pre and post-tx Ab pattern. Pre-tx serum showed presence of several Non-HLA antibodies. Interestingly, in post-tx serum, these Ab decreased in MFI. However, despite immunosuppression, a two-fold MFI increase of glutathione S-transferase T1 (GSTT1) Ab was observed. Further investigation revealed that the Ab against GSTT1 was C1q binding and most likely the potential cause for C4d positivity observed in the allograft biopsy. Based on the overall scenario and

response to the treatment, we presume that this is a case of an ATN and AMR mediated through complement activating Non-HLA Ab.

P030

HOW DO DIFFERENT TESTING PLATFORMS INFLUENCE NOVEL ALLELE CHARACTERIZATION? A CASE STUDY OF A NOVEL DPB1 ALLELE

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A renal transplant patient sample was submitted in order to characterize an unresolved HLA-DPB1 allele. Sanger sequence-based typing (SBT) resulted in DPB1*04:02:01 and a number of equally possible candidates for the second allele, each with a single nucleotide mismatch. Removing all exons except exon 2 from analysis resulted in a perfect match with homozygous DPB1*04:02:01G. However, several heterozygous positions leading to non-synonymous mutations were present in the excluded exons, contradicting this apparent homozygosity. One next-generation sequencing (NGS) platform then typed the sample as DPB1*04:02:01 for the first allele and various ambiguous alleles for the second. However, this was due to low coverage in a 19-nucleotide region of exon 2 rather than a mismatch. The low coverage pattern was replicated on a repeat run. DPB1*414:01 with one mismatch in exon 2 was called as the second allele when the minimum read depth was lowered. Interestingly, none of the possible second alleles were part of DPB1*04:02:01G. Upon testing on a second NGS platform, DPB1*04:02:01, DPB1*463:01:01 was obtained with novel mutations in exon 4 and introns. Comparing this result to the Sanger SBT data revealed the same heterozygous mismatch in exon 4. A third NGS platform verified this typing and associated mismatches. Therefore, if potential intronic mutations are ignored, the typing may be reported in two ways: as DPB1*04:02:01, DPB1*463:01 with a mutation in exon 4, or as DPB1*04:02:01, DPB1*414:01 with a mutation in exon 2. The first combination may lead to the assumption of homozygosity for treatment purposes, since DPB1*463:01 is part of DPB1*04:02:01G. This is currently the practice in the HLA field when exons 3 and 4 are not included in analysis. Ignoring heterozygous positions in exons that make up regions outside the antigen binding groove could lead to development of antibodies. This may be an important consideration in the renal transplant setting, depending on patient-donor matching and determination of immunogenicity.

P031

UNACCEPTABLE ANTIGEN LISTING SHOULD NOT BE BASED ON LUMINEX SINGLE ANTIGEN TESTING ONLY

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Unacceptable HLA antigens are largely determined by testing sera with Luminex single antigen (LSA) beads. Table 1 shows the MFI values for the five DQB7 beads in three pre-transplant specimens performed on a patient with a cPRA of 99%. DQA1*05 and DQA1*06:01, but not DQB7, were listed as unacceptable antigens. A deceased donor with DQA1*01,03 and DQB5,7 became available and was virtual and flow cytometry crossmatch compatible. On post-op day 14, the patient presented with graft dysfunction. A biopsy contained evidence of antibody mediated rejection (AMR), which was supported by microarray. A donor specific antibody (DSA) screen revealed a greater than 10 fold increase in the previously negative DQB7 beads (DQA1*02:01/DQB1*03:01 and DQA1*03:01/DQB1*03:01) in addition to the previously positive DQA1*05:03, DQA1*05:05, and DQA1*06:01 beads and was consistent with a DSA to DQB7. C1q testing was positive for DQA1*05 only, which was not donor specific. The patient was aggressively treated for AMR but a repeat biopsy on post-op day 25 contained a thrombotic microangiopathy consistent with AMR. The allograft was removed on post-op day 75 due to non-function. Investigation into this case revealed a previous failed transplant with a DQB7 positive donor at another institution, which was unknown to our laboratory. Although the DQA1* typing was not available for the first donor, DR/DQB linkage analysis favors DQA1*05 consistent with the pre-transplant LSA and post-transplant C1q results. Based upon the clinical course, we hypothesize DQB7 specific memory B cells, formed in response to the first donor, facilitated accelerated AMR, which may have been avoided by listing DQB7 as an unacceptable antigen. In conclusion, the results of the LSA assay are only one component of the patient's immunological profile. Decisions regarding the listing of unacceptable antigens must additionally take into consideration previous immunizing events, like a failed transplant, and this information must be provided to the histocompatibility laboratory for optimal patient care.

P032

EDTA ELUCIDATES UNEXPECTED CROSSMATCH RESULTS

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Introduction: Pretreatment of serum with ethylenediaminetetraacetic acid (EDTA) eliminates interfering components and provides a clearer, more accurate antibody (Ab) profile in luminex single antigen (SA) antibody detection assays. Some patient sera do not show obvious signs of interference but have high background (i.e. binding to NC bead devoid of HLA antigen). We report the case of a highly sensitized patient with hidden reactivity to class I antigens revealed by EDTA, but confounded by treatment to reduce high background. **Methods:** DC is a 64 yo Asian male who received a deceased donor (DD) kidney transplant (Tx) in 1984, but since 2005 has had MPGN and is currently listed for a second kidney Tx. His antibody profile has remained stable the past few years (cPRA 49-57%). His serum has been historically treated with Adsorb Out™ (AO) to remove high background. The strongest Ab reactivity in AO treated sera has been against class I: A3, A11, A25, A26, A29, A43. However, recent flow crossmatches (XMs) against two DD cells were strong B and T cell positive in the absence of any strong or discernible DSA, with delta channel shifts (DCS) of 350-400. One DD typed for A1, the other A11 so we suspected an epitope/CREG reactivity since the actual DSA levels to A1 and A11 were low to moderate (MFI=616-3,155), which did not correlate with the strong positive flow XMs. We decided to treat the patient's sera with EDTA, instead of the routine AO treatment, for the SA assay and discovered a significant increase in A1 Ab (MFI=23,274) and A11 (MFI=20,622). In fact, all Abs previously detected by AO treatment, displayed significantly higher MFI levels after EDTA treatment. Although the background reactivity to the NC bead was higher in EDTA treated compared to AO treated sera, it was within acceptable limits of the assay. We then EDTA and AO treated this patient's serum and found no significant difference compared to the EDTA alone treated sample (A1 MFI=23,226, A11 MFI=21,672). **Conclusion:** Despite what may appear as clear, consistent patient Ab data over the course of months to years, it is imperative to continually monitor Ab profiles carefully, to reveal possible epitopes and changing trends. Ab testing by SA assays must also be interpreted in concert with XM data to ensure proper unacceptable antigens are in UNET and to ultimately provide an optimal Tx outcome.

P033

THE EFFECT OF PRONASE ON B-CELL FLOW CYTOMETRY CROSSMATCH RESULTS: A CASE STUDY.

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Cross matching is crucial in pre-organ transplantation and FCXM is the most sensitive cell-based XM technique. Routinely in our laboratory, we pre-treat the cells with pronase to improve the sensitivity and specificity of the B-cell FCXM. Pronase treatment of B-cell is used to disrupt the Fc and CD20 to minimize nonspecific binding of immunoglobulins and interference with Rituximab respectively. Pronase-treated T cells are tested in a single tube T-cell/B-cell technique and it has been shown that pronase treatment is likely to give false-positive reactions in the T-FCXM test. T cells express fewer Fc receptors than B cells for that, pronase may expose cryptic epitopes. This

phenomenon of falsely incompatible T-cell FCXM with pronase in the absence of DSA has been well documented and has been frequently seen in our laboratory. In this study, we are presenting a case of incompatible B-cell FCXM after pronase treatment. An 18-year-old Caucasian male with an end-stage renal disease secondary to Joubert syndrome was considered for renal transplantation. Prior antibody screening and single-antigen bead testing disclosed anti-HLA class I antibodies with 6% cPRA and the absence of anti-HLA class II antibodies. His mother was the potential living related donor and the recipient did not carry any DSA against his mother HLA antigen. Crossmatch was performed between the donor lymphocytes and the patient serum after incubating the isolated lymphocytes with pronase. An initial FCXM showed unexpected B-FCXM incompatibility with both current serum and a historic serum. The concurrent T-FCXM was compatible. One month later, we repeated the XM with and without pronase-treatment. Unexpectedly, pronase treatment caused incompatible B-FCXM, whereas non-treated cells were B-FCXM compatible. In all cases, the T-FCXM was compatible. The take-home message of that case that cautious should be taken in clinical labs using pronase treatment in single tube T/B FCXM, to avoid false-positive reporting of results and alternative methods for blocking Fc receptors in FCXM should be explored.

P034

WHEN BONE MARROW TRANSPLANT RECIPIENTS BECOME DECEASED ORGAN DONORS - A CASE REPORT

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Blood samples for ABO and HLA typing were received from the OPO on a potential deceased donor. The donor medical records indicated AMS due to hemorrhagic stroke, no significant PMH and no recent transfusions. ABO typing was performed by serological agglutination and HLA typing was performed by SSP/melt curve analysis. Results indicated that the donor was blood group O and had HLA typing consistent with the donor's reported race. The results were reported to the OPO and organ allocation proceeded. Upon review of the UDRAI, a transplant program requested repeat HLA typing on lymph nodes (LN) as the next of kin indicated that the donor received a BMT 15+ years ago for aplastic anemia prior to residing in the USA. The LN results were concordant with the initial blood HLA typing. The repeat indication and testing results were reviewed by the lab general supervisor with the OPO's AOC since DNA from LN was expected to also have a predominant BMT-donor origin as well. To ensure the correct HLA type of the allocated organs, buccal swab and muscle biopsy samples were requested for repeat testing. Additional HLA typing of these samples confirmed the previous results, indicating that the organ donor most likely received a fully HLA-matched BMT. The confirmed results were reported to the OPO; however, the HLA lab recommended re-allocation as an AB blood group donor since 30-40% of BMTs are ABO incompatible. The OPO reallocated and informed all transplant programs involved that the deceased donor's tissue ABO typing could not be verified as group O. In conclusion, the HLA lab should be knowledgeable about ABO as it relates to all types of transplantation and provide consultation regarding results and testing limitations. This case also demonstrates the HLA lab can only give proper advice when provided important donor related information. Furthermore, effective communication is vital to managing increasingly complex donors and transplant cases.

P035

IMPORTANT ROLE OF HLA LABORATORY IN THE DIAGNOSIS OF HLA ASSOCIATED DISEASES

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A 43 years old female patient was introduced to our polyclinic and the treating physician referred her to our HLA-lab for HLA-B27 typing. According to the ordering physician the patient is suffering from back pain, stiffness in the morning, degenerative changes, facet Arthropathy and her hip was also affected, hence we were suspicious that the patient was suffering from ankylosing spondylitis. The blood pressure was normal, BMI 29, WBC 6,84^9/L, HB 146 g/dl, Glc 5.5 mmol/L, PTH 56.6 ng/L, anti gliedin 3.88 mg/L, CRP 3.46 mg/L. According to published studies HLA-B27 is present in 85% of patients with ankylosing spondylitis. This means that HLA-B27 positive individuals are approximately 85 times at more risk to develop ankylosing spondylitis compared to the general population. However, in this case our HLA-typing, which was performed for HLA-Class I by using SSO for low/intermediate

resolution, showed a negative result for HLA-B27. The negative HLA-typing results for HLA-B27 in this female patient do not totally exclude the presence of ankylosing spondylitis because 15% of affected patients do not carry this HLA allele. However, the absence of HLA-B27 in this patient makes the diagnosis of ankylosing spondylitis unlikely. This interpretation is supported by the fact, that Ankylosing spondylitis predominantly affects male patients and not females in the age > 30 years old. In addition, the described clinical symptoms could be caused by many other various diseases. At our lab we usually performed the testing of all HLA class I alleles even when only single allele requested. In this case we have observed that the patient has beside HLA-B27 HLA-B51 also, which is very strongly associated with Behcet's disease. Behcet's disease has similar clinical symptoms to ankylosis spondylitis. Our positive results for HLA-B51 directed the treating physicians to perform further investigations to confirm Behcet's disease instead of ankylosing spondylitis. The presented case highlights the important role of HLA laboratory in the diagnosis of HLA associated diseases.

P036

PROLONGED THERAPEUTIC APHERESIS INCREASES THE SUCCESSFUL RATE OF ABO INCOMPATIBLE RENAL TRANSPLANTATION

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A 41 years old female patient with ESRD. She is in the waiting list for one year only, but she could not tolerate the dialysis and she had recurrent severe infections on her Jugular Permcath. At the same time the patient has multiple class I & II HLA-antibodies and considered as a highly sensitized patient who is difficult to be transplanted. All these issues caused her not only physical but various psychological difficulties as well. Only one sister of her 5 siblings was fully HLA matched to her. However, this donor has BG A while patient has BG O. Due to the difficult situation of the patient, she was prepared for the ABO incompatible RTX. At that time the Anti-A titer was 1:64 and according to our ABOi RTX protocol the isoantibody must be reduced to </=1:8. Therefore plasmapheresis was planned for 4 sessions, but the titer could not be reduced to less than 1:32 after these first 4 sessions. We decided therefore to extend the plasmapheresis and the patient received additional 3 sessions of plasmapheresis. However, the Anti-A isoantibodies titer was reduced to 1:16 which is higher than our target. Now the decision should be made either to continue with this ABOi RTX and plasmapheresis or to stop the procedure and leave the patient on dialysis. After discussing this situation with the patient and in the transplant committee we decided to continue with the ABOi RTX. Fortunately, after 3 additional sessions Anti-A was reduced to the target level 1:8. In total the patient received 10 sessions of plasmapheresis in the pre-transplant preparation setting. On 13/12/2017 the transplantation was performed without complications. The induction therapy was with ATG and 6.4 mg/kg was the total accumulative dose that she received. The post-operative course was excellent, as the ABOi kidney functioned immediately and without any delay. To avoid possible ABO incompatibility reactions in the acute post-transplant phase, we continued plasmapheresis after transplant and the patient received further 3 sessions of plasmapheresis. No any signs of rejections were observed in the entire post-transplant period. However, the patient developed mild urinary tract infection (UTI) without affect to the renal function. The UTI was treated successfully. The patient followed up after 2 months on Feb, 2018 and her Creatinine was in the normal range of 104 umol/L (reference range 62-106 umol/L). She is medically stable with no signs of rejection or any other complication. It is to be mentioned that the B-cell HLA flow Crossmatch was weak positive by negative DSA and negative T cell Crossmatch. This was explained by the treatment of Rituximab as a prophylaxis using its anti-CD20 effect to block the building of ABO antibodies and thus avoiding hyper acute rejection. The patient received Rituximab in a dose of 500 mg IV.

P037

PRE-TRANSPLANT HLA DP10 DONOR SPECIFIC ANTIBODY OF UNKNOWN SIGNIFICANCE IN HEART TRANSPLANT A CASE STUDY

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There is minimal data in the literature on the impact of pre-formed HLA DPB1 Donor Specific Antibodies in heart transplant patients. Accurately assessing these antibodies and the risk they pose would hopefully broaden the donor pool available to this group of patients. We have reviewed a case of heart transplantation across strong C1q binding donor specific antibody (DSA) to the HLA DP epitopes 84DEAV and 96K2. A 64y/o female with a history of breast

cancer (1997), coronary artery disease, hypertension, hyperlipidemia (2002), presented with congestive heart failure. Sensitization history includes two children but no transfusions prior to transplant evaluation. Her initial HLA antibody results were reported as 0% cPRA for Class I and Class II (Luminex Single Antigen). There were HLA-DP antibodies present below the reporting cutoff (<10,000 MFI). Antibody testing was done 10 days post LVAD implantation. Unfortunately, her cPRA levels increased dramatically (to 70% Class I and 48% Class II). The Class II antibodies were largely HLA-DP, likely to the 84DEAV and 96K epitopes. Epitope analysis was initially performed using the HLA-DPB Compatability Wizard.

The patient received a heart offer. Virtual crossmatch revealed a single DSA (Anti-DP10 > 20,000 MFI). A prospective CDC crossmatch was performed and was negative. The patient's auto crossmatch was also negative. The patient was transplanted with this donor. A retrospective flow crossmatch was performed with pronase treated cells. It was T-cell negative, B-cell positive. Due to the positive flow crossmatch, the heart team performed one session of PP/IVIG on POD 1. This case provides insight to the role of high level C1q binding pre-formed donor specific HLA-DPB antibody, where the antibody is not confounded by additional DSA to other loci. It supports the concept that pre-existing HLA-DP antibodies may not represent a significant barrier to transplantation.

P038

DONOR SPECIFIC ANTI-A TITER FOR ABO INCOMPATIBLE (ABOi) LIVING KIDNEY TRANSPLANTATION

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To increase the donor options for our active living kidney donor program, candidates who are blood group B may receive a living donor transplant from a non-A1 or non-A1B donor. ABO incompatible donors (ABOi) are no longer an absolute contraindication for kidney transplantation as long as the recipient titer against ABO A is low. It is well known that there are several variations of non-A1 blood groups, and therefore measurement of anti-A titer against commercial A1 or A2 cells may not provide an accurate measurement of donor reactive IgG antibody to blood group A antigens. Therefore, we have initiated a strategy of assessing anti-A titers using red cells from the prospective non-A1 donor being evaluated. The candidate will have initial non-A1 titers performed with commercially produced A2 red cells as well as red cells from the intended living donor. This will help identify the rare instance of a more or less immunogenic ABO A subgroup. Recipient sera is serially diluted with saline from 1:1 to 1:32 in a standard tube assay. One drop of RBC suspension (50 ul) is added to 2 drops (100 ul) of each sera dilution. Tubes are incubated at RT for 30 minutes, centrifuged and agglutination macroscopically observed and recorded. This provides measurement of the IgM non-A1 titer present in the recipient. Tubes are then incubated at 37C for 30 minutes, washed 4 times with saline, IgG added, then centrifuged and agglutination recorded again to provide IgG non-A1 titer results. To be considered compatible for non-A1 or non-A1B donors, the IgG titer obtained with A2 reagent cells should be no higher than 1:16, and no greater than 1:8 with the intended living donor. Titers must be repeated within 48 hours prior to transplant. Consistent with initial testing, IgG non-A1 titer results at the time of transplant are considered acceptable if the titer is no greater than 1:16 against commercial A2 red cells, and 1:8 against the red

cells of the intended deceased donor. As of the date of this submission we have performed two such ABOi transplants using this testing methodology, with no rejection episodes occurring thus far.

P039

THE BUMPY ROAD TO EPIC BEAKER IMPLEMENTATION

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Our lab has reported discrete results into Epic EMR since the initial Epic Go Live allowing for access to electronic data for clinical and research use. YNHH initiated the LIS update from SoftLab to Epic Beaker in 2016. This update dramatically changed our workflow. Initially, the Epic Beaker team set up our lab with a workaround to report results back into Epic without completing the standard Beaker sample workflow. Generic sample labels and incomplete specimen collection information often led to confusion and samples were redrawn or orders were automatically canceled in Epic before results were posted to the EMR. In 2017, a project was initiated to incorporate the HLA lab into the Epic Beaker workflow. This project faced three primary challenges. First, Beaker lacked support for Epic order panels with multiple tests and sample types, which were initially set up to improve efficiency when ordering multiple concurrent tests. As a solution, these panels were split into orders based on the various tube types necessary to perform the test and order sets were created in Beaker. The second challenge was the Beaker required update of the HL7 orders and results interfaces to support the data transfer between Epic and HistoTrac. The final hurdle was that every result message needed to be updated with the Beaker sample ID. This prompted the HLA Lab to consolidate our Epic orders removing organ specificity and reducing orders from 28 to 10 unique order codes. Furthermore, Beaker enforced a one to one relationship between orders and results. To accommodate this rule, SystemLink added status logic to the results message. All results are sent as preliminary until the last outstanding test result is signed off in HistoTrac and sent as final. Prior to the HLA lab Go Live the Epic team trained the sample collection staff to process our orders with the standard Beaker workflow, updated the clinicians with an Epic splash screen message, and trained the HLA staff to receive samples in Beaker before processing in HistoTrac. All Epic outstanding orders, standing orders and preference lists were updated by the Epic consolidation team. Post-Go Live optimization has included filing results for all pre-Go Live outstanding tests in HistoTrac, and identifying sample collection sites needing updated order transmittal rules which add the Beaker sample ID at collection. As a result of the Epic Beaker implementation order specific labels are printed for all samples, sample status is updated in real time, and samples are processed with the standard Beaker workflow.

P040

POST-TRANSPLANT DE-NOVO DONOR SPECIFIC ANTIBODY TO HLA-B8, OF UNKNOWN CLINICAL SIGNIFICANCE

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Clinical History: A 62 year-old male with chronic glomerulonephritis underwent kidney transplant from a donation after cardiac death donor in Jan 2017. Cold ischemia time was 8 hours. The recipient had no donor specific antibody detected in any pretransplant sera tested since 2012. Flow-cytometric crossmatch (FCXM) was negative with the day of transplant sample. He received induction with Thymoglobulin, methylprednisolone, mycophenolate mofetil, and tacrolimus. Post-op day (POD)#1 creatinine was 7.90 mg/dl. He experienced delayed graft function, but otherwise no complications. He was discharged on POD#7 with a creatinine of 2.82mg/dl. On POD#8 his creatinine was 1.7 mg/dl. De novo HLA-B8 antibody (14,365 MFI) was detected on a Thymo-absorbed and heat inactivated sample from the same day. The patient began therapeutic plasma exchange (TPE) and IVIG treatments on an every other day schedule. The kidney biopsy showed no evidence of antibody-mediated rejection. The Anti-HLA-B8 levels decreased to ~2,000MFI. To understand the nature of this antibody, the FCXM was repeated using the POD#8 sample and was negative (T cell channel shift=36 and B cell=65). Therefore TPE/IVIG was stopped after 2 cycles. An incidental cavitary lesion was identified in the mid lobe of his right lung post-transplant. Quantiferon gold test was intermediate positive, but AFB culture was negative. 15 months post transplant, he continues to have HLA-B8 antibody 1500-3000 MFI, a stable creatinine ~1.2mg/dl, no proteinuria, and protocol biopsy in March 2018 showed no evidence of rejection. Discussion: The single antigen bead (SAB) assay is a very sensitive and specific antibody assay. During the manufacturing process, HLA antigens can become denatured and unfolded. This artificial unfolding allows for unmasking of cryptic epitopes on the recombinant HLA antigens. These cryptic epitopes may

cross-react with epitopes found in microorganisms or allergens and may generate antibodies that react with denatured HLA proteins. False positive SAB assay may lead to unnecessary procedures in patients, and may limit patients' to access to organ transplantation. Therefore, it is of utmost importance to recognize these artifacts and when possible utilize different platforms of testing to confirm the presence of artifactually denatured antibodies.

P041

RENAL ALLOGRAFT HYPERACUTE REJECTION: REVIEWING THE ROLE OF HLA AND NON-HLA ANTIBODIES

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A 35 y/o Caucasian female with bilateral nephrectomies due to Von Hippel-Lindeau disease received a first right kidney transplant from a 14 y/o male deceased donor. At 0.5 day post kidney transplant (POD), ultrasound suggested renal vein thrombosis. Allograft biopsy at the time of thrombectomy showed extensive endothelial injury with diffuse thrombotic angiopathy and microangiopathy. There was moderate microvascular inflammation, though C4d was negative. Overall findings were suggestive of hyperacute rejection. Despite thrombectomy and apheresis, the allograft was not viable and was removed on POD 2. We retrospectively re-analyzed HLA donor specific antibody (DSA) and cross-match (XM) results. Additional retrospective testing included endothelial cell XM using surrogate endothelial cell lines (1.5 fold > negative control as +), non-HLA auto-antibodies (AuAb) against different targets associated with allograft rejection (1.5 fold > negative control as +), and angiotensin II type-I receptor antibody (AT1R-Ab) >17 U/ml as positive and 10-17 U/ml as borderline positive. Pre-kidney transplant HLA-B7 DSA was 3000 mean fluorescence intensity, but both T/B cell flow cytometry XMs were negative. HLA-B7 DSA reduced to 2100 mean fluorescence intensity at POD 1. Pre- kidney transplant AT1R-Ab was positive, but also declined to borderline positive at POD 1. Both pre and post-kidney transplant endothelial cell XM were consistently positive. Six endothelial targets and 1 chronic kidney disease related AuAbs were positive pre- transplant, but 6 out of 7 decreased to borderline level POD 1 (Table 1). Positive endothelial cell XM combined with weak HLA- B7 DSA and multiple strong direct/indirect endothelial target antigen specificities suggest the possible involvement of AuAbs in severe acute endothelial injury related allograft dysfunction.

P042

ACCELERATED ANTIBODY MEDIATED REJECTION IN A HEART PATIENT WITH WEAK DONOR SPECIFIC ANTIBODIES PRESENT PRE-TRANSPLANT

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We present a case on a 46 year old Caucasian female patient with hypertrophic obstructive cardiomyopathy. The patient's sensitization history included 3 pregnancies and a flu vaccination 2 months prior to transplant. The pre-transplant single antigen bead assay revealed weak reactivity to the Bw6 epitope including B7 (1,149 MFI) and B8 (775 MFI). The patient received a heart transplant in which B7 and B8 were crossed. As expected, the retrospective flow crossmatch was weakly positive. The patient was treated with Simulect on the day of transplant (Day 0) and with anti-thymoglobulin on Day 3 (*Figure 1A*). On Day 7, the patient went into cardiac arrest and was placed on extracorporeal membrane oxygenation (ECMO). The antibody screen on Day 7 revealed a large increase in donor specific antibodies (DSA) to B7 (21,694 MFI) and B8 (21,460 MFI). Positive ERG, CD68, and C4d staining on the Day 7 biopsy corroborated antibody mediated rejection (AMR). The patient was treated with plasma exchange, IVIG, Rituxan, and Cytoxan. At Day 14, ECMO was discontinued. DSAs declined to levels observed at pre-transplant (*Figure 1B*). Although antibody and biopsy results demonstrated a reduction in AMR, the patient became neutropenic and developed fever, bacteremia, a necrotic leg wound, and a fungal infection in the brain. She progressed to brain death. This case describes an amnestic response to weak DSA which contributed to cardiac arrest. Augmented immunosuppression used to treat an anamnestic AMR is associated with a significant risk of fatal infectious complications.

P043

ASSESSMENT OF DONOR SPECIFIC ANTIBODY BY SINGLE ANTIGEN BEAD / EPITOPE ANALYSIS COMBINED WITH FLOW CYTOMETRY CROSS MATCH FACILLITATES BETTER PATIENT MANAGEMENT AFTER KIDNEY TRANSPLANTATION

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Post transplant epitope analysis of anti - donor specific antibody (DSA) using single antigen bead (SAB) assays when combined with flow cytometry cross matches (FCXM) may provide enhanced ability to direct the management of a patient's immunosuppressive therapy. This proposal is illustrated by the following case report. A 57 year old female (HLA, A2, A31, B51, B53, DR13, DR15) received a deceased donor kidney transplant (TX) (Donor HLA, A1, B13, B37, C6, DR7, DR15, DQ2, DQ6). The patient was sensitized (cPRA 97%) from a prior kidney TX. Pre-TX DSA was detected to B*37:01, MFI = 645 with a possible epitope 156DA, the sum of which is the MFI- score (MFI-s) = 4515). T and B cell FCXMs were negative. The patient did not receive plasmapheresis (PPE) prior to TX and DSA was not detected one day after transplantation. On day 8, DSA was detected to the A*01:01 SAB (MFI=591) with and MFI-score of 3992 to the possible epitope 163R that includes A1. The patient's DSA increased to 2451, A*01:01, MFI-s of 14,936, on day 12 but the creatinine (Cr) decreased from 4.1 to 2.5 mg/dL over the next 14 days so treatment was withheld. On day 30 an increase in Cr from 2.3 mg/dL to 3.7 mg/dL was noted. SAB testing indicated DSA to B*37:01, as well as the A1 bead. A C3d test and a FCXM test was performed (with frozen donor cells).The DSA did not bind C3d and the FCXM was negative. Typically the FCXM would be positive with this level of DSA (14,936). In view of the negative FCXM, further epitope analysis was performed as the patient's post transplant serum likely contained antibodies with several overlapping specificities. In this case the donor antigen A1 did not express the epitope that was broadly expressed on other beads, 163R, but rather an epitope that was restricted to A1 alleles only 163RG with an MFI-s = 2787. This explained why the FCXM was negative. PPE, IVIG and Rituxan therapy was initiated but only 5 PPE were given since the DSA was of relatively low strength. The patient's DSA was undetectable 10 days later and her Cr stabilized to 2.1 mg/dL. This case illustrates how post-transplant assessment of anti-HLA antibody with SAB/ epitope analysis, combined with

FCXM, can provide important information on the specificity and strength of the DSA for use in the management of recipients that develop antibodies after kidney transplantation.
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P044

THE USE OF HLA-DQ EXON 3 DATA IN SSO ANALYSIS CAN PRODUCE DISCREPANT HLA TYPING RESULTS

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Herein we document two cases of discrepant typing results obtained when data from both exon 2 and 3 is used to analyze LabType SSO results for HLA-DQ using the HLA Fusion software. The first case is an ASHI PT sample (HT-184) tested by LabType SSO and analyzed using HLA Fusion 4.1. For this sample, 39% of survey participants reported HLA-DQA1*05:05, 26% HLA-DQA1*05:02 and the remaining labs reported either a new allele, HLA-DQA1*05:01G or P. By sequence based analysis (SBT) the correct typing is HLA-DQA1*05:02,*01:01. When using LabType SSO and analyzing with HLA Fusion 4.1, including both exon 2 and 3, the typing obtained is HLA-DQA1*05:05/09/11 when bead #56 is excluded. The software flags bead #56 as a false positive bead. No typing is obtained without excluding bead 56. HLA-DQA1*05:05 was the most common response on the PT survey indicating several labs may be analyzing data using Fusion and excluding bead #56 from the analysis. The correct typing HLA-DQA1*05:02 can only be obtained when HLA-DQA1 exon 3 is excluded from the analysis despite the fact that the probe attached to bead 56 binds to DQA1*05:02 but not DQA1*05:05. The issue encountered with this sample stems from the fact that HLA-DQA1*05:02 does not have an exon 3 sequence in IMGT. As such, the probe pattern for exon 3 has not been established for this allele. The software automatically rules out alleles with no exon 3 sequences from the possible allele string when the analysis includes both exon 2 and 3 rather than flagging it as a possibility and indicating exon 3 sequence is not available for this allele. The second case is a patient typed by SBT as HLA-DQB1*06:11, *03:03. When the sample is tested using LabType SSO and analyzed using HLA Fusion 4.1 the typing obtained is HLA-DQB1*03:03, *06:112N. The correct typing, HLA-DQB1*03:03, *06:11 can only be obtained when HLA-DQB1 exon 3 data is excluded from the analysis. Again this is because the sequence for exon 3 is not available in IMGT for HLA-DQB1*06:11:01. The two cases presented are representative of others we have encountered and reinforces the importance of software validation in the laboratory. Regardless of vendors and methodologies most software have limitations and impute some HLA typing data. As such, a variety of typing samples with specific allele combinations should be used in validation studies.

P045

KIDNEY TRANSPLANT WITH HISTORIC BUT NO CURRENT DSA -A CASE STUDY

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Presence of donor-specific HLA antibodies (DSA) at the time of transplant is associated with poor outcome. DSA may be present in historic serum samples but not at the time of a kidney offer. Going forward with the transplant holds the risk of a memory B-cell response upon re-exposure to a specific HLA antigen or epitope. A patient with X-linked Alport syndrome was investigated for a kidney transplant. Anti-HLA class II antibodies were observed following pregnancy, which were specific to her husband’s mismatched DR and DQ antigens. A year later, she received a blood transfusion and had an episode of aseptic peritonitis while on dialysis. Class I HLA antibodies appeared but were observed over a period of 7 months. Thereafter, anti-HLA antibody screening using FlowPRA beads and identification using Luminex single antigen beads showed disappearance of class I antibodies but persistence of a single anti-HLA-DR antibody developed after prior pregnancy. Three years later, a kidney from a DCD donor was offered and transplanted despite presence of historic DSA against 2 HLA-B antigens. A CDC crossmatch proved negative using a current serum whereas a flow cytometric crossmatch using 3 DSA-containing historic sera gave positive but inconsistent results (T+B+, T-B+, T-B-). One week after transplant, historic DSA reappeared (3000 MFIs). Despite absence of biopsy-proven rejection, short-term treatment comprising plasmapheresis, rituximab and low-dose IVIg was initiated. DSA quickly disappeared from circulation with only an HLA-DR-specific reactivity from the previous pregnancy. Without any further desensitization treatment, the patient has not demonstrated re-appearance of DSA nor experienced antibody-mediated rejection (AMR) as demonstrated

upon for-cause and protocol biopsies, up to 8 months posttransplant at the time of abstract submission. This case demonstrates that, although DSA may reappear following re-exposure to a sensitizing HLA antigen/epitope, acceptable transplant outcome can be achieved without AMR. Time between DSA development and transplant could be a factor in identifying acceptable HLA mismatches.

P046

UNDER ASSESSMENT OF DSA STRENGTH IN SINGLE ANTIBODY BEAD ASSAYS MAY LEAD TO POOR OUTCOME IN KIDNEY TRANSPLANTATION

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Solid phase anti-HLA antibody tests have become an important tool in predicting the compatibility of donor-recipient pairs for kidney transplantation. Additionally, single antigen bead (SAB) assays provide valuable information for the management of post-transplant immunotherapy. However, there can be limitations to SAB assays, for example, the presence of donor specific IgM antibodies and/or binding of high levels of compliment may block binding of donor specific IgG antibodies (DSA) to the SAB. This could possibly result in an under estimation of DSA. The following case is an example of this phenomenon. A 48 years old female patient (HLA Type: A1 A29 B53 B72 DR8 DR13) was transplanted with a deceased donor kidney (HLA Type: A2 A33 B53 B58 DR8 DR11 DQB7 DQA04 DQA05 DPB01:01 DPB04:02) in 2017. The patient was presensitized (pregnancy) to a donor antigen A2 (MFI of 4 A2 beads =3904) with an associated epitope 144TKH (MFI=5101, includes A68, A69). On day 3 post-transplant, there was no detectable antibodies to donor antigens due to the sponge effect of the transplanted kidney. On day 6 antibodies to A2 (MFI=5000) and the associated epitope 144TKH (MFI=8946) reappeared. Additionally, a low level of de novo antibodies to B*58:01 (MFI=716) and DPB1*04:02 (MFI=864) was detected. On day 12, antibodies were detected to only A*02:01 (MFI=615), while a significant increase in B58 antibody was observed (MFI=9025). At this time, a biopsy indicated antibody mediated graft rejection; creatinine was 10 mg/dl. Accordingly, the patient's antibody was further evaluated by an AMOS one wash CDC cross match with frozen cells and sera from day 6 and 12. On day 6, CDC-XM was negative, but on day 12 the CDCXM was positive. The level of DSA to class I of MFI 9640 would typically not give a positive CDC cross match. To determine if there was interference of IgM and/or compliment, the 12-day serum was treated at 63C for 13 minutes to inactivate these molecules. The heat-treated serum demonstrated an MFI to A*02:01 of MFI=20,567 compared to an MFI of 615 before heat inactivation. Unfortunately, treatment for antibody-mediated rejection was not initiated soon enough to save the kidney. Although it is rare to observe IgM and/or compliment interference with SAB assays (4 out of 3020 cases, 0.1%), post-transplant assessment of DSA should be performed with heat inactivated sera or other methods to eliminate the under estimation of the strength of IgG antibodies.

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P047

A COLD CASE: WHEN SINGLE ANTIGEN BEADS ASSAY DO NOT RESPOND

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Case history: We evaluated a live donor (D1) and recipient (P1) pair for potential kidney donation however their flow crossmatches (FXM) continuously result in B-cell positive. P1 was tested negative by single antigen beads and no donor specific antibody (DSA) was evident. Patient has been transfused once in 2014 and treated with adalimumab for ankylosing spondylitis in the past. Methods: Autologous FXM of D1 was conducted. A series of FXM with surrogate donors was conducted. Supplemental single antigen beads, FlowPRA beads and Luminex-based mixed screening beads were also conducted but results are questionable. Results: After the first allo FXM, a repeat was done to rule out B cell positive FXM whether it was reproducible. Since patient was screened negative by single antigen beads assay and supplemental beads, absence of DSA could not explain why B cell FXM can be positive. When patient serum subject to two other surrogate donors (D2 “O” and D3 “A”) interestingly B cell FXM has become negative. To rule out blood type issue and non-HLA factors, autologous FXM of D1 and allo-FXM with zero PRA serum (P2 “A”), and with positive PRA serum (absence of DSA, P3 “A”) were performed and all were found negative, but remained positive with diluted serum of P1. Patient P1 was tested again in 3 months however the same result remains. The final FXM was then focused on testing with surrogate donors (D4 and D5) who carry the

same class II antigens as D1. The result became positive with D5. Although no clear evidence can confirm the antibody to DR11 to be allele specific, the collective results infer that suspected DSA DR11 is not distinguishable by FlowPRA screening beads and mixed beads and not detectable by single antigen and supplemental beads. Conclusion: This is the first case in our experience to discover such a situation with “cold bead” or specificity-unproven DSA. Transplantation is not recommended. Correlation between different assays and diligent testing to clarify unreasonable positive FXM is very important especially in cases of autoimmune disease.

P048

PERSISTENT DETECTION OF ANTI-HLA B8 BY IGM AND C1Q TESTING IN THE POST-TRANSPLANT SETTING

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A female patient presented for follow-up care approximately ten years post-cardiac transplantation. HLA antibody testing revealed multiple class I and class II donor specific antibodies (DSA), including anti-HLA A1 (MFI>2500), DQ6, and DQ7/DQ8/DQ9 antibodies (DQ antibodies have MFIs>15,000). Per our current testing protocols, the sample was reflexed to C1q to determine if any DSAs were complement- fixing. As expected, based on antibody strength, the class II DSAs were C1q positive, while anti-HLA A1 was C1q negative. Interestingly, C1q testing revealed reactivity for anti-HLA B8 which was not previously detected by our standard IgG testing methodology. While anti-HLA B8 was not a DSA to the previous transplant, we wanted to determine the underlying cause for the positive reactivity seen with C1q testing: prozone effect or IgM? Since our laboratory routinely treats all sera tested for HLA antibodies with EDTA prior to testing, we thought prozone was unlikely as previous studies performed in our lab demonstrated the removal of this effect. Nonetheless, to rule out prozone with another method, IgG testing was repeated using a series of dilutions (Neat, 1:4, 1:16, 1:64, and 1:256). This testing did not yield positive reactivity for anti-HLA B8, and therefore prozone effect was excluded, indicating that EDTA methodology is robust to overcome prozone effect. To confirm the isotype of this antibody, IgM testing was conducted in three sera using the same assays as the IgG testing which produced positive results. In conclusion, anti-HLA B8 was determined to be complement-fixing and of the IgM isotype. Unexpectedly, our results supported the absence of an IgM-IgG isotype class switch for the period of testing performed in our lab spanning 17 months. *RLU and JJX contributed equally to this work.

P049

DEFINING THE TUMOR ORIGIN IN LUNG POST-TRANSPLANT RECIPIENT USING HLA TYPING

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Here we present a case to show that HLA typing can be used to identify tumor origin in transplant recipients. A 61-year-old man with a past medical history of idiopathic pulmonary fibrosis received right lung transplant on 7/25/2016. He was admitted to the hospital on 12/25/17 with complaints of worsening shortness of breath as well as a non-productive cough and wheezing for a few days prior to arrival. Examination of the lungs revealed a poorly differentiated adenocarcinoma involving the right lung. The patient's left lung had metastatic adenocarcinoma, interstitial fibrosis, fibroblastic foci, and interstitial chronic inflammation. There was also metastatic tumor identified in the liver, vertebrae, adherent to the adventitia of the aorta, esophageal, and gastric serosa. The patient expired 548 days post-transplant. After the finding of cancer in the donor's lung was reported to UNOS, other centers that shared this donor requested additional testing on cancer to determine if it was donor-derived cancer. HLA typing performed on DNA isolated from the formalin-fixed paraffin-embedded tumor tissue sample fully matched the patient's original HLA typing. This finding indicated that tumor did not arise from the donor's lung. This case shows that HLA typing can be used to identify an origin of malignant tumor developed in transplant recipients, in particular, when no fresh tissue or DNA from both donor and recipient is available for microsatellite analysis.

P050

UNUSUAL DP ALLELES ARE BEING FOUND ON A REGULAR BASIS AND MAY NOT BE "UNUSUAL"

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An African American deceased donor was typed by Luminex rSSO (One Lambda) as A23, 30; B45, 52; Cw6, 16; DR7, 18; DR 52, 53; DQ 2, 4; DPB1*01:01, 425/522. Follow-up re-typing of the DP locus by SSP showed DRB1*01:01, 354:01/375:01/584:01. The second DP did not share any alleles between the SSP and rSSO strings, making it impossible to call a type for the second allele. Interestingly, another African American donor showed the same unusual strings by rSSO and SSP within a few weeks of the first typing. The occurrence of these unusual and discrepant strings (SSP vs rSSO) in two patients of the same ethnicity suggested that this may not be a rare allele and identification of this allele is needed. The sample results were sent to the manufacturer who reported that DPB1*584:01 was a shared allele with both methods and was the probable allele. However, our Luminex analysis did not show DPB1*584:01 as an ambiguity with either of these samples. NGS was requested to confirm the DP type. This case demonstrates some of the difficulties laboratories face when performing DP typing, especially on deceased donor samples. More and more "unusual" DP typings are being found on a regular basis. We do not yet have a clear understanding of which DP antigens are unusual or rare. Analysis software for a particular lot may not be updated in a timely fashion to include the latest alleles that should be in the list of ambiguities. Confirmation of these unusual antigens is also not possible during the time constraints of performing deceased donor typing. An even greater problem may be that the DP antibody testing is drastically behind in identifying antibodies to an ever-growing number of possibly "common" DP antigens. Even if we were able to confirm the DP in this sample as DPB1*584:01, we would not be able to identify specificity against this particular antigen, using the currently available DP antibody reagents.

P051

LOW-LEVEL ANTIBODY AGAINST SHARED EPITOPES - ARE THEY RELEVANT?

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This case illustrates the importance of considering low-level antibody reactivity against HLA shared epitopes. A 58 year African American female patient with ESDR due to DM type 2 was evaluated for simultaneous kidney-pancreas transplantation. Although the patient had 4 pregnancies, based on the One Lambda Single Antigen (SA) results, the patient had only weak (<2,000 MFI) antibodies against Cw4, DR53, and some DP antigens. The patient received an offer from a deceased donor. Virtual crossmatch was Negative (donor-specific antigens are shown in blue in Figure 1A). Unexpectedly, Flow cytometry crossmatch was borderline positive, even though the strongest DSA A2 was only ~500 MFI. To investigate this discrepancy, phenotype bead assay (LSPRA) was performed, which clearly detected the presence of weak antibodies against A2, A68, and A69, which are known to share an

epitope 144TKH (Figure 1B). Reactivity against A2 was additionally confirmed using surrogate crossmatches. We concluded that due to epitope sharing the single antigen bead assays may not have reflected the true strength of the patient's antibodies and listed A2, A68, and A69 as unacceptable antigens in UNOS. The patient was successfully transplanted on 9/26/17 avoiding pre-existing DSAs. Interestingly, 10 days after transplant A2, A68, A69 increased to ~20,000 MFI (Figure 1C). Since these specificities were not DSAs, no further action required. However, this case underscores that although low-level antibody against a shared epitope may not be effectively detected by solid-phase platforms, but still may result in positive crossmatch and increase the risk of AMR post-transplant. This case shows that setting positive cut-offs solely based on MFI values may be misleading if a patient has antibody against shared epitopes and that any discrepancy between virtual and cell-based crossmatch needs to be investigated. This case also highlights that use of epitope analysis and multiple assays help to evaluate immunological risk post-transplant more accurately.

P052

ANTIBODY MEDIATED REJECTION IN A CARDIAC TRANSPLANT CASE; TO C OR NOT TO C

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Background: Due to the less sensitive nature of the historical assays and lack of data, and the low expression level of the HLA-C antigens, the role of pre-transplant HLA-C antigens in solid organ transplantation is less clear. Herein we describe a cardiac transplant case were patient was transplanted in the presence of donor specific antibody (DSA) to HLA-C antigen and a negative flow cytometric crossmatch; however, shortly post-transplant they developed antibody mediated rejection (AMR) due to C1q-fixing DSA to the HLA-C antigen. **Method:** IgG and C1q-fixing HLA antibodies were detected using the Single Antigen luminex solid phase assay (One Lambda, Thermo Fisher). While the sole sensitizing antigen could not be determined, HLAMatchmaker was utilized to identify antibody specificities to a shared epitope, 76VRN. Flow cytometric crossmatches (FCXM) were performed on negatively

selected (StemCell Technologies™) pronase-treated lymphocytes. T cell FlowDSA crossmatch (One Lambda, Thermo Fisher) was performed at the Southwest Immunodiagnostics, Inc., Laboratory. **Results:** Regardless of the presence of relatively strong DSA to HLA-C antigens, MFI ranging from 6,000-14,000, T and B cell FCXM were negative. To further understand the clinical relevance of antibodies to the HLA-C antigens, sera was sent out for FlowDSA crossmatch testing. Two surrogate donors, one with DSA to C7 and one with DSA to C7 and C8, were both T cell positive by FlowDSA crossmatch. Nevertheless, due to clinical urgency patient was transplanted in the presence of pre-transplant DSA to HLA-C7 (MFI 12,600) and a negative FCXM. Approximately 10 days post-transplant patient developed clinical and biopsy proven AMR. Single antigen solid phase assay showed elevated DSA to C7 (MFI 19,500) and C17 (MFI 6,500). In agreement with the biopsy results, the post-transplant C1q Single Antigen assay was positive for C7 as well as the antigens carrying the shared epitope 76VRN. **Conclusion:** Pre-formed HLA-C antibodies to shared epitopes can cause acute AMR. The FlowDSA crossmatch enhanced detection of such deleterious antibodies more efficiently than the conventional flow crossmatch assay. Six months post-transplant the patient is stable; however, the long term clinical relevance of such antibodies on the graft outcome remains to be determined.

P053

DP DONOR SPECIFIC ANTIBODIES PRE-RENAL TRANSPLANT: MEAN FLUORESCENCE INTENSITY AND EPITOPE PATTERN ALONE IS NOT PREDICTIVE OF CROSSMATCH RESULT OR CLINICAL OUTCOME.

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The clinical significance of HLA-DP donor specific antibodies (DSA) detected with solid phase assays in recipients of renal transplants remains poorly defined. Recent literature has elucidated that many patients have a HLA-DP antibody pattern of reactivity against broad cross-reactive epitopes which may be more clinically significant. Typically, a DSA that reacts at a mean fluorescence intensity (MFI) of greater than 5000 results in positive crossmatch. We present two cases with pre-transplant HLA-DP DSAs at high MFI (>5000) directed against cross-reactive epitopes identified on virtual crossmatch which resulted in negative and positive flow crossmatch respectively. Case 1 is 57 yr old male who received a deceased donor renal transplant in 2015. He was known to have a HLA-DP9 DSA DED epitope reactivity pattern at 7,333 MFI detected by Luminex bead assay against his donor on peak serum. Pre-transplant crossmatch was negative and he proceeded to transplant. Current creatinine is stable at 150 umol/L and there are no episodes of rejection. Case 2 is 67 yr old female who remains on the deceased donor list awaiting renal transplant with a cPRA of 99%. She has had 3 offers where she has had a HLA-DP1 or HLA-DP3 DSA DEAV epitope reactivity pattern at 10,000 MFI against her donors resulting in flow B-cell positive crossmatch. Crossmatch with donor cells known not to express DP DEAV epitope antigens have been negative. The current cases illustrate that HLA-DP DSAs at high MFI with epitope reactivity may not necessarily result in a positive flow crossmatch. The differential crossmatch results for these two recipients may be related to variable HLA-DPB1 antigen expression in the donor rather than MFI and/or epitope pattern reaction in the recipient. Avoidance of transplant in the context of high MFI HLA-DP DSA epitope cross-reactivity pattern may not be necessary and can be associated with acceptable clinical outcome in the context of a negative pre-transplant crossmatch. HLA-DPA DSAs that have an epitope pattern of reactivity resulting in positive crossmatch are still avoided. Adding DP DSAs to the allocation algorithm without the opportunity to perform flow crossmatch does not allow for accurate immunological risk assessment and may prevent the opportunity for successful transplant.
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P054

UNEXPECTED QUIESCENCE OF EXPECTED MEMORY RESPONSE AGAINST PRIOR KIDNEY ALLOGRAFTS - NOVEL LESSONS LEARNED FROM AN 11 YEAR OLD RECIPIENT OF 3 KIDNEY TRANSPLANTS

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We present an 11 yr old girl who received 3 kidney transplants (KTx). She received 1st-KTx from a living donor at age 4 due to the end-stage renal disease. She had no HLA antibodies (Ab) at pre-1st-KTx, and thus pre-tx crossmatch (XM) and donor-specific HLA antibodies (DSA) were -ve. Six weeks later, the allograft was removed due to torsion (twisting of blood vessels) and thrombosis (formation of a blood clot). The C4d staining of Tx-biopsy was -ve indicating no antibody-mediated rejection (ABMR). She was relisted for the 2nd-KTx. Two-mo post tx-nephrectomy sample revealed Abs to all 10 mismatched (MM) donor's HLA types (and associated CREG) resulting in 97% CPRA. After a 3 yr wait, she received 2nd-KTx from a deceased donor (DD) with -ve XM and DQ8 DSA (MFI=1961). Shortly, she developed complications by BK viremia/nephropathy and immunosuppression was reduced, which potentially increased alloimmune activation. At 2-mo post-2nd-KTx, she developed de novo DSAs directed against 4 mismatched HLAs of 2nd-KTx (1600-14600 MFI). She was treated with multiple rounds of eculizumab, plasmapheresis and IVIG until Dec. 2015, when she presented with an acute increase in serum creatinine to 8.7 mg/dL. KTx biopsy showed acute ABMR. She was relisted for 3rd-KTx on Feb. 2016 with a cPRA 100%. After 2 yr wait, she received 3rd-KTx on Feb. 2018 from a DD with -ve XM and no DSA. The 3rd donor was mismatched by 8 HLA antigens - 1 of them was a repeat-MM with 1st donor and another was repeat-MM with both 1st and 2nd donors. However, Abs to 5 mismatched HLAs of the 3rd-KTx (B8, B37, Cw6, DP2, DP4) found in the historical sera (MFIs 1000-16,000) were completely -ve in multiple sera tested during 2 mo post-3rd-KTx (Fig.). No rejection was noted. Lessons learned from this case were: A 4-yr old human immune system can recognize and respond to alloantigens, and can trigger HLA-Abs production efficiently. However, acquired memory response, an integral component of adaptive immunity is not fully developed until 11 yr of age. These findings have vital implications in pediatric KTxs.

P055

TO BE A DSA OR NOT TO BE A DSA, WE NEED TO KNOW!

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Aim: The accuracy of HLA antibody testing is very important for entering UNOS Unacceptable donor antigens, evaluating organ offers by virtual and prospective crossmatch, and evaluating donor specific antibodies (DSAs) pre and post transplant. It is known that Single Antigen Beads (SAB) often have problems with false positive reactions, when the manufacturing process results in 3-5% denatured HLA determinants on every bead, exposing cryptic, non HLA antigens. This case study demonstrates that SAB false positive HLA antibodies can often be ruled out or verified by using additional tests and analyses, including FlowPRA Screen and LSPRA (phenotype bead) assays and epitope and donor HLA allele analysis, which will reduce the risk of denying lifesaving organ transplants based on false positive HLA antibodies. **Methods:** Deceased donor pronased mononuclear cells from blood, were Flow crossmatched against three sera from a 64% CPRA patient. The results were T cell Negative on all three sera, B cell low positive on one of the sera. By SAB the sera had three potential weak DSAs (B62/2,100 MFI, DRB3* 02:02/1,000 MFI, DPB1*01:01/A1*01:03/2,500 MFI, and DPB1*01:01/A1*02:01/1,000 MFI), LSPRA assay, crossmatch results, and epitope and donor alleles analysis, were used to rule out or confirm the potential DSAs. **Results:** The three potential DSAs were ruled out, see the table below. The B cell Low Positive in serum 1 did not make sense, the potential DSAs were stronger in serum 3, the reactivity was nonspecific, which had been observed before.

Serum:	**1**		**2**		**3**		
Cells:	T	B	T	B	T	B	
FlowXM	Neg	LowPos	Neg	Neg	Neg	Neg	**DSA?**
B62	1,000		1,000		2,100		**No, Phenotype**
DRB3*02:02	660		700		1,000		**No, Phenotype**
DPB1*01:01/A1*01:03	1,700		1,700		2,500		**Not Donor Allele,**
DPB1*01:01/A1*02:01	900		730		1,000		**No Epitope Share**

Conclusions: It is unacceptable that alone, the SAB assay false positive DSAs will deny patients lifesaving organ transplants, when there are tests and analysis methods which can often detect and rule out these antibodies. The SAB assay is a very important test, but unless other testing platforms, like Phenotype beads and FlowPRA screen, are used to identify the false positive antibodies, the SAB assay will lose organ offers for our patients or trigger unnecessary DSA treatments post transplant.

S.S. Geier: *2. Consultant; Company/Organization; LABSNE Clinical Consulting Director. 7. Other (Identify); Company/Organization; UNOS Histocompatibility Committee and MPSC ad.*

P056

EXTENDING THE SEQUENCES OF 569 INCOMPLETE UNCOMMON ALLELES

Ana M. Lazaro-Shiben, Lihua Hou, Misti Persaud, Elizabeth Enriquez, Noriko Steiner, Bin Tu, Carly Masaberg, Jennifer Gerfen, Kanthi Kariyawasam, Jennifer S. Ng, Carolyn K. Hurley, C.W.Bill Young DoD Marrow Donor Recruitment and Research Program. Georgetown University, Rockville, MD.

Aim: The aim of this study was to complete full gene sequences for previously reported HLA class I sequences for the IPD-IMGT/HLA database. **Methods:** We identified 569 class I alleles in ~99,000 individuals typed for a U.S. donor registry that had incomplete sequences in the IPD-IMGTHLA database. The alleles had been typed by a combined Sanger SBT-SSOP workflow focused on exons 2+3. Single locus alleles were amplified with primers annealing in the 5' and 3' UTR and the sequences of exons and introns determined with an Illumina Miseq using an in-house protocol. **Results:** Most (423, 74%) of the 569 alleles were found in a single cell. The majority of the class I full length sequences were identical to the most similar HLA allele (e.g., C*01:70 differed by only a single exon 2 nucleotide when compared to C*01:02:01:01). Two novel HLA-A alleles with exon 2/3 variation were not detected by SBT/SSO due to inability to phase key polymorphic nucleotides [A*03:150, *26:01:01G vs.

A*03:01:01:01+A*26:149 (new) ; A*02:01:55+*33:03:01G vs. A*02:01:01:01+*33:03:33 (new)]. Overall, new alleles were found in 18 (3.2%) samples; most in the targeted incomplete allele. Most (14, 78%) showed variation in intron and UTR regions. Some of the incomplete alleles (49/569) could not be characterized because the allele combination did not allow phasing of the sequences (e.g., C*07:01:01:01+ *07:27:01 had no polymorphic nucleotides within a span of 2298 bp (introns 2-7)). **Conclusions:** Class I gene sequences outside exons 2+3 are conserved. Genotypes with novel alleles can be missed by typing assays that do not provide comprehensive phasing. Increasing the knowledge of full sequences for all alleles will improve the accuracy of HLA typing.

	HLA-A	HLA-B	HLA-C
Confirmatory sequences submitted	160	199	143
New alleles	4 (2.2%)	9 (4.1%)	5 (2.9%)
Exon - Nonsynonymous	1	0	0
Exon - Synonymous	1	2	0
Intron / UTR variation	2	7	5
Unphased	14	14	21

J.S. Ng: *4. Scientific/Medical Advisor; Company/Organization; Georgetown University.* ***C.K. Hurley:*** *4. Scientific/Medical Advisor; Company/Organization; Georgetown University.*

P057

LONG RANGE NEXT GENERATION DNA SEQUENCING OF HLA FROM A REGISTRY POPULATION FROM THE NETHERLANDS

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Aim: Long range next generation sequencing was used to characterize the HLA class I (HLA-A,B,C) and class II (DRB1, DRB3/4/5, DQB1) alleles of a hematopoietic stem cell registry population (N=643) from The Netherlands (Matchis). **Methods:** The in-house typing protocol included several new strategies to reduce errors, add automation, and balance the yield of all loci when 96 samples are sequenced in one 600 cycle paired-end Illumina MiSeq sequence run. **Results:** Of the 22 new class I alleles, three contain a nonsynonymous substitution in an exon (1, 4 or 7). The remainder are single nucleotide intron variants. Thirty two class I alleles are incomplete in the IMGT/HLA database. The majority of the alleles in the population are common; the three most common alleles at each locus are listed in the table. Alleles not previously listed as common or well-documented include mainly 4th field variations; exceptions included single copies of B*51:20, C*07:100, and DRB1*04:92. Alleles encoding nonsynonymous substitutions compared to the primary allele in the G group include, as examples, A*23:17 (1 vs. 20 A*23:01:01:01), B*44:27:01 (1 vs 83 B*44:02:01:01), C*07:06 (3 vs 190 C*07:01:01:01), DRB1*14:54:01 (44 vs 3 DRB1*14:01:01), DQB1*02:02:01 (84 vs 175 DQB1*02:01:01). Non-expressed alleles were not observed. DRB3/4/5 associations were identified. **Conclusions:** Long-range sequencing of HLA alleles from world-wide populations will continue to build our understanding of HLA diversity and its impact on the immune response.

Most common HLA alleles				
HLA-A	HLA-B	HLA-C	HLA-DRB1	HLA-DQB1
A*01:01:01:01	B*07:02:01:01/03	C*07:01:01:01	DRB1*03:01:01	DQB1*02:01:01
A*02:01:01:01	B*08:01:01:01/02	C*07:02:01:03	DRB1*07:01:01	DQB1*03:01:01
A*03:01:01:01	B*15:01:01:01	C*03:04:01:01	DRB1*15:01:01	DQB1*06:02:01

C.K. Hurley: *7. Other (Identify); Company/Organization; Intellectual property sequencing Thermo Fisher.*

P058

THE IMPORTANCE OF COMPLETE HLA-DQB1 SEQUENCES WHEN TYPING BEYOND EXON 2

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Aim: This study examined the variation observed when typing exons 2+3 of HLA-DQB1 using reverse sequence specific oligonucleotides (rSSO) as compared to typing exon 2 alone in volunteers from an unrelated hematopoietic stem cell donor program. New alleles and those with incomplete sequences were characterized with next generation sequencing (NGS) to update the IPD-IMGT/HLA database. **Methods:** Samples were typed for HLA-DQB1 using One Lambda's SSO LabType with additional phasing probes for exon 2 (~50,000 donors) or for exons 2+3 (~15,000). Full-length HLA-DQB1 sequences (40) were obtained using an Illumina platform and in-house protocol. **Results:** Overall, 38 incomplete DQB1 alleles were identified and sequenced with NGS, including 6 that could only be detected by rSSO with exon 3 probes. The majority of these alleles appear only 1-2 times in the sample set and were not listed as common or well-documented in Mack et al. 2013. The addition of exon 3 in rSSO typing complicated analysis since many alleles lack sequence data for exon 3, and the software assumes no probe binding if there is no known sequence. An example is DQB1*06:08 (~10), which binds to one of the exon 3 probes and therefore is not assigned (i.e., false positive). Two alleles showed discrepant results between rSSO and NGS due to novel sequences. The full-length sequences obtained for all alleles sequenced demonstrate high conservation of the regions outside of exon 2. **Conclusions:** There are advantages and disadvantages from screening exons 2+3 of HLA-DQB1. It elucidates greater variation not identified from screening exon 2 alone but has a more complicated analysis due to incomplete DNA sequences. Updating the IPD-IMGT/HLA database is critical for improved accuracy.

C. Masaberg: *7. Other (Identify); Company/Organization; Intellectual property typing workflow, One Lambda licensing.* ***C.K. Hurley:*** *7. Other (Identify); Company/Organization; Intellectual property typing workflow, One Lambda licensing.*

P059

ANALYSIS OF HLAcII PEPTIDOMES PRESENTED BY DENDRITIC CELLS (DCs) FROM HEALTHY DONORS AND HEMOPHILIA-A (HA) PATIENTS WITH OR WITHOUT FACTOR VIII (FVIII) INHIBITORS AFTER EX VIVO ADMINISTRATION OF DIFFERENT THERAPEUTIC FVIII PROTEINS (tFVIIIs)

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Aim: T cells specific for tFVIIIs in HA patients can induce neutralizing FVIII antibodies called inhibitors. DCs that present tFVIII derived peptides in their HLAcII repertoire may activate T cells. We employed DC protein processing and presentation assays (DCAs) to quantify tFVIII peptides in HLAcII peptidomes and used these as measures of the immunogenic potential (IP) of tFVIIIs as a function of their associated HLAcII/tFVIII peptide density. **Methods:** We used DCs from 28 healthy donors and 5 HA patients [2 inhibitor (Inh) positive and 3 Inh negative] in 3 DCAs. In DCA 1, DCs from healthy donors 1-12 were given an equimolar mix of 5 recombinant (r) FVIIIs. In DCA 2, DCs from healthy donors 13-24 were given a plasma derived (pd) FVIII + vWF or 1 of 2 rFVIIIs (BDT or FL) ± vWF. In DCA 3, DCs from healthy donors 25-28 and HA patients 1-5 were incubated with pdFVIII or FL, BDD, or BDT rFVIII, all + vWF (Fig. 1A). After DC lysis and DR, DQ and DP isolation, we identified tFVIII peptides by LC-MS/MS. We performed a log linear analysis of the DR bound tFVIII peptides to identify determinants of Inh risk

(Fig. 1B-E) with random effects to control for potential donor- and DCA-clustering. Space limits prevent description of the DQ and DP peptides.

Results: The IP of tFVIIIs was greater in HA patients than in healthy donors and in Inh positive than in Inh negative patients (Fig. 1B-E). We found that BDT + vWF and FL ± vWF had lesser and greater IP, respectively, than pdFVIII + vWF; the IP of BDD + vWF was comparable to that of pdFVIII + vWF. The rFVIII mix had the greatest IP. The higher IP in HA patients versus healthy donors and in Inh positive vs Inh negative patients reflect significant correlations of 0.91 and 0.52 with the presence/absence of the FVIII Inh risk allele DRB1*15:01. **Conclusions:** DC protein processing and presentation is affected by HLAcII repertoires but not by HA patient or FVIII Inh status after accounting for correlation with DRB1*15:01. Relative to pdFVIII, rFVIIIs have different IP with BDT and FL being significantly less and greater, respectively.

T.E. Howard: *4. Scientific/Medical Advisor; Company/Organization; Haplomics Biotechnology.* ***M. Hofmann:*** *5. Employee; Company/Organization; CSL Behring.* ***L.V. Dinh:*** *5. Employee; Company/Organization; Haplomics Biotechnology.* ***J. Powell:*** *5. Employee; Company/Organization; CSL Behring.* ***E. Maraskovsky:*** *5. Employee; Company/Organization; CSL Limited.*

P060

CHARACTERIZATION OF 108 GENOMIC DNA REFERENCE MATERIALS FOR 11 HLA LOCI: A GeT-RM COLLABORATIVE PROJECT

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Aim: Improve availability of publicly available, renewable, and well-characterized genomic DNA reference materials to support molecular HLA typing assay development, validation and verification, quality control, and proficiency testing. **Methods:** The Centers for Disease Control and Prevention's (CDC) Genetic Testing Reference Materials Coordination Program (GeT-RM) together with three clinical laboratories and the Coriell Cell Repositories characterized genomic DNA from 108 cell lines for HLA-A, B, C, DRB1, DRB3, DRB4, DRB5, DQA1, DQB1, DPA1, and DPB1. DNA was isolated by the Coriell Cell Repositories and distributed to the 3 laboratories. Methodologies used were: PCR-SSO - LABType® SSO (One Lambda, Canoga Park, CA), SSP - Olerup SSP® (Stockholm, Sweden), SBT -AlleleSEQR® HLA-SBT (Abbott Molecular, Des Plaines, IL) and NGS - HoloType HLA™ kits (Omixon Inc., Budapest, Hungary). Each of the 11 genes was tested by 2 or 3 methods. Consensus genotype was the NGS result, which provided the highest level of resolution, and was consistent with the results obtained by the parallel testing using other methods. **Results:** HLA typing results using SBT, SSP, SSO and NGS were 99.95 % concordant. Of the 1905 alleles typed, only one discrepancy was observed. The discrepant result was found to be due to an error in SBT/SSO typing. Five unique novel alleles with differences in exonic regions were identified and three were submitted to the WHO Nomenclature Committee. Ambiguities persisted in 50/1905 allele calls, most due to alternative cis/trans combinations of exons 2 and 3 of DPB1 (n = 21 pairs: 42 alleles) and 8 caused by polymorphisms in a non-covered region (exon1) of the DRB1and DPB1 genes. Thirty HLA-A, 54 -B, 30 -C, 36 -DRB1, 4 -DRB3, 4 -DRB4, 3 -DRB5, 19 -DQA1, 17- DQB1, 10 -DPA1 and 29 -DPB1 alleles are represented in the panel. These alleles cover a high percentage of HLA specificities present in five ancestry groups in the USA population. **Conclusions:** These genomic DNA samples are publicly available from the National Institutes of General Medical Science (NIGMS) Repository at the Coriell Cell Repositories.
M. Bettinotti: *3. Speaker's Bureau; Company/Organization; One Lambda Thermo Fisher.* ***D. Ferriola:*** *7. Other (Identify); Company/Organization; Licensing proceeds of the HLA-NGS protocol by CHOP to Omixon.* ***J.L. Duke:*** *7. Other (Identify); Company/Organization; receiving proceeds from the licensing of the HLA-NGS protocol by CHOP to Omixon.* ***D. Monos:*** *4. Scientific/Medical Advisor; Company/Organization; Omixon. 7. Other (Identify); Company/Organization; receiving proceeds from the licensing of the HLA-NGS protocol by CHOP to Omixon.*

P060A

MICA TYPING BY NGS REVEALED THE ASSOCIATION OF MICA GENE POLYMORPHISM WITH SPECIFIC IgE PRODUCTION IN CEDAR POLLEN ALLERGY

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Aim: We have found the association of Japanese Cedar Pollinosis (JCPsis) with SNPs near MICA gene by SNPs typing in HLA gene region. In this study, the association was clarified by MICA gene typing using amplicon sequenceing method by NGS. **Methods:** DNAs from 178 JCPsis patients, 185 healthy controls were tested allele types of MICA and HLA by HLA and KIR typing Kit (Scisco Genetics Inc) on MiSeq (Illumina Inc,). Serum samples from them were titrated for the presence of specific IgE antibodies to Japanese cedar pollen(JCP), orchard grass pollen, mugwort pollen, & house dust mite by radioallergosorbent test. **Results:** Though all controls were selected not to have any symptom of pollinosis, only 101 in 185 individuals were specific IgE negative to all allergens tested, and 84 individuals were positive to one or more allergens including JCP. Therefore, the control group was divided into two groups, specific IgE negative and positive to allergens. The significant association of MICA with JCPsis was found only in analysis using IgE negative control group. IgE positive control group showed no association. MICA*008((P=0.0036, odds=0.5) & MICA*027 (P=0.016, odds=0.55) showed strong reverse association. **Conclusions:** MICA*008 is lacked a part of trans-membrane region and produce soluble protein. Furthermore, **008 and 027** has the haplotype of 175Gly, 213 Ile, 251Arg which are reported to relate with MICA shedding. This haplotype associate with JCPsis strongly (P=0.0000399, Odds=0.46). It is speculated that soluble

MICA may suppress IgE production by binding NKG2D and inactivating NK cell, and suppress pollinosis. This speculation is also supported by the report that NKG2D is critical for allergy.

P061

ALLELE-LEVEL GENOTYPING OF KIR2DL4 IN LARGE EUROPEAN POPULATION SAMPLES REVEALS HIGHLY SIGNIFICANT HETEROZYGOTE EXCESS FOR 9A/10A ALLELIC VARIANTS

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Aim: KIR2DL4 is an evolutionarily conserved framework member of the human killer-cell immunoglobulin-like receptor (KIR) gene family. It is unique amongst KIR genes in that it may elicit both activation and inhibition signals. Moreover, KIR2DL4 alleles are polymorphic for a frameshift mutation in the transmembrane domain that leads to a truncated cytoplasmatic tail. Alleles with a 10A homopolymer in exon 7 encode receptors that are expressed on the cell surface of NK cells. In contrast, alleles with a 9A frameshift mutation have been shown to produce soluble secreted KIR2DL4 receptors. Here, we investigate allele and genotype frequencies of 9A and 10A alleles in large European population samples. **Methods:** In 2016, DKMS Life Science Lab has established an exon-based NGS workflow for KIR genotyping. Between 09/2017 and 03/2018, we have performed successful allelic-resolution genotyping for KIR2DL4 for approximately 380,000 potential bone-marrow donor samples originating from Germany (DE), Great Britain (GB), and Poland (PL). These samples where checked for presence of the 9A frameshift mutation. **Results:** Overall, the defective 9A variant was slightly underrepresented in DE (AF_{9A} = 0.496, n = 242,983) and GB (AF_{9A} = 0.475, n = 31,100) but overrepresented in the Polish sample (AF_{9A} = 0.537, n = 101,998). The fraction of homozygous individuals (10A/10A or 9A/9A) ranges from 44.4% (DE) to 44.9% (PL). Comparing expected and observed genotype frequencies indicates a highly significant deviation from Hardy-Weinberg Equilibrium ($P < 10^{-9}$), and a consistent heterozygote excess across all three populations, with inbreeding coefficients ranging from FIS = -0.104 (GB) to -0.115 (PL). **Conclusions:** The high frequency of 10A alleles in all three populations indicates no negative selection against a lack of cell surface expression of KIR2DL4. Rather, the strong signature of heterozygote excess across populations may best be explained as a result of overdominant selection (i.e., "heterozygous advantage"). This suggests that the presence of both, cell-surface-expressed KIR2DL4 receptors and soluble secreted KIR2DL4 receptors confers a selective advantage to humans.

P062

CODING AND NON-CODING VARIATION IN 60 NOVEL FULL-LENGTH SEQUENCES OF KIR2DL1

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Aim: KIR2DL1 is a member of the killer-cell immunoglobulin-like receptor (KIR) family, which is an important factor of the human immune system. KIR genes are key regulators of natural killer cell activity and partly bind to proteins of the human leukocyte antigen (HLA) family, e.g. HLA-B and -C in the case of KIR2DL1. Likely due to the complexity of the KIR locus with its extensive genetic variation, only little is known about the impact of allelic variation. Here, we identified novel KIR2DL1 alleles by routine high-throughput exon-based KIR genotyping and subsequently created full-length reference sequences. We analysed coding and non-coding variation to identify possible systematic differences between alleles. **Methods:** We sequenced whole 16 kb amplicons of KIR2DL1 using shotgun sequencing (Illumina MiSeq) and single molecule real time (SMRT) sequencing (PacBio Sequel). Using the R package DR2S, we combined phase information from SMRT sequencing with the accuracy of shotgun sequencing to generate phased full-length sequences. **Results:** We successfully generated 60 distinct KIR2DL1 allele sequences from 45 specifically selected samples. This includes 41 novel alleles and 17 distinct alleles that were previously only partially characterised. The sequence analysis revealed three deep allelic groups separated by 164 variable positions. Allelic variants of two groups harbour mutually exclusive nucleotides, while a third group of alleles may harbour nucleotides of both the other groups. The three groups are mostly separated by non-coding SNPs, indels, and a T homopolymer of fixed length in one and variable length in two groups. Some separating variants exist in most exons and alter the amino acid sequence of the leader peptide, the extracellular D1- and D2-domains containing the HLA binding sites as well as the cytoplasmatic domain. **Conclusions:** Our sequencing efforts resulted in a 4-fold increase in known full-length sequences of KIR2DL1, enabling further research on this KIR gene. We gained insights into systematic differences at the sequence level which might be responsible for or indicative of medically relevant

allelic differences. Especially variation in the D2-domain has been shown to be involved in binding to HLA proteins and may as such be clinically relevant.

P063

KILLER-CELL IMMUNOGLOBULIN-LIKE RECEPTORS GENOTYPES & HAPLOTYPES AMONG KUWAITI FAMILIES

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Aim: Inhibitory and activating immunoglobulin like receptor (KIR) family is a major determinant of NK cells activity against cancer. Specific KIR genotypes and/or haplotypes have been reported to influence the outcomes of hematopoietic cell transplantation and in association with a number of autoimmune disorders. The repertoire of KIR genes varies within and between populations, creating a diverse pool of KIR genotypes. The aim of this study was to characterize the genotypes and haplotypes of the KIR genes among 25 Kuwaiti Arab families. **Methods:** A total of 75 individuals from 25 Kuwaiti Arab trios families (including father, mother and a child) were enrolled in this study. KIR genotyping was performed using Real-time PCR based KIR genotyping kit (Linkage Biosciences) from peripheral blood. Sequence specific primer amplification combined with a melting curve analysis method was used to identify 14 KIR genes and 2 KIR pseudogenes including both full length and deleted forms of 3DP1 and 2DS4. Interpretation and reporting of genotype with SureTyper ™ software**. Results:** The percentage for the presence of the KIR genes was found to be: KIR2DL1 97.2%, KIR2DL2 56.3%, KIR2DL3 88.7%, KIR2DL4 100%, KIR2DL5 62%, KIR2DP1 97.2%, KIR2DS1 35.2%, KIR2DS2 56.3%, KIR2DS3 42.3%, KIR2DS4 95.8%, KIR2DS5 31%, KIR3DL1 95.8 %, KIR3DL2 100%, KIR3DL3 100%, KIR3DP1 100%, KIR3DS1 36.7%. The framework genes were present in 100% for all 75 individuals. The highest frequency of 97% is in 2DP1 and 2DL1 gene and lowest frequency of 31% among KIR genes is in 2DS5. Only 32% of the tested subjects were of AA haplotype genotype, making 68% Bx haplotype genotype. **Conclusions:** Findings of this study are crucial to characterize and compare KIR gene and haplotype frequencies with other populations and also to provide a new data on KIR genotypes and haplotypes of the Kuwaiti Arab population based on family study to understand the population's genetic makeup. Further, majority of Kuwaiti patients have a several healthy haploidentical relatives who could potentially donate, therefore donor activating KIR-based donor selection is an area of current interest.

P064

EVALUATION OF MIA FORA NGS FLEX HLATYPING KIT

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Aim: As the HLA database expanding, traditional SBT method results in more and more ambiguities and requires increasing additional testing. We evaluated Mia Fora FLEX11 NGS typing kit with automation intending to replace SBT with NGS. **Methods:** 108 DNA samples were selected. 92 and 16 samples were previously typed by SBT and rSSO/SSP, respectively. All DNA were prepared from blood except 4 from buccal swab and 3 from B cell line. DNA were extracted 2009-2017 with $OD_{260/280}$ 1.51-2.05. NGS typing was performed following manufacturers automated protocol using Mia Fora FLEX11 kit. **Results:** 313 unique alleles were tested in our validation cohort including 269 CWD alleles, 38 rare alleles, 3 null alleles and 7 new alleles. All DNA samples were successfully typed with typing results accurate to the resolution of third field according to WHO nomenclature. 11 ambiguities were identified which were all with DPB1. 7 potential new alleles were identified: 2 HLA-B, 1 DRB5, 1 DQA1 and 3 DPA1. NGS typing was 99.5% (1499/1506) concordant with previous typing at allele level. Discordance was observed with DQB1, DRB3/4/5 and DPA1 (Table 1). Allele dropout was observed in 1 out of 23 DQB1*06:02 and 2 out of 11 DRB4*01:01 in the validation cohort. Both dropouts can be predicted by allele association with DQA1 or DRB1. Sample repeat rate was 11% (18/161). Four samples failed due to software glitch with the Window 10 version but corrected when switched to the Linux Version. Most other failure was due to insufficient data which can be resolved by repeating test. The only failure that could not be resolved by repeat was DRB4*01:01 dropout.

Table 1: Mismatches between NGS typing and previous typing

Sample ID	NGS Result	Previous Typing Result
21	DQB1*06:02 drop out	DQB1*06:02 by SBT
24	DRB4 drop out	DRB4*01:01 by rSSO
74	DRB4 drop out	DRB4*01:01 by rSSO
23	DRB3*01:01:02/DRB3*01:14	DRB3*01:01 (DRB3*01:15 masked) by SSP
78	DRB3*01:01:02/DRB3*01:14	DRB3*01:01 (DRB3*01:15 masked) by SSP
7	DRB5*02new	DRB5*01:06 by rSSO
234	DPA1*02new	DPA1*02:03 by rSSO

Conclusions: Comparing to SBT method, Mia Fora typing produced high resolution results with ambiguities only presented with DPB1 locus. Little or no additional testing was required. Mia Fora typing had a high concordance rate with previous typing results. A reasonable failure rate was observed where most could be resolved by repeating test except for some DRB4*01:01 dropout.

P065

POLYMORPHISMS IN PRO- AND ANTI- INFLAMMATORY CYTOKINE GENES AND HIV INFECTION IN NORTH INDIANS

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Aim: Various candidate gene based studies on available specialized cohorts viz. viremic controllers, elite controllers, exposed uninfected individuals, rapid and slow progressor individuals support role of immunogenetic factors in defining HIV/AIDS outcome. In this context, the differential genetic influence of cytokine genes on immune response network can affect the establishment and spread of HIV infection; however information is limited on Indian populations. Therefore, here we aimed to explore the largely unknown genetic influence of various pro (IL-1, IL-2, IFN-γ etc.) and anti inflammatory (IL-4, IL-10, TGF-β etc.) cytokine gene polymorphisms on HIV infection, particularly in North Indian population. **Methods:** We enrolled a total of 157 HIV patients, 130 healthy and 27 exposed uninfected healthy controls from the North Indian states after obtaining the institutional ethical approval and participants' informed consent. Evaluation of 22 functionally relevant single nucleotide polymorphisms in 13 cytokine and their receptor genes was performed by genotyping using PCR-SSP based approach. Appropriate softwares and statistical tests were used (chi square and Fishers Exact test wherever applicable) for comparison. **Results:** Frequencies of most of the analyzed variants were statistically indifferent between HIV patients and uninfected healthy controls at the allelic, genotypic and haplotypic levels. Significantly higher allelic frequencies of IL-1α -889 T and IL-4 -1098 T were observed in HIV patients, while IL-1α -889 CC, IL-4 -1098 GG and IL-6 nt565 AA genotypes were observed significantly lower as compared healthy uninfected controls. **Conclusions:** This study represents the first report that highlights the influence of various cytokine gene polymorphisms in context of HIV infection in the North Indians. The observed associated variants are known to have functional relevance towards modulating the inflammatory mileu.

P066

HLA-A*02 REPERTOIRE IN THREE DEFINED POPULATION GROUPS FROM NORTH AND CENTRAL INDIA: PUNJABI KHATRIES, KASHMIRI BRAHMINS AND SAHARIYA TRIBE

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Aim: The allelic family of HLA-A*02 with a repertoire of approximately 951 known alleles represents the predominant and most heterogeneous group at the HLA-A locus. This remarkable diversity signifies the evolutionary relevance of this allelic family. Its population specific diversity is attributed to environmental factors and pathogen pressure and can be harnessed in biology and medicine; particularly in disease association and HLA

based vaccine approaches. We therefore investigated the HLA-A*02 repertoire in three defined population groups including a Central Indian tribe Sahariya (ST, N=100) and two North Indian caste populations *viz* Punjabi Khatries (PK, N=250) and Kashmiri Brahmins (KB, N=160). **Methods:** Luminex based high resolution rSSO method was utilized for HLA genotyping. Results were also confirmed using high resolution PCR-SSP and/or next generation sequencing (NGS) based approach. **Results:** HLA-A*02 was observed with an overall high phenotypic/ allelic frequency in these populations. However, within A*02 repertoire, differences were observed among the three population groups evaluated. A total of 6 alleles were observed (A*02:01, *02:03, *02:05, *02:06, *02:07 and *02:11) in the caste groups (PK and KB) compared to four (except *02:05 and *02:07) in the tribals (ST). A striking observation was the high occurrence of A*02:11 i.e. 33-38% in caste groups and extremely high, more than 80% in tribals. Globally, this allele is rarely observed and is present with low frequencies in limited ethnic groups. The primordial A*02:01 allele, which is the representative A*02 allele in most ethnicities was observed as the second most predominant allele (~30% in caste groups and ~10% in tribals). **Conclusions:** Extreme high prevalence of A*02:11 in Sahariya Tribes may be representation of ancient Indian genetic pool and in caste populations the observed A*02 repertoire may be a consequence of genetic drift, natural selection and/or admixture from different races.

P067

A SIMPLE SEQUENCING BASED METHOD FOR IDENTIFICATION OF DPB1 RESIDENT SNP rs9277534 DEFINING TH LEVEL OF DPB1 EXPRESSION

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Aim: Hematopoietic cell transplantation from unrelated donors carries the risk of acute graft-versus-host disease (GvHD). HLA-DPB1 matching has recently emerged as an important part of donor selection. HLA-DPB1 classification by T-cell epitope is currently used to identify permissive and non-permissive donor-recipient combinations. In addition, DPB1-linked SNP rs9277534 has been recently implicated in the severity of GvHD following transplantation with non-permissive DPB1 mismatching. Variant rs9277534A was associated in low expression, whereas rs9277534G was associated with high expression of DPB1. Recipients with the high expression variant who received transplant from DPB1-mismatched donors with low expression variant had high risk of GvHD. A simple and low-cost SNP rs9277534A/G test can assist optimal donor selection when multiple HLA suitable donors are available. **Methods:** A simple Sanger sequencing-based method for identification of rs9277534 variants was developed. M13-tagged PCR primers were designed by applying NCBI Primer-BLAST tool to the fragment of DPB1 3'UTR containing the targeted SNP. Amplified fragments were sequenced with M13 sequencing primers. The targeted nucleotide position, n150 from the sequence start, can be visualized using any available sequencing analysis software and clearly shows the variant of SNP ("A", "G" or "A/G"). **Results:** Using the published list of A/G variants and a large set of samples with different combinations of DPB1 alleles, we defined a number of additional low- and high-expression DPB1 alleles. The published association of individual DPB1 alleles and rs9277534 variants was confirmed. **Conclusions:** The extended list of low- and high-expression DPB1 alleles will provide a basis for assessing the suitability of DPB1-mismatched donors in the situation when a DPB1-matched donor is not available. The developed Sanger sequencing-based method for identification of rs9277534 variants can be used to identify the level of expression of DPB1 alleles previously not assessed.

P068

THE NOVEL RECOMBINANT HLA-A*11:271 STORY: SEROLOGY TO THE RESCUE OF THE MISNOMENCLATURE

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Aim: The wide spread use of NGS for HLA typing has resulted in exponential growth of novel alleles reported to the IMGT. However, the majority of these novel alleles have not been serologically characterized which has significant clinical implications in solid organ and HCT, particularly pertaining to assignment of donor specific HLA antibodies. **Methods:** The identified novel allele had been originally and unsuccessfully tested by rSSOP, SSP, Sanger SBT and Real-Time PCR. Subsequently, the sample was tested by NGS using Mia Fora reagents and analysis software (Immucor). The subject was redrawn for serologic typing using Terasaki trays (One Lambda). A family study analysis was conducted to determine the segregation of the novel allele carrying haplotype by descent.

Results: With the exception of NGS, none of the molecular methods produced a result of the A locus. NGS revealed a novel HLA-A allele that appears to result from a recombination between A*11:01:01:01 (ex 1 and partial ex 2) & A:01:01:01 (starting from ex 2) as depicted in the figure of the nucleotide and amino acid partial sequence alignments and the NGS coverage plot. The Mia Fora software assigned the HLA A locus typing as A*33:03:01 and the second allele as novel with the closest reference allele match as A*01:01:01:01. The haplotype assignment function of Mia Fora identified the 2 haplotypes of the subject carrying the novel allele which was confirmed by a family study. The novel allele was confirmed by NGS using TruSight HLA typing kit (Illumina) and submitted to IMGT and assigned the name A*11:271. Serological typing was performed and showed positive reactions with 2 operationally monospecific A1 antisera and one polyspecific (A1 & A36) sera, but with neither of the 2 operationally monospecific A11 antisera on the typing tray.

Conclusions: This report presents a cautionary note that in the absence of serological characterization of HLA alleles, the current IMGT nomenclature may not reflect the clinically relevant antigen specificity of these alleles.

P069

UTR SNPS RS111686073 and RS73410010 INDEPENDENTLY MODIFY EXPRESSION OF TAPASIN IN BLACKS

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Aim: The transmembrane glycoprotein tapasin (TAPBP) mediates the binding of MHC-I and TAP1/2 of the peptide loading complex in the endoplasmic reticulum and aids in optimal peptide loading. TAPBP absence in mice alters both MHC-I expression and T cell development, and virally mediated inhibition of *TAPBP* gene transcription impairs antigen presentation in CMV infection. Owing to its importance in antigen presentation, we hypothesized that TAPBP expression may also affect HIV pathogenesis, and gene variants indicating TAPBP expression could then serve as markers for HIV outcome. We sought to identify single nucleotide polymorphisms (SNPs) affecting TAPBP expression and ascertain the mechanism. **Methods:** A scan of the *TAPBP* gene identified two variants, rs111686073 (G/C) and rs73410010 (A/G), found only in blacks that significantly and independently correlated with TAPBP expression via qPCR in three South African cohorts (FRESH, SK and CAPRISA002). Luciferase expression constructs containing the UTRs of TAPBP were generated and examined in HeLa cells. For rs111686073, electrophoretic mobility shift assays (EMSAs) were performed to determine if the transcription factors predicted to bind by Alibaba2 mediated the change in TAPBP expression. **Results:** Both rs111686073 ($p = 0.0003$-0.0006) and rs73410010 ($p < 0.0001$-0.0001) were found to cause significant changes in TAPBP mRNA levels in the tested cohorts. Luciferase assays confirmed that rs111686073 altered expression, with the G variant conferring higher expression than the C variant in HeLa cells ($p = 0.0004$), and the SNP was predicted (Alibaba2) and confirmed via EMSA to be a binding site for transcription factor AP-2a. **Conclusions:** The SNPs rs111686073 and rs73410010 are independently and significantly associated with TAPBP expression, and rs111686073G results in increased expression over the C variant, likely due to binding affinity of AP-2a. The lower expression of TAPBP may alter TAPBP-dependent HLA-B expression and antigen presentation, and affect CD8+ T cell development. Tapasin expression and tapasin-dependence of HLA-I alleles will be considered in future studies of HIV pathogenesis.

P070

A NOVEL NGS WORKFLOW REVEALS MICA ALLELE FREQUENCIES BASED ON 350,000 GERMAN SAMPLES

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Aim: The MICA and MICB molecules serve as ligands of the activating NKG2D receptor expressed on natural killer (NK) cells. Recent studies indicate effects of MIC genotypes on hematopoietic stem cell transplantation (HSCT) outcome. To provide MIC allele information for donor selection, we developed an NGS-based high-throughput genotyping workflow for MICA and MICB and applied it for genotyping registry donor samples. **Methods:** Exons 2 and 3, as well as most of exons 4 and 5 of MICA and MICB are amplified in a multiplexed PCR reaction. The PCR products are sequenced on Illumina HiSeq or MiSeq instruments. The data are processed by an updated neXtype software version to provide allele-level genotyping information. **Results:** Using this NGS based workflow, we genotyped 350,000 donors registered in Germany and report on the observed MICA allele frequencies. Due to the restricted sequence coverage, 9 alleles encoding distinct proteins cannot be resolved. However, the unprecedented depth of the study allowed us to estimate allele frequencies for 49 of the 84 described MICA alleles distinguished at the protein coding level. In addition we identified novel alleles in 0.2% of the samples. The 13 (31) most abundant alleles account for a cumulative allele frequency of 99% (99.99%).

Conclusions: This newly developed NGS-based genotyping approach offers the opportunity to analyze the genetic diversity of MICA and MICB in large cohorts at high-resolution.

P071

LIMITED EXON SEQUENCE MISMATCHES OUTSIDE THE ANTIGEN RECOGNITION DOMAIN IN A COHORT OF 4,646 HIGH RESOLUTION 10/10 HLA-MATCHED DONOR AND RECIPIENT

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Aim: In hematopoietic stem cell transplantation (HCT), HLA allele matching between donor and recipient has traditionally focused on the polymorphic antigen recognition domain (ARD). Mismatching at the ARD is known to influence outcomes. This study investigated the genetic differences in the non-ARD regions among ARD-matched donor-recipient pairs to determine possible association with the transplant outcomes. **Methods:** We compared the full-length HLA Class I allele sequences (HLA-A, -B, -C) and partial-length (partial intron 1 through partial intron 3) Class II allele sequences (HLA-DRB1, DQB1) for 4,646 high-resolution 10/10 HLA-matched HCT donor-recipient pairs. We developed a comprehensive HLA allele sequence comparison pipeline, which identifies and annotates the mismatched positions between two alleles by their functional region and their protein sequence differences using IMGT/HLA Database (v3.31.0). **Results:** For HLA Class I alleles, 95.4% of the ARD matched alleles have identical sequences outside the ARD, including introns and non-ARD exons. 0.3% of the mismatches were synonymous variants from the ARD region while 0.2% and 0.1% of mismatches found from non-ARD exons were synonymous and nonsynonymous variants, respectively. The intronic variation accounted for 4.2% of the mismatches. Similarly, for HLA Class II alleles, 0.3% of mismatches were synonymous ARD variants, and the mismatches in the non-ARD exons were also very rare (synonymous: 0.3%; nonsynonymous: 0.2%). However, a high degree of polymorphism was observed in the intronic regions of the Class II genes with only 77.3% of the allele pairs having identical sequences. 0.2% and 4.6% of Class I and Class II allele pairs, respectively, showed both exonic and intronic mismatches. **Conclusions:** HCT donor/recipient pairs matched at high resolution for HLA-A, B, C, DRB1 and DQB1 have limited coding variation outside of the ARD. Intronic variation was observed at a higher rate for HLA Class II in our study compared to prior publications (17.3% vs. <2% in Hou et al HI 2017) and may be due to differences in typing methodologies and sequencing of highly repetitive intronic regions. Assessment of non-ARD mismatches and impact on clinical outcome will require larger datasets due to the low frequency of coding variant mismatches.

P072

NGS CHARACTERIZATION OF EXTENDED HLA HAPLOTYPES IN JAMAICAN FAMILIES FROM THE CARIBBEAN BONE MARROW REGISTRY: A STUDY OF THE 17TH INTERNATIONAL HLA & IMMUNOGENETICS WORKSHOP

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Aim: Family studies are the gold standard for studying HLA haplotype segregation. This information is critical when studying haplotype associations among populations where few members of the population have been typed, rendering bioinformatics algorithms less reliable. Here, we characterize HLA haplotypes in 5 Jamaican families identified through the Caribbean Bone Marrow Registry. **Methods:** Twenty Jamaican individuals of 5 families were tested by NGS HLA genotypes at loci A, B, C, DRB345, DRB1, DQA1, DQB1, DPA1 & DPB1. We initially attempted full gene sequencing but amplification failed repeatedly due to suboptimal DNA quality from buccal swabs. Typing was performed using Scisco Genomics kit on the MiSeq. Identified haplotypes were compared to haplotype frequency ranking among the 5 major US populations (Caucasians (CAU), African Americans (AFA), Hispanics (HIS), Asian Pacific Islanders (API), and Native Americans (NAM)) using the NMDP Haplostats. Haplostats provides data on 6-locus 2-field resolution, which could give a higher estimate than the 9-locus 3-field

haplotypes described here. **Results:** Typing was successful in all individuals and all loci except for failed DPB1 typing on 2 subjects due to low DNA quantity. Analysis of 20 members identified 17 unique haplotypes. The most common haplotype among CAU, HIS, and NAM and 2nd most common in AFA (Haplotype A in table 1) was observed in 3 families. Another haplotype in 2 families (B) was uncommon among CAU, HIS & AFA (ranked 20466, 23219 & 8463, respectively) and not reported in other populations. Overall observed haplotypes were more common in AFA than other population and 2 haplotypes (C & D) were seen only in AFA. One haplotype (E) was seen only in API (ranked 5660). One haplotype (F) was not reported for any population. **Conclusions:** Although the number of tested families is small, this data suggest that the Jamaican population is unique and diverse, and supports the proposition of establishing a local/regional BMT registry to maximize the chances of identifying matched donors.

P073

THE COMMON UNCOMMON EXTENDED HLA HAPLOTYPE: DO TWO WEAK ASSOCIATIONS MAKE IT STRONG?

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Aim: Genetic recombination occurs at specific hotspots within the MHC, leading to widely dispersed block-like structures; the alpha block containing HLA-A, beta block (HLA-C and HLA-B) and delta block (HLA-DR and HLA-DQ). Linkage disequilibrium (LD) is much stronger within than across these blocks. The HLA-A locus (most telomeric) has weaker LD with extended HLA haplotypes. Additionally, a recombination hotspot exists between HLA-DP genes and other class II loci, which leads to an HLA-DPB1 match rate of only 10-15% in 10/10 HLA-matched donor/recipient pairs. This report describes an extended 9 locus HLA haplotype found in 2 HCT and 1 kidney transplant recipients and their potential donors (8, 2 and 1 donors, respectively). **Methods:**

1. Case review of 2 unrelated HCT recipients, both European Caucasian (CAU) and their related and unrelated potential donors
2. Family study of HCT recipient and a sibling to identify the haplotype by segregation
3. Determine frequency of observed haplotype in different populations through Haplostats.org (6 locus at 2 field resolution could yield higher estimate than the 9 locus 3 field resolution haplotype described here)
4. Searching lab database for incidence of this haplotype

Results: All identified cases shared the following haplotype HLA-A*31:01:02~C*07:02:01~ B*07:02:01~DRB5*01:01:01~DRB1*15:01:01~DQA1*01:02:01~DQB1*06:02:01~DPA1*01:03:01~DPB1*04:01:01. Although most alleles in this haplotype are fairly common, with HLA-A*31:01 least common (~5%), Haplostats indicated that the haplotype HLA-A*31:01~C*07:02~B*07:02~DRB5*01:01~DRB1*15:01~DQB1*06:02 ranks 61898th & 170477th in CAU & African Americans (AFA), respectively and is not seen in other populations. Searching our laboratory database for subjects A31 positive, 382 subjects out of 8,340 typed between 2015 & 2017 were identified. Of those, 40 were positive for the haplotype HLA-A*31:01~ C*07:02~ B*07:02~DRB5*01:01~DRB1*15:01~DQB1*06:02 and of these 17 were typed for HLA-DPB1. All were positive for the entire extended 9 locus haplotype and were either CAU (n=6) or of unknown ethnicity (n=11). **Conclusions:** Although the 6 locus haplotype portion of this extended haplotype seems fairly uncommon, the extended 9 locus subset of that haplotype seems to be conserved especially in Caucasians.

P074

HAPLOTYPE AND ALLELE FREQUENCIES IN DIFFERENT POPULATIONS FOR HLA-E

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Aim: HLA-E has been reported to impact the outcome of hematopoietic stem cell transplantations (HSCT). Starting July 2017, DKMS added HLA-E to the default typing profile for newly enrolled potential donors for HSCT. Since then, DKMS Germany has typed more than 390,000 samples for HLA-E together with the HLA loci A, B, C, DRB1, DQB1, and DPB1. In this work, we present 7-locus haplotype and allele frequencies for donors from Germany (N=325,955), Turkey (N=10,649), Poland (N=4,144), Russia (N=2,896) and Italy (N=2,035). **Methods:** HLA-E is typed by our well-establish high-throughput approach relying on an amplicon spanning exons 2 and 3. Amplicons are sequenced by Illumina MiSeq or HiSeq 2500 instruments without fragmentation, preserving phase information. Reads are analysed by our in-house typing software neXtype. Typing results comprise the high-resolved alleles HLA-E*01:01:02, 01:03:03, 01:04, 01:05, 01:07 and 01:08N and three G-groups: HLA-E*01:01:01G, 01:03:01G and 01:03:02G. Upon enrollment with DKMS Germany, potential stem cell donors provide information on their self-assessed ethnic background by statement of their respective country of origin. Using our open-source software Hapl-o-Mat, we computed frequencies on g-group resolution level for both, alleles and 7-loci haplotypes. **Results:** The most common allele group in all five populations is HLA-E*01:01g with a fairly similar frequency ($f_{Germany}$ = 55.5%, f_{Turkey} = 53.4%, f_{Poland} = 56.9%, f_{Russia} = 57.0%, f_{Italy} = 53.0%). Frequencies for HLA-E*01:03g are ($f_{Germany}$ = 44.4%, f_{Turkey} = 46.6%, f_{Poland} = 43.1%, f_{Russia} = 43.0%, f_{Italy} = 46.9%). In all populations, except Russia, HLA-E*01:05 is the third most common allele. In the Russian population only the two dominant alleles were observed. **Conclusions:** Our approach provided reliable HLA-E typing data for a large number of samples. In all considered populations, identical allele groups are dominant with similar frequencies.

P075

EXTENDED HLA HAPLOTYPES IN A GREEK POPULATION BY NGS TECHNOLOGY

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Aim: HLA alleles are observed in specific haplotypes due to Linkage Disequilibrium (LD) between certain alleles. Recent improvements in Next-Generation Sequencing (NGS) technologies accommodate the tight linkage disequilibrium between genes as well as the high degree of polymorphism of the MHC. In order to assign HLA haplotypes at allele level (4-fields) in a Greek population, in the context of 17th IHWS, 25 families including parents and siblings (95 subjects), all of Greek origin, were typed at 11 loci (HLA-A*, -B*, -C*, -DRB1*, DRB3/4/5*, -DQA1*, -DQB1*, -DPA1* and -DPB1*) by NGS technology. **Methods:** Instrument and Chemistry: Illumina MiSeq, v2 chemistry, 300 cycles (2X150) and standard flow cell size. Illumina TruSight HLA v2.0 and Omixon Holotype HLA kits were used. HLA haplotype count and frequency (%) were estimated according to the number of parental haplotypes identified in the whole cohort. Additionally, haplotype count in families (N=25) and subjects (N=95) were calculated. **Results:** HLA-A*01:01:01:01-C*07:01:01:01-B*08:01:01:01-DRB3*01:01:02:01-HLA-DRB1*03:01:01:01/02-DQA1*05:01:01:02-DQB1*02:01:01-DPA1*01:03:01:02-DPB1*04:01:01:01/02 haplotype was found in 3 members of 2 families (haplotype frequency 2%).All other haplotypes were observed once, giving haplotype frequency = 1% **Conclusions:** It is confirmed that the HLA-A*01-Cw*07-B*08-DRB1*03:01-DQB1*02 haplotype, defined by low-resolution molecular techniques, is the most common in Greeks, as in all Caucasians. These results are useful as reference data (controls) in disease association studies as well as in population genetics.

P076

A COMMUNITY RESOURCE USING GENE FEATURE ENUMERATION TO GENERATE ACCURATE ALLELE CALLS AND SEQUENCE ANNOTATIONS FOR HLA AND KIR

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Aim: A publicly available service for accurately annotating and assigning allele names to consensus sequences would be a valuable community resource. We have developed such a service for HLA and KIR consensus sequences. **Methods:** Gene feature enumeration (GFE) notation was developed as a way to retain sequence variation outside of the current nomenclature. We generated GFE notation for every sequence in the IPD-IMGT/HLA and -KIR Databases, and loaded the results into a neo4j graph database (neo4j.b12x.org), which includes KIR, HLA, GFE and sequence feature nodes. Our allele-calling tool (ACT) annotates sequences and converts those annotations to GFE notation. The ACT uses these GFE notations and the graph database to make allele calls by finding alleles that share similar GFE notations. A RESTful service web-interface makes the ACT easy to use and allows for cross-platform compatibility (act.b12x.org). We used 118,585 Be the Match® donors typed at high resolution with consensus sequences available for HLA-A, B, C, DRB3/4/5, DRB1, DQB1 and DPB1 to test the ACT. We compared the allele calls generated by the ACT to the lab-reported allele calls using the same IPD-IMGT/HLA release as the labs and IPD-IMGT/HLA release 3.31.0. To test the ACT with KIR, we took all the KIR sequences that were characterized in IPD-KIR release 2.7.0 and made allele calls with the ACT using release 2.6.0. **Results:** The allele calls made by the ACT matched the lab reported typing 100% of the time for class I and 99.5% of the time for class II when using the same IMGT release as the lab. When using the 3.31.0 IPD-IMGT/HLA release, the ACT extended third field assignments to the fourth field for 98% of donors for at least one locus. On average, the ACT took 3 seconds to annotate a sequence and make an allele call for class I and 9 seconds for class II sequences. The ACT made accurate allele calls for all KIR sequences that were characterized in 2.7.0. **Conclusions:** Accurate allele calls and sequence annotations can be made when using our GFE-based ACT. The ACT can also be used to extend the nomenclature for allele assignments made with previous IPD-IMGT/HLA releases. This service will allow anyone to easily convert HLA and KIR consensus sequences into detailed sequence annotations and allele names.

P077

IMPUTING ALLELIC VARIANTS FOR FIVE HUMAN LEUKOCYTE ANTIGEN GENES USING A HIGH-THROUGHPUT GENOTYPING ARRAY

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Aim: To assess the feasibility of imputing human leukocyte antigen (HLA) alleles in African cohorts based on high-throughput genotyping of single-nucleotide polymorphisms (SNPs) in the human major histocompatibility complex (MHC). **Methods:** For two African cohorts representing eastern and southern Africa, genomic DNA samples extracted from peripheral blood were first used for genotyping *HLA-A*, *HLA-B*, *HLA-C*, *HLA-DRB1* and *HLA-DQB1* alleles, all with 4-digit resolution through a combination of PCR-based genotyping techniques, including direct sequencing of locus-specific amplicons. SNP genotypes were further defined by a widely-used commercial array (the Illumina ImmunoChip). Paired HLA and SNP data from the first 400 individuals in each cohort served as a reference (the training set) for validation in the rest of samples. All imputation procedures were done using the HIBAG algorithm. **Results:** A total of 7,539 SNPs spanning the extended human MHC passed various quality control procedures and then merged with fully-resolved HLA alleles in 490 Rwandans and 1100 Zambians. Overall, SNP-based imputation of 2-digit allele groups at the five target loci showed success rates between 93.9% and 98.3%, while success rates in imputing 4-digit alleles ranged from 85.4% to 97.2%. Two major HLA-DRB1 allele groups (DRB1*04 and DRB1*10) in Zambians were the only ones with suboptimal imputation probabilities (<95%). The 4-digit alleles with suboptimal imputations were found at all five loci and in both cohorts, even for relatively common variants like HLA-B*58:01 and HLA-DRB1 alleles *01:01, *08:02, *11:02, *13:01 and HLA-DQB*06:04. Having HLA and SNP data from both cohorts as the overall reference (a combined training set) did not improve success rates in either cohort. **Conclusions:** In the era of high-throughput genomics, SNP-based imputation

of HLA variants may offer a quick and affordable alternative for initial assessment of 2-digit allele groups. However, reliable imputation of 4-digit allele remains difficult even for some common alleles of broad interest

P078

HLA-DRB3/4/5 ALLELE AND HAPLOTYPE FREQUENCIES IN KOREANS AND ITS APPLICATION TO COUNTING DR EPLET MISMATCH

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Aim: Allele level interpretation of HLA DR-3/4/5 is encouraged as low-expression loci mismatch can affect the outcome of organ transplantation. Here we assess the HLA-DRB3/4/5 allele frequency in Korean population and report the occurrence rate of post-transplantation HLA-DR donor specific antigen (DSA) according to eplet mismatches including HLA-DRB1 and HLA-DRB3/4/5. **Methods:** High resolution genotyping of HLA-DRB3/4/5 at 6-digit level was done using blood samples from 150 unrelated healthy Koreans. Regarding assessment of the effect on organ transplantation, there were 272 patients with pre- and post-op panel reactive antibody (PRA) results during September 1st, 2012 and August 31th, 2017. Their post-transplantation de novo DSA occurence was analyzed by HLA-DR eplet mismatches of HLA-DRB1 and HLA-DR3/4/5. **Results:** Four HLA-DRB3 alleles, three HLA-DRB4 alleles and three HLA-DRB5 alleles were found at 6-digit level (Table). Total 32 HLA-DRB1-DRB3/4/5 haplotype combinations were found (data not shown). The number of eplet mismatch with no post-transplantation de novo DSA was seven for HLA-DR and cumulative DSA frequency was observed upto 9.8% (Image). **Conclusions:** HLA-DRB3/4/5 allele frequency and HLA-DRB1-DRB3/4/5 haplotype frequency were analyzed at 6-digit level for the first time in Koreans. Our result demonstrates that degree of HLA-DR eplet mismatches of all HLA-DR loci could affect generation of post-op de novo DSA. These results will be useful for PRA interpretation and understanding the impact of low-expression loci on organ transplantation.

HLA-DRB3, -DRB4, and -DRB5 allele frequencies in Koreans (N = 150)

Locus	Allele	Serology	Frequency (%)
DRB3	*01:01:02*	DR52	6.00
	02:02:01	DR52	17.00
	03:01:01	DR52	12.30
	03:01:03	DR52	0.30
DRB4	*01:03:01*	DR53	27.00
	01:03:02	DR53	8.70
	01:03:03	DR53	0.30
DRB5	*01:01:01*	DR51	6.70
	01:02	DR51	2.00
	02:02	DR51	1.30

P079

HIGH THROUGHPUT HLA-B27 IMMUNOPHENOTYPING; CONVERSION TO A 96 WELL PLATE

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Aim: Spondyloarthropathies are more commonly diagnosed in patients that are positive for HLA B27. The Immunology lab in Hamilton Regional Laboratory Medicine Program (HRLMP) currently performs 200

samples/week of HLA B27 Immunophenotyping test by conventional manual tube (5 ml) based flow cytometry method using the Becton Dickinson (BD) HLA-B27 kit. Testing is transferring to the Histocompatibility lab to utilize existing flow cytometry equipment. The aim of our study was to modify the BD HLA-B27 test for processing on a 96 well plate to improve testing efficiency and maintain cost effectiveness. **Methods:** Phenotype testing-BD HLA-B27 Kit (Catalog No 340183). PCR-SSP/RSSO One Lambda Micro SSP and/or Labtype HD or XR kits. Immunophenotyping acquired and analysed by BD HLA-B27 Clinical software on the FACSCanto II. Phase 1-Test Modifications-96 wells plate was used and the lyse solution step was modified from a single 10 minute incubation in 2ml of lysing solution to two 5 minute incubations in 120ul and 200ul of lysing solution. Wash volume of Phosphate Buffered Saline (PBS) was reduced from 2ml to 200ul. All other aspects of the test were as per package insert. Phase 2-Side-by-Side Testing-performed on 55 samples (sample age 1-3 days). Samples were from the HLA testing population with confirmed PCR-SSP/RSSO B locus typings. Phase 3-Blind Comparison Study-20 samples tested by the tube method in the Immunology lab were compared to the modified plate method done in the HLA Lab. Discrepant results confirmed by PCR-SSP. **Results:** There was good correlation between the standard tube method and the Hamilton plate method. Paired T test does show statistical difference between the methods, however, the homoscedastic T Test does not indicate statistical difference in their variances. Despite the mean statistical difference, as per the package insert recommended interpretation there were no false positive or false negative results in the side-by-side study as verified by PCR. **Conclusions:** The 96 well plate method produced reliable results using package insert recommended cut-off guidelines. The modified method appeared to be a very useful and a high throughput cost effective method for HLA B27 testing.

P080

CORRELATION BETWEEN THE REJECT OF HAPLOIDENTICAL TRANSPLANTS IN PATIENTS AFFECTED BY HEMOGLOBINEPATHIES AND THE PRESENCE OF DONOR-SPECIFIC ANTI-HLA ANTIBODIES

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Aim: Allogeneic haematopoietic stem cell transplantation is the treatment of choice for both pediatric and adult patients with onco-haematological diseases and non-malignant disorders. It is well known that an HLA-identical family donor is available in about 25% of cases and it is not always possible to promptly identify a voluntary donor in international registries. In the absence of a HLA-matched donor, the transplantation strategy is increasingly orienting towards the choice of allogeneic hematopoietic stem cell transplantation alternatives, such as the use of haploidentical family donors. The aim of this study was to determinate the presence in the recipients of donor specific anti-Human leukocyte antigen of class I and/or class II antibodies an correlate their potential role with the high rate of graft rejection observed. **Methods:** The plasma or the sera of 23 patients affected by hemoglobinepathies, treated with a haploidentical transplant, were analyzed using the Mixed Antigen assay kit and subsequently, in case of positivity, with the Single Antigen beads assay kit (One Lambda, California) by Luminex technology. **Results:** In the group of 23 transplanted patients affected by hemoglobinopathies, 9 rejected the graft. Among these, 3 (33.3%) showed the presence of DSA, while only 1 out of 14 patients with complete chimerism (7.1%) was positive for Donor-specific HLA Antibody. The presence of non-specific donor anti-Human leukocyte antigen antibodies class I and/or II was also demonstrated in 6 of the 23 patients analyzed, 3 of which were included in the group of 9 who rejected the transplantat (33.3%). **Conclusions:** Our data showed that the presence of specific-donor anti-HLA antibodies represents a parameter associated with a higher percentage of rejection in haploidentical hematopoietic stem cell transplantation performed in patients affected by hemoglobinopathies, although a wider cohort of patients needs to be analyzed before drawing any conclusion. *With the contribution of Berloni* ***T. Galluccio:*** *1. Grant/Research Support; Company/Organization; IME Foundation. 2. Consultant; Company/Organization; Andreani Marco. 4. Scientific/Medical Advisor; Company/Organization; Andreani Marco. 5. Employee; Company/Organization; Galluccio Tiziana.* ***A. Di Luzio:*** *1. Grant/Research Support; Company/Organization; IME Foundation. 2. Consultant; Company/Organization; Andreani Marco. 4. Scientific/Medical Advisor; Company/Organization; Andreani Marco. 5. Employee; Company/Organization; Di Luzio Andrea.* ***M. Battarra:*** *1. Grant/Research Support; Company/Organization; Andreani Marco. 2. Consultant; Company/Organization; Andreani Marco. 4. Scientific/Medical Advisor; Company/Organization; Andreani Marco. 5. Employee; Company/Organization; Mariarosa Battarra.*

P081

ST14 PROTEASE RESISTANT PEPTIDES, FROM GLIOBLASTOMA MULTIFORME MUTANT PROTEINS, REPRESENT HIGHER BINDING AFFINITIES AS POTENTIAL HLA CLASS I EPITOPES

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Aim: We previously identified a set of the most frequently mutated cytoskeleton- and extracellular matrix-related proteins (CECMPs) in numerous cancer datasets. Mutations in the cytoskeleton and extracellular matrix can induce resistance or sensitivity to proteolysis. We aim to elucidate the effects of protease cleavage on exposing new epitopes for potential HLA Class I binding. **Methods:** Here, we use a bioinformatics approach to assess the impact of amino acid (AA) substitutions on the sensitivity of CECMPs to the ST14 protease (matriptase), a transmembrane serine protease previously implicated in cancer development. Furthermore, to assess tumor specimen immunogenicity, we identified T-cell receptor (TCR) V(D)J recombinations in GBM exome files and mapped overlaps in patient survival between 1) protease sensitivity, 2) HLA Class I binding affinities of new epitopes, and 3) V(D)J recombination reads. **Results:** Results indicated that AA substitutions in the glioblastoma multiforme CECMPs are skewed toward increased resistance to the ST14 protease, in comparison to the wild-type peptide sequence. Furthermore, the protease resistant AA substitutions represent relatively high binding affinities to HLA class I proteins, when assessing the binding specificities using HLA class I alleles matched to the source of the mutant AA. In contrast, the protease sensitive AA substitutions create epitopes that represent lower binding affinities with HLA class I. Moreover, samples representing AA substitutions that increased protease sensitivity also represented reduced overall and disease-free survival periods for patients with glioblastoma. Although the presence of TCR V(D)J recombinations alone did not represent any significant survival differences, the overlap between ST14-protease sensitive mutant barcodes and the TCR V(D)J recombination read positive barcodes represented significantly reduced survival. **Conclusions:** These results may provide indications of the value of the overlap of protease sensitive and resistant, mutant peptides and immune activity as a biomarker for prognosis in Glioblastoma multiforme further elucidating on the role of histocompatibility in cancer development and prognosis.

P082

HLA DISEASE ASSOCIATION TESTING BY NGS: IS IT SUPERIOR TO OTHER METHODS?

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Aim: HLA associations have been linked to more than 100 diseases and are also important targets for pharmacogenomic risk assessment for drug hypersensitivity reactions. Traditionally, sequence-specific oligonucleotide probe (SSOP) genotyping have been used to identify HLA alleles associated with diseases. Other methods include real-time PCR with melting curve analysis used to determine the presence of HLA-DQB1*02, DQB1*03:02 and DQA1*05 for celiac disease, DQB1*06:02 for narcolepsy, B*27 for ankylosing spondylitis, and B*57:01 for abacavir hypersensitivity. The diagnostic value of HLA disease association testing is only as good as the accuracy of the methodology used. The increasing number of discovered HLA alleles has generated a growing list of ambiguities that cannot be ruled out with the current assays. **Methods:** We proposed to evaluate the NGS-based HLA-B and DQ Monotype kits (Omixon) for HLA-disease association testing using a cohort of 72 samples. Method comparison was performed with both SSOP genotyping (One Lambda), and real-time PCR with melting curve analysis (laboratory-developed). **Results:** Multiple discrepancies were observed between NGS and the existing methods. One sample was called DQB1*03:02 positive by the real-time PCR assay but was actually homozygous for DQB1*03:03, an allele not associated with celiac disease. DQB1*03:02 and 03:03 only differ by one base pair and that difference lies in the reverse primer. Likewise, a DQB1*03:04 allele was called DQB1*03:02 positive by the melting curve assay. A similar situation occurred with the B*57:01 real-time PCR assay; a sample was called weak positive but was in fact homozygous for B*57:03, an allele not associated with abacavir hypersensitivity. Limitations of the SSOP genotyping method included an ambiguous false positive DQB1*03:02, but NGS genotyped it as DQB1*03:40, an allele not known to be associated with celiac disease. The NGS kit correctly genotyped all samples without ambiguity. **Conclusions:** Our study demonstrates that HLA typing by NGS is the most accurate method for identifying alleles associated with disease or drug hypersensitivity. The kit allows for testing of 96-192 individual patients per run, and HLA B and DQ samples can be combined, making it a viable approach for high volume clinical diagnostic laboratories.

P083

THE HLA GENETIC STRUCTURE OF AN ARGENTINIAN REGISTRY POPULATION REFLECT A DIVERGENT DEMOGRAPHIC HISTORY

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Aim: Argentina represents an amalgamation of European, indigenous Amerindians, Mestizos, and non-European descendants but the contributions from each ethnic group remain relatively unexplored from a genetic perspective. We applied NGS to determine the distribution of HLA allele and haplotype frequencies in a large collection from the INCUCAI-Argentinian unrelated donor BMT registry. **Methods:** The cohort consists of individuals (>36,000) with ancestry from 70 ethnic groups. To facilitate analyses individuals were classified into 10 broad ethnic groups; Iberian, Mixed-Hispanic, Mediterranean, Central/Western Europe, East European, English, French, Middle Eastern, API, and 'other' pertaining to individuals with unreported or African ancestry. Various parameters of intra-population diversity were assessed including heterozygosity, LD, and selective neutrality. Haplotype frequencies were estimated using the EM algorithm. Inter-population variance was determined using Fst distances which were used to construct phylogenetic dendrograms. **Results:** The most frequent alleles at the class I loci were: *A*02:01:01G*; *C*04:01:01G*; *B*51:01:01G* in 9 groups, *B*07:01:01G* and *B*18:01:01G* were most common in East Europeans and English individuals. *DQB1*02:01:01G* and *DQB1*03:01:01G*, *DRB1*07:01:01G* were frequent across all groups. At the HLA-B locus, Mixed-Hispanics exhibited the greater diversity (178 alleles), and the lowest diversity was observed in the French (51 alleles). Native-American ancestry was evident as indicated by the presence of B*15, B*35, B*39, B*40, B*48, B*51, B*52 rare alleles which had the highest phenotype frequency of 20% in Iberians suggesting that patients with indigenous ancestry may find it difficult to find a suitable donor outside of Argentina. Haplotypes with distinct HLA associations common and specific to particular ethnic groups were observed. Genetic distances indicated that the Argentinian populations shared a strong HLA affinity with European populations. **Conclusions:** The divergent HLA signatures in the Argentinian populace allude to the different migratory events of Europeans to the new world. Our data provides well-defined HLA profiles of the Argentinian registry and this information may be useful to increase the accuracy of donor selection for BMT patients.

P084

TYPE 1 DIABETES DQA1~DQB1~DRB1 HAPLOTYPES IN AN ADMIXED BRAZILIAN POPULATION

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Aim: The development of type 1 diabetes (T1D) and its chronic complications may have a genetic background. We aimed to describe HLA haplotypes in patients with T1D from different urban geographical regions of Brazil. **Methods:** This was a cross-sectional, nationwide survey conducted in 14 public clinics from 10 Brazilian cities. HLA Class II Typing were performed with medium to high-resolution PCR-RSSO (LabType SSO, One lambda Inc.) in 561 T1D patients. Common and Well Documented (version 2.0) was used to defined alleles, and ambiguities were solved by Next Generation Sequencing (NGS) (Holotype HLA Assay, Omixon Inc.). HLA haplotypes were determined in Arlequin v.3.5 software. **Results:** We have determined 125 haplotypes from T1D patients. Top 10 haplotypes are shown in Table 1. Haplotypes from South Brazil T1D patients were significantly different when compared to Southeast and North ($p<0.01$). Four haplotypes associated to European descent (Noble and Valdes, 2011) were among them, although in distinct frequencies (Table 1), and only one protective haplotype (01:02~06:02~15:01 - 12.0%) was determined among Brazilian T1D (0.8%). **Conclusions:** Our study showed that T1D patients presented new haplotypes which could be associated with T1D. Further characterization of genomic

ancestry, as well as extended haplotypes analysis, should be able to offer a more definitive risk for unfavorable outcome of Brazilian T1D individuals.

Ten more frequent Type 1 Diabetes Haplotypes in Brazilian patients in comparison to European Descent

HLA-DQA1~DQB1~DRB1 Haplotype	T1D Brazilian frequency	T1D European descent frequency[#]
05:01~02:01~03:01	27.6%	34.1%
02:01~02:02~07:01	5.9%	
03:01~03:02~04:02	5.0%	3.5%
03:03~03:02~04:05	4.8%	
03:01~03:02~04:01	4.2%	28.1%
03:03~02:02~09:01	3.6%	
01:01~05:01~01:01	3.3%	
01:01~05:01~01:02	2.5%	
03:01~03:02~04:05	2.5%	2.5%
01:02~06:04~13:02	2.2%	

- Noble JA & Valdes AM. *Curr Diab Rep* **11**, 533-42 (2011).

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P085

IMPROVING HLA LABORATORY WORKFLOW MANAGEMENT AND "GOING PAPERLESS"

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Aim: Using an electronic workflow in the lab has many benefits, including the efficient use of physical space. We sought to identify a solution that in addition would allow us to analyze and annotate raw data from patient testing and archive previous paper files to maintain a continuous and accessible patient record. We implemented Docuware, a cloud-based document management software, which we use along with Histotrac for clinical testing. **Methods:** We worked with Docuware to customize and adapt their existing software to fit our complex HLA laboratory testing workflow. The software allows for two-way travel within the workflow as the review process and reflex testing is performed. An important function is the ability to direct certain documents to a specific individual for the next step in the workflow. Docuware has built-in annotation tools, enabling seamless and trackable review. In addition, a full text search function was added to allow for easy recall of results from specific instrument runs for QA purposes. **Results:** An example of the workflow for antibody testing is shown in Figure 1. Test requisitions are scanned and indexed with patient and sample information. All documents associated with the sample (e.g. raw data from testing and final reports) are converted to PDFs and attached. This “packet” travels through the workflow with the use of electronic “stamps” and signed final reports can be exported as PDFs for easy distribution to individual providers. **Conclusions:** An electronic system dramatically reduces paper waste and allows for remote access to raw data. Files can be viewed at any step during testing, and all results for a given patient can be easily collated and compared for clinical and QA purposes. While other tools exist for annotation of results, such as Adobe Acrobat, Docuware is specifically designed for this purpose, and is easily customizable to the lab’s specific needs, rather than adapting procedures to fit an existing tool.

P086

UNOS UPLOAD TOOL (UUT): AN EASY AUTOMATED SYSTEM FOR UPDATING AND CONFIRMING UNACCEPTABLE ANTIGEN RESULTS RECORDED IN UNET FROM LABORATORY RESULTS

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Aim: UNet is a data repository for transplant candidates and donors and is managed by the United Network for Organ Sharing (UNOS). Our laboratory updates UNet daily with unacceptable antigens for transplant candidates. We have developed and validated the UUT to automate generation of unacceptable antigen data for both upload and receipt confirmation of values recorded in UNet. **Methods:** Unacceptable antigens are calculated from median fluorescent intensity (MFI) from HLA Class I and II LabScreen assays (One Lambda), analyzed on the Luminex 3D, and interpreted using Fusion (One Lamda). Data is stored in HistoTrac (SystemLink) for consultant sign off and reporting to the medical record. UUT interrogates HistoTrac and can be modified to query other databases (E.g. Fusion) using Excel Power Query and VBA. MFI and prozone data is organized to show recurrent patient data over time, highlighting significant signal changes and shifts above or below reporting cutoffs. After data review a file is generated based on unacceptable antigen criteria for export to UNet. Following upload to UNet, a report of unacceptable antigens for all active patients is exported from UNet. This report is imported to the UUT for confirmation of successful import and highlights changes in unacceptable antigens since the last time the patient was recorded. **Results:** UUT was validated both retrospectively with historic data of patients with complicated histories as well as concurrently with live clinical data. UNet was manually reviewed to confirm that patient data was recorded correctly. Prior to implementing this tool our process required two people and ~5 minutes per patient. With this tool the process is now performed by one person at ~1.2 minutes per patient. **Conclusions:** Use of the UUT in the automated upload of data to UNet reduces both workload and the potential for transcription errors while helping to highlight unexpected changes in patient data over time that could indicate technical artifacts.

P087

ALLELE FREQUENCY OF THIOPURINE S-METHYLTRANSFERASE (TPMT) IN HONG KONG POPULATION

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Aim: Azathioprine, 6-Mercaptopurine and 6-Thioguanine are commonly used in the management of acute leukemia, autoimmune diseases and as the immunosuppressants in organ transplantation. These drugs belong to the class of thiopurine which can inhibit DNA synthesis and cell proliferation of fast growing lymphocytes by suppressing de novo purine synthesis. Thioguanine nucleotides (TGNs) are the active metabolites in which accumulation of high level cytotoxic TGNs can lead to myelosuppression. It has been demonstrated that Thiopurine S-methyltransferase (TPMT) is the key enzyme responsible for catalytic conversion of TGNs to non-cytotoxic methyl-thioguanine. The TPMT gene is polymorphic and TPMT protein variants with reduced enzyme activity are associated with inefficient removal of TGNs. In this study, we aim to investigate the allele frequency of the most common TPMT variants (TPMT*2 and *3C) in Hong Kong population. **Methods:** Genomic DNA from whole blood was extracted. The presence of TPMT*2 and *3C mutant alleles were determined using Amplification-Refractory Mutation System (ARMS) PCR method. Beta-2-microglobulin was used as the internal control. All mutant alleles were confirmed by Sanger's sequencing. Hong Kong healthy individuals (n = 300) were included in this study. Rare TPMT alleles were not determined. **Results:** TPMT*1/3C genotype was detected in 9 out of 300 healthy subjects while TPMT*2 allele was absent. The allele frequency of TPMT*3C in Hong Kong population is 1.5%. **Conclusions:** This study provides the first analysis of the allele frequency of TPMT variants in Hong Kong population and our finding is in accordance with the reported frequency range in Chinese population. TPMT*3C is the most common TPMT variant in Hong Kong subjects. The establishment of TPMT genotyping assay for patient requires thiopurine therapy will provide additional information to guide the reduction of drug dose to minimize the risk of myelosuppression.

P088

EFFECT OF LEUKOREDUCTION OF TRANSFUSED BLOOD PRODUCTS ON DECEASED DONOR HLA TYPING

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Aim: Deceased donor HLA typing is predicated on the assumption that the blood sample contains only DNA from the organ donor. Prior to procurement, most organ donors receive at least one unit of red blood cells (RBC), with some receiving >20, as part of life support or in order to maintain organ viability. Non-organ donor DNA acquired from transfusion may result in incorrect and/or ambiguous HLA typing, which could have dire consequences for the organ recipient. We investigated the impact of RBC transfusion on HLA typing with leukoreduced (LR) and non-LR RBCs. **Methods:** To replicate the average total blood volume (TBV) of 5L, an *in vitro* model of 5mL was used. Varying volumes of LR and non-LR donor RBCs (e.g. 0.25mL *in vitro* = 1 RBC unit *in vivo*) were mixed to simulate different transfusion scenarios (Table). Typing was performed by real time PCR using Linkage Biosciences HLA typing kit (11 loci+: HLA - ABCDRDQDP SABR™ 384 Kits) and software (SureTyper™). **Results:** For non-LR scenarios of 10 and 2 RBC units, PCR was unable to assign a definitive HLA type (i.e."no call") for multiple loci as >2 antigens per locus were detected. In the 1 non-LR RBC scenario, some loci had donor-specific positive wells that if ignored resulted in recipient typing. In contrast, both the 16 and 19 LR-RBC transfusion scenarios, which approach replacement of TBV, resulted in non-ambiguous recipient typing. Even when DNA concentration was low (3ng/µl), recipient typing was assigned despite well drop-outs. **Conclusions:** Our results show that HLA typing for deceased donors is differentially impacted by RBC LR status. Whereas non-LR RBCs can result in ambiguous or incorrect recipient typing, LR RBCs do not appear to preclude definitive typing results. Given that approximately half of the U.S. blood supply is non-LR, determining LR status has testing and clinical implications. If LR RBCs are given, regardless the volume, HLA typing should not be affected and there is no reason to delay typing for additional DNA material (e.g. lymph node, pre-transfusion sample).

Transfusion Scenarios

Non-LR donor scenario	Volume of recipient blood	Volume of donor blood	DNA concentration
10 units	2.5 ml	2.5 ml	47 ng/µl
2 units	4.5 ml	0.5 ml	77 ng/µl
1 unit	4.75 ml	0.25 ml	41 ng/µl
LR donor scenario			
16 units	1.0 ml	4.0 ml	13 ng/µl
19 units	0.25 ml	4.75 ml	3 ng/µl

P089

SUPPRESSION OF A *HLA-B* ENCODED MICRORNA UPREGULATES THE EXPRESSION OF HEAVY CHAIN IGA *(IGHA1)* TRANSCRIPT IN HUMAN PRIMARY B- CELLS

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Aim: Our previous work has shown that the *HLA-B* encoded miR-6891-5p influences the expression of about 200 transcripts, including the transcript encoding for the heavy chain of IgA, in a B cell line. We showed that the expression of heavy chain of IgA was up-regulated when miR-6891-5p activity was suppressed (doi: 10.3389/fimmu.2017.00583). In this work we aimed to determine whether increased expression of heavy chain of IgA transcript is also possible by suppressing miR-6891-5p activity in human primary B-cells. **Methods:** Human primary B-cells from a total of 12 human volunteers were analyzed. One of the samples was from an IgA deficient patient (<10 mg/dL). Human primary B-cells were transduced with either the scrambled or the miR-6891-5p antisense construct expressing lentiviruses. Total RNA was purified from these cells and qPCR was performed to analyze *IGHA1* expression. The results are shown in fold increase of the antisense vs the scrambled mi-RNA-5p construct. We assessed whether suppression of miR-6891-5p activity can increase expression of heavy chain of IgA in human primary B-cells. **Results:** Suppression of miR-6891-5p activity in human primary B-cells led to *IGHA1* up-regulation in 11 antisense miR-6891-5p transduced samples. The average upregulation of the 11 samples was 2.48 fold. (range of increase 1.04-7.88X). The B cells of the IgA deficient sample upon transduction with antisense miR-6891-5p showed a 6.75 fold upregulation relative to scrambled control. We are in the process of evaluating more IgA deficient subjects. **Conclusions:** We show that the suppression of miR-6891-5p activity can upregulate *IGHA1* expression in primary human B-cells. This upregulation was higher in the IgA deficient sample compared to average of the 11 non-IgA deficient samples. MicroRNAs are known for fine-tuning gene expression. Modifying the activity of the miR-6891-5p, a *HLA-B* intronic sequence, in an IgA deficient subject restores the expression levels of *IGHA1* transcript in primary B cells.

P090

IMPACT OF CHEMICAL CONTAMINATION ON HLA TYPING ACCURACY OBTAINED BY REAL TIME PCR

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Aim: RT-PCR offers many advantages for HLA typing. Rapid turnaround time is the most appreciated for laboratories performing time-sensitive testing related to deceased donors. This technology reduces hands-on time, provides an alternative to gels, avoiding use of ethidium bromide and detects the presence of common null alleles. DNA concentration and 260/280 absorbance ratio are well-established parameters known to affect the quality of results. However, the impact of the 260/230 ratio has not been considered or properly studied. Here, we describe how 260/230 ratio can interfere with accuracy of typing obtained by RT-PCR. **Methods:** RT-PCR typing was

performed according to manufacturer instructions using LinkSeq SABR 1580 kits, K3476-AR and K3499-DR in the QuantStudio 6 Flex instrument. Samples were analyzed using Suretyper v.5.0.5 software. **Results:** Troubleshooting samples exhibiting multiple dropout reactions, DNA quality was assessed by 260/280 and 260/230 ratios. Reagents used to isolate nucleic acids, including guanidinium thyocianate (GT), have absorbance near to 230nm. GT is present in high concentrations in the cartridges used for DNA isolation. Downstream applications, including PCR, are known to be affected by RNA preparations exhibiting low 230/260 ratios. However, there is not much information about the impact of DNA preparations with low 230/260 ratios on typing results obtained by RT-PCR. 260/230 ratio was measured for 10 samples, where multiple reactions either failed or had weak amplifications. In all cases, ratios were less than 1. We compared with 260/230 ratios of samples with robust amplifications and all ratios were above 1.5 and above 1.7 in eight samples. The problematic DNA samples were washed with ethanol and retyped. The 260/230 ratios increased above 1.6 for all samples and the typing obtained had no amplification problems. Finally, we study the effect of the proportion between blood and elution buffer volumes loaded in the DNA isolation cartridges. We found that it could also affect the typing and established a safe range where results are not impacted. **Conclusions:** DNA preparations containing chemical contaminants and the proportion between blood and elution buffer volumes can affect the accuracy of HLA typing results obtained by RT-PCR.

P091

TYPELOADER FOR WINDOWS: AUTOMATED FULL LENGTH SUBMISSION OF NOVEL ALLELES

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Aim: Many of the alleles submitted to the IPD-IMGT/HLA and IPD-KIR databases are restricted to the coding sequence or even, for HLA, to the exons encoding the antigen recognition domain. This impedes the establishment of long-read-based genotyping technologies. It is desirable that novel alleles are characterized and submitted in full length, and that known alleles are extended to cover the complete gene sequence. However, the manual annotation and submission of full-length sequences to the IPD-IMGT/HLA and ENA databases is a time-consuming and error-prone task. In 2016, we developed and published TypeLoader, a tool that takes the full-length sequence of a novel HLA allele in FASTA format as input, automatically annotates it, and creates all files necessary for submission. This reduced the manual effort for submission by over 95%. It was implemented as a web application to run on a Linux server and has been used at the DKMS Life Science Lab to successfully submit more than 1000 novel HLA alleles. **Methods:** To enable a more widespread use of TypeLoader by other labs, we have adapted the tool to make it available as a Windows standalone application. We replaced the server-dependent web form with a convenient GUI (graphical user interface) using Python3 and PyQt5, which support most common operating systems. An internal SQLite database was added to store a wide range of metadata about each allele, which are accessible via the GUI. As a further improvement, TypeLoader now automatically detects null alleles generated by premature stop codons or frameshift mutations, and features enhanced integration with ENA's automated submission API. TypeLoader also implements experimental support for annotation and submission of KIR alleles. **Results:** The new TypeLoader can easily be used on standard PCs without dependencies on other software. With the added internal database, TypeLoader can be used as a lab's central information platform on their full-length sequences meant for submission. **Conclusions:** We hope that the increased convenience and scope of TypeLoader will foster the submission of more full-length sequences to the IPD-IMGT/HLA and IPD-KIR databases, ultimately promoting the widespread use of full-length sequencing for genotyping of both HLA and KIR.

P092

VALIDATION OF THE AUTOMATED NGSGO LIBRARY PREPARATION WORKFLOW FOR HLA TYPING ON THE BIOMEK 4000

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Aim: The GenDx NGSgo workflow for HLA typing using Next-generation sequencing (NGS) consists of four practical steps: amplification, library preparation, sequencing and data analysis. Out of these four steps the library

preparation is the most labor intensive and most interspersed by incubations. To decrease hands-on time and reduce the risk of human error, NGSgo library preparation can be automated from start to finish using a liquid handler workstation. Here we describe the results of the automation of the NGSgo library preparation for Illumina on a Beckman Coulter Biomek 4000 workstation. The workflow was fully automated from amplicon pool to finalized library, making it completely hands-off to accommodate overnight runs and allow the operator to step away from the machine. **Methods:** The Biomek setup included a thermal cycler, 96-well plate magnet, two peltiers devices for cooling 96-well plates and 1.5ml tubes, a gripper tool, reagent reservoirs and liquid waste disposal. Pipetting was performed by two pipet tools. This setup allowed for flexible processing of 4 to 24 samples per run, with any number of loci. Per run only three boxes of pipetting tips were used for 24 samples. Panels with variable numbers of samples and replicate panels were processed with the Biomek. Subsequently, the pooled libraries were sequenced on a MiSeq system and the resulting data was analyzed with NGSengine software (GenDx). **Results:** MiSeq run parameters were indicative of a high-quality sequencing runs. Read depth, mappability and noise levels of the sequence data presented here showed that the automated workflow resulted in high quality reads and accurate typings. Results from the replicate panel showed a 100% typing match. **Conclusions:** - NGSgo library preparation for 4 to 24 samples can be performed on the Biomek 4000 platform, without manual intervention. - The automated workflow results in high-quality reproducible libraries. - Performing automated NGSgo library preparation as described here reduces hands-on time from 1.5 hours to 10 minutes as compared to manual execution.
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P093

NEXT GENERATION SEQUENCING - EVOLUTION TO A WORKFLOW MODEL FOR EXCLUSIVE PRIMARY HLA TYPING OF HEMATOPOIETIC STEM CELL AND SOLID ORGAN TRANSPLANTATION

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Aim: Our lab has established the NXType™ kit on the Ion-Chef and Ion-S5 workflow for high resolution HLA typing. This work was aimed to evaluate the analytical performance of the method for accuracy, precision, sensitivity, specificity, reportable range, repeatability, and reproducibility. We also determined optimal run size and workflow within the lab to strike a balance between suitable number of reads per locus and number of samples to avoid low coverage and dropouts. Using NGS as primary typing method for hematopoietic stem cell (HSC) and the solid organ (SO) programs was assessed. **Methods:** Class I and HLA Class II were amplified with NXType™ or ALLType™ primer sets. Automated template preparation and chip loading were performed in the Ion Chef™ system and run on Ion S5™. Data was analyzed with TypeStream™ v1.1.0.11 and TypeStream™ Visual NGS Analysis Software. **Results:** We found that the optimal run size was a minimum of 24 samples, a 3-fold increase of previous capacity. To maximize the use of chip space the number of samples/run was increased with confirmatory solid organ typings. We determined that running 24 samples with SSOP or NGS required similar manpower and instrument time. Although lab work was less with SSOP, analysis time and effort was greater than with NGS. After two years of experience with NGS HLA typing (~ 1,500 samples), we considered using NGS as the primary typing method for the HSC and the solid organ programs. Our historical data showed only 2 cases where NGS gave non-concordant results with SSOP or SSP. However, these samples were flagged by the software and both had associations that were red-flagged during analysis. The repeat rate for SSOP was 2.8% while for NGS was 0.3%. All NGS repeats were technical failures and not ambiguity resolution. We evaluated the ALLtype kit, which includes DQA1 necessary for solid organ initial typing, and a liquid handler for automation of library preparation. In addition, we implemented the TypeStream Visual (TSV) analysis software that includes serological equivalents. **Conclusions:** We confirmed that NGS is a robust methodology for HLA typing. It provides results first time is run, reducing reflex testing. Combining automation with ALLType™ kit and TSV software, the analysis will be used as the primary method and SSOP as a reflex method for HSC and SO.

P094

PRINTING TO PAPERLESS: IMPROVING REPORTING OF HIGH COMPLEXITY IMMUNOGENETICS SINGLE ANTIGEN BEAD DATA TO THE ELECTRONIC MEDICAL RECORD- DESIGN OF A STATE OF THE ART REPORTING SYSTEM AND PROCESS IMPROVEMENT

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Aim: The UCLA Immunogenetics Center (UIC) uses HistoTrac for transplant patient data storage, analysis and reporting to UCLA Health System's EMR EPIC:CareConnect. Currently, paper reports are uploaded from UIC to EPIC as a scanned PDF. To improve reporting workflows from HistoTrac to the EMR, we developed and mapped components in EPIC:CareConnect to allow numeric reporting of Single Antigen Bead (SAB) reports from HistoTrac. **Methods:** A new State of the Art reporting workflow (Figure 1a) was designed and validated in the test environments. The build was completed for multiple orderables: SAB Class I, Class II or Class I/II combo, and modifications- titration and C1q (specificity, strength/MFI's and interpretative comments). **Results:** After migration of the reporting workflow to the live environment, baseline system performance data evaluated over a 9 week period showed that ~4.5/100 tests were resulting in EPIC as "Preliminary" or "In Process" instead of "Final Result" with only common cause variation (Figure 1b). Classic quality improvement methods were employed to improve the process with the aim of reporting 100% of tests as "Final Result". An audit system was developed and a Pareto Analysis identified two errors accounting for 79% of common system level failures- Status/Timing Errors, and HL-7 Warning Errors. We used the Model For Improvement for system transformation. Status/Timing and HL-7 Warning Errors were completely resolved through the process improvement. Through the audit process, the remaining common system level failures due to technical errors in ordering/reporting, result delays, and HL-7 Fatal errors are identified and resolved. Continuous monitoring reviled a system level shift with only ~1.9/100 tests resulting in EPIC as "Preliminary" or "In Process" instead of "Final Result" (Figure 1b). **Conclusions:** The study demonstrates that high complexity SAB bead data can be numerically reported to the EPIC EMR from HistoTrac. Through the process improvement, 100% of tests result to EPIC as "Final Result".

P095

ANTIBODIES TO HUMAN PLATELET ANTIGENS: WHEN DO THEY CAUSE AN INCOMPATIBLE CROSSMATCH

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Aim: Our center evaluates approximately 100 platelet refractory patients each year. Previously, we have correlated single antigen bead MFI with platelet crossmatch results in an effort to optimize our process for selection of HLA-compatible platelets. However, we continue to struggle to appreciate when antibodies to human platelet antigens (HPA) are also contributing to the platelet refractory state. In this study, our aim is to understand the relationship between HPA antibody testing (PakPlus, Immucor) and platelet crossmatch (CaptureP, Immucor). **Methods:** One patient sample with a singular & strong specificity, HPA-1a, was identified based on PakPlus testing. This sample was also negative for HLA antibodies based on PakPlus testing. The plasma was then diluted and the PakPlus result, optical density (OD), was determined at each dilution. Ten antigen-positive platelet units were crossmatched with the battery of plasma dilutions. The strength of the platelet crossmatch was graded by one technologist, blinded to the dilution, with 4+ being strong positive to negative. **Results:** The patient sample demonstrated a fairly linear dilution of HPA-1a, beginning with a neat result of OD 1.33. All 10 antigen-positive platelet units were positive at OD 0.42 or greater, yet negative at OD 0.04. There was platelet-specific variability between OD 0.3 and OD 0.06 (see table 1). **Conclusions:** These results demonstrate that HPA antibodies cause crossmatch positivity at low OD. The variability seen may be due to homozygosity versus heterozygosity of antigen expression. These initial results suggest that a PakPlus result as low as OD 0.06 may cause incompatibility in the platelet crossmatch and could be considered when selecting platelets for the platelet refractory patient.

Table 1. Reactivity of platelet crossmatch at different PakPlus OD

OD	PLT 1	PLT 2	PLT 3	PLT 4	PLT 5	PLT 6	PLT 7	PLT 8	PLT 9	PLT 10
1.33	1+	2+	1+	1+	1+	n/a	n/a	n/a	n/a	n/a

0.89	n/a	1+	1+	n/a	1+	1+	1+	1+	1+	w+
0.72	1+	w+	1+	1+	w+	1+	1+	w+	w+	w+
0.42	n/a	w+	1+	n/a	w+	w+	1+	w+	w+	w+
0.30	w+	neg	1+	w+	w+	w+	w+	w+	w+	w+
0.12	neg	neg	1+	w+	w+	neg	w+	w+	w+	w+
0.06	neg	neg	neg	neg	neg	neg	w+	neg	neg	neg
0.04	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg

P096

COLLECTION & STORAGE OF BLOOD SAMPLES FOR CFDNA EXTRACTION: IS THE MORE ALWAYS THE MERRIER?

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Aim: Analysis of circulating donor derived cell-free DNA (ccfDNA) promises a non-invasive method of assessing allograft rejection and possibly eliminating the need for surgical biopsies. In this study we compared the concentration and purity of ccfDNA in specimens collected in ACD (BD Vacutainer) tubes and Cell-Free DNA BCT (Streck) tubes and processed after different storage times. **Methods:** Blood samples from 4 healthy donors were drawn into collection tubes (3 tubes each for analysis at different time points). Samples were stored at room temperature and processed at 18, 24, and 72 hours. The samples were processed using Promega LV ccfDNA Custom kit according to the manufacturer protocol. Tubes were centrifuged at 2000g for 10 minutes and an average of 4 ml of plasma was separated from the cells. Samples were extracted on the Maxwell RSC, and eluted in a volume of 65 ul. Samples were measured by Qubit (Thermo Fisher) & ProNex DNA QC Assay (Promega) to determine DNA concentration. The level of genomic DNA (gDNA) contamination was determined by the ProNex Assay and confirmed by the TapeStation D1000 High Sensitivity kit (Agilent Technologies). The ProNex Assay uses a multiplexed probe-based qPCR assay to detect quantity and quality of genomic DNA. It detects 75bp, 150bp and 300bp genomic DNA sequences. The presence of 300bp sequences indicates contamination with gDNA. **Results:** DNA concentration by the Qubit showed a wide range of quantity. The quantity of 75bp and 150 bp DNA fragments as shown by the qPCR assay indicate the amount of ccfDNA in each sample. The ratios of smaller fragments to 300 bp in each sample indicate the level of gDNA contamination. The Streck specimens had consistently high ratios up to 72 hours. The ACD specimens had much lower ratios, decreasing by 72 hours. The presence of gDNA contamination was confirmed by TapeStation traces and one such example at 72 hours is shown below.

Conclusions: ACD tubes consistently exhibited higher gDNA contamination, as reflected by lower ratios in qPCR, which increased over time.

P097

QUANTITY, INTEGRITY & PURITY: A COMPARATIVE STUDY OF DIFFERENT GENOMIC DNA ASSESSMENT METHODS

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Aim: DNA quantity and quality are critical for the performance of downstream applications. There are different methods to determine these characteristics and each provide data to evaluate the DNA present in the sample.
Methods: In this study, four methods were used to evaluate gDNA from 20 blood and buccal swab sources: measurement by Nanodrop 2000 (Thermo Fisher), quantitation by Qubit 3.0 (Thermo Fisher), evaluation by the Tapestation 4200 (Agilent Technologies), and assessment by the Promega ProNex DNA QC Assay (Promega).
Results: The Nanodrop provides the quantity of DNA , but can overestimate or underestimate the quantity in an unpredictable fashion. The Qubit accurately provides quantity of DNA present.

We utilized the TapeStation to measure fragment sizes and to obtain a DNA integrity number (DIN) that reflects the level of degradation.

The ProNex DNA QC assay determines varying amplifiable gDNA sequences of 75bp, 150bp, and 300bp, providing information about quantity and quality of gDNA present in the sample. There is also an internal positive control which can test for the presence of a PCR inhibitor. Four samples (2 ,3 ,6, and 9) exhibited degradation with the TapeStation assay, which was also reflected in the qPCR ratios.

Conclusions: All four methods evaluated provide varied information about the quantity, quality and purity of DNA specimens. Ultimately, the appropriateness of assay selected depends on the downstream application.

P098

A NOVEL FLOW CYTOMETRIC ASSAY TO SIMULTANEOUSLY DETECT ATG CONCENTRATION AND CYTOTOXIC EFFECT IN RENAL TRANSPLANT PATIENT

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Aim: To develop a new flow cytometry assay for detecting ATG concentration and immunokilling effect to monitor immunotherapy in transplantation. **Methods:** CDC: ASHI standard protocol; Flow-ATG: 50ul patient sera or ATG spiked AB sera with and without complement inactivation were incubated with $0.2X10^6$ PBMC for 15' at 37 C. After 3 washes, the cells were resuspended in 110 ul stain mix containing PE-anti Rabbit IgG, FITC-CD3, PE-Cy7-CD19, and 7-AAD and continuously incubated at RT for an additional 10'. The cells were washed again and acquired on a Canto II flow cytometer. ATG binding (MCS) and cell death % were calculated on total lymphocyte, T , B, and non-T/B lymphocyte populations. **Results:** ATG exerted a dose dependent effect in cell binding and killing on multiple immune cell populations including T, B lymphocytes, and non-T/B cells. T cells were more sensitive to ATG killing than non T cells (Fig 1). In the binding assay, ATG at 0.4 ng /ml showed significant bindings on T and B lymphocytes and non-T/B cells. The killing effect of ATG was started at 0.25ug/ml and reached a maximum at 1.25ug/ml (100% death rate) on T and non-T cells. After complement inactivation (56C/30'), cytotoxic effect of ATG on immune cells was only slightly decreased (Fig 2), which indicated the killing of ATG was mainly resulted from mechanisms of antibody-dependent cellular cytotoxicity (ADCC) and apoptosis under our testing system. Five samples from kidney transplant patients undergoing ATG treatment were tested by both CDC and Flow-ATG. The quantitative dynamical changes of ATG binding and killing were observed by Flow-ATG. Compared to the traditional CDC assay Flow-ATG is much more sensitive and also able to measure the concentration of ATG in the same reaction. **Conclusions:** The newly developed Flow-ATG is able to simultaneously detect ATG binding and killing effect on T, B cells, and non-T/B cells. The assay is simple, sensitive (0.4ng/ml), quantitative, and can be used for monitoring clinical ATG immunotherapy in bone marrow and solid organ transplantations.

P099

COMPARISON OF SSOP VERSUS NGS FOR TYPING OF HLA-A, B, C, DRB1, DRB3/B4/B5, DQA1, DQB1, DPA1, DPB1: TOWARD SINGLE PASS HIGH RESOLUTION HLA TYPING IN SUPPORT OF SOLID ORGAN AND HEMATOPOIETIC CELL TRANSPLANT PROGRAMS

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Aim: Many laboratories use a 2-tier HLA typing process: a first pass intermediate resolution and then high resolution, when clinically necessary. SSP or qPCR provide rapid typing for deceased donor workup; SSOP is widely employed for higher volumes; and Sanger sequencing has been the gold standard for high resolution. However, SSOP and Sanger SBT often yield ambiguous results and resolution is expensive and time consuming. Next generation sequencing offers a single pass technology to achieve unambiguous HLA allele assignments. We compared commercial SSOP and NGS systems with respect to accuracy, turnaround time, effort and resolution level. **Methods:** Five laboratories submitted coded, blinded samples, previously typed by SSOP at each institute, to a separate lab for NGS of HLA-A, B, C at exons 1-7, DRB1, DRB3/4/5, DQA1, DQB1, DPB1 at exons 1-4, and DPA1 at exons 2-4. For up to 24 samples by SSOP or up to 48 samples by NGS, benchwork is completed by one technologist on day 1 with results available for reporting on day 2. **Results:** At the SSOP resolution level, results were concordant except for 11 SSOP assignments in 8 specimens due to false probe reactions at DRB1, DRB5, DQA1, DQB1, and DPB1. NGS identified 21 novel sequences: one with a multi-exon deletion and 20 with SNP polymorphisms in HLA-C, DPA1, DPB1, DQA1, DRB1, DRB3, DRB4, and DRB5 alleles. Across the highly polymorphic HLA-A, B, C, DRB1 loci, only 1% of intermediate resolution, IR, SSOP results were unambiguous.

Even higher resolution, HR, SSOP by 1 lab gave only 17% specific allele assignments. In contrast, over 99% of NGS results were specific unambiguous genotypes. Across all loci, NGS typing was specific except for certain discrete diploid ambiguities, also found in SSOP. **Conclusions:** This study provides a compelling rationale for implementing NGS for single pass HLA analysis with accurate, 2 day turnaround, which includes the high resolution, unambiguous genotyping critical for unrelated donor HCT and that may be required for sensitized patients with allele specific antibodies.

Percent resolution to a specific allele						
Typing technology	Sample Numbers	HLA-A	HLA-B	HLA-C	DRB1	Mean % allele resolution
Lab 1: IR SSOP	120	1.7%	2%	0.8%	1.7%	1.5%
Lab 2: IR SSOP	50	0	1%	0	2%	0.75%
Lab 3: IR SSOP	50	0	0	0	2%	0.5%
Lab 4: IR SSOP	19	0	5.3%	0	0	1.3%
Lab 5: HR SSOP	50	20%	25%	5%	19%	17%
Lab 6: NGS	289	98.3%	98.5%	100%	100%	99.7%

A.G. Smith: *2. Consultant; Company/Organization; Scisco Genetics Inc.* ***S.E. Pereira:*** *4. Scientific/Medical Advisor; Company/Organization; Scisco Genetics Inc.* ***C. Pyo:*** *4. Scientific/Medical Advisor; Company/Organization; Scisco Genetics Inc.* ***W. Nelson:*** *5. Employee; Company/Organization; Scisco Genetics Inc.* ***M. Bettinotti:*** *3. Speaker's Bureau; Company/Organization; One Lambda ThermoFisher.* ***M.Z. Askar:*** *3. Speaker's Bureau; Company/Organization; Immucor. 4. Scientific/Medical Advisor; Company/Organization; Illumina.* ***D.E. Geraghty:*** *6. Stock Shareholder; Company/Organization; Scisco Genetics Inc.*

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COMPLETE SINGLE MICA GENE SEQUENCING-BASED TYPING USING NEXT GENERATION SEQUECERS

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Aim: The MICA gene located in the HLA gene cluster region close to HLA-B is highly polymorphic. It is associated with solid organ transplant rejection and bone marrow transplant GVHD. To test a novel Next Generation Sequencing (NGS) based MICA high resolution typing method, we used both Sanger Based Typing (SBT) and NGS in a parallel double blind study. **Methods:** Genomic DNA samples from 54 healthy Han people in southern China were genotyped by both SBT and NGS methods. Well established MICA SBT was performed using an amplicon covering the MICA gene from exon 2 to exon 5 (2.2kb) and MICA-Seq specific software. NGS was performed with a PCR product that includes the entire length of the MICA gene from mid 5'UTR to the end of the 3'UTR (12.6 Kb). Library preparation was performed with the standard library preparation method and reagents from Omixon's V2 Holotype HLA kits and sequenced on an Illumina MiSeq using paired-end 2x250 bp V2 chemistry. MICA typing was assigned using Omixon Twin software and GenDx's NGSEngine. **Results:** 2.04 million reads with an average quality score ≥Q30 were generated. 93.3% of reads produced were high quality and used for MICA consensus assembly with an average coverage depth of 300 across the exonic regions. 26 MICA genotypes consisting of 17 unique alleles among 54 samples were identified, and were 100% concordant between NGS and SBT. MICA NGS was able to resolve all ambiguities obtained by MICA SBT. Alleles initially typed as ambiguous MICA*008:01/04 by SBT were determined to be MICA*008:01:01, *008:01:02 and *008:04. MICA*008:04 was the most frequent at 19.4% (21/108 alleles) by NGS. In addition, 4 samples typed ambiguously as MICA*002:01/MICA*008 or MICA*023/MICA*035, and 3 samples typed as MICA*002:01/MICA*010 or MICA*022/MICA*025 by SBT were resolved by NGS. Furthermore a new *008:01 allele was found with 5 differences in intron 1 and 1 difference in intron 5. This new allele was represented three times in the population of 108 allele calls. Additionally we were able the complete the sequencing of exon 1 for *009:01 and *033 and exon 6 for *007:01 and *033 alleles. **Conclusions:** A NGS based MICA typing method has been established and is capable

of resolving ambiguities observed with SBT, identifies new alleles and allows the completion of IMGT reported incomplete allele sequences.

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CHIMERISM MONITORING BY NEXT-GENERATION SEQUENCING: A MULTIPLATFORM COMPARISON

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Aim: Accurate monitoring of the chimeric status after stem cell transplantation is essential for early detection of relapse and at the moment mainly performed by STR or qPCR. The major disadvantages of STR are the laborious data analysis and poor sensitivity. The qPCR technique enables a much quicker workflow and better sensitivity. However a disadvantage is the need of a pre-transplant sample for each monitoring event. Performing chimerism monitoring by NGS will eliminate these limitations while allowing for a multiplexed setup, reducing the amount of needed lab work and DNA. The workflow can be combined with routinely applied NGS HLA typing in clinical laboratories. **Methods:** PCR amplification primers have been designed for a set of biallelic markers. These markers were tested on a number of artificial chimeric samples in a range between 0.01-100% of positive DNA mixed with negative DNA. Amplicons were applied in the respective NGSgo library preparation workflows and run on Illumina MiSeq, Ion Torrent S5 and Oxford Nanopore MinION systems. NGS data was analysed with customized analysis tools designed to quantitate the two variants of each marker. **Results:** Each marker was tested on a small panel of non-chimeric samples (Coriell Institute) and resulted in clear homo- or heterozygous patterns for all markers. Results of the different platforms on the artificial chimeric samples in a range of 0.01%-100% were compared. Sensitivity was determined for each sequencing platform. A read depth threshold was established for each of the platforms to achieve a minimum sensitivity of 0.05%. Results including difference between sequencing platforms will be discussed further. **Conclusions:** The results demonstrate that NGS-based chimerism monitoring is feasible for a wide range of NGS platforms, with a high sensitivity and wide dynamic range.

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PERFORMANCE OF A MULTIPLEX PCR AMPLIFICATION FOR NGS-BASED TYPING OF 6 HLA GENES

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Aim: Accurate NGS-based HLA typing heavily depends on a reliable PCR amplification of HLA genes. Most protocols apply a method in which each HLA gene is amplified separately, after which amplicons are pooled and processed in a library preparation procedure. We have developed a reliable multiplex PCR for HLA-A, B, C, DRB1, DQB1 and DPB1, bringing down the number of amplifications from six to only one reaction per sample. **Methods:** HLA gene-specific amplification primers, based on NGSgo-AmpX primers (GenDx), are combined in one PCR using the GenDx-LongMix mastermix. Amplicons are processed in the NGSgo® workflow (GenDx) for library preparation and sequenced on a MiSeq (Illumina) with application of paired-end sequencing (2x151 bp). Data is analysed in HLA typing software NGSengine®. **Results:** A multiplex PCR was developed and optimized such that high quality NGS data was obtained and reliable HLA typings were obtained for a large validation panel (n = >96). When comparing the data of the multiplex PCR data with the singleplex PCR, slightly elevated noise levels were observed due to formation of hybrid reads in Class I genes. With an optimized PCR protocol noise levels were reduced to acceptable levels. In addition, read depth difference between genes were minimized for efficient flow cell use. **Conclusions:** A multiplex PCR for HLA-A, B, C, DRB1, DQB1 and DPB1 has been developed, that is fully compatible with the HLA typing workflow with NGSgo® for Illumina. PCR artefacts have been minimized with a new protocol that generates high quality data and supports reliable NGS-based HLA typing.

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THE PROMISE OF COST-EFFICIENT FULL-LENGTH HLA CLASS I GENOTYPING: ADVANCES USING NANOPORE SEQUENCING

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Aim: Nanopore based sequencing has seen rapid advancement in the recent years with iterations in pore structure, sequencing chemistries and base callers. This has led to increasingly accurate sequencing of extremely long DNA molecules. Since current HLA genotyping algorithms are not optimized for nanopore data we developed a genotyping algorithm based on read grouping and subsequent mapping to a reduced reference database. **Methods:** High raw sequencing errors present a challenge for genotyping based on direct mapping to a reference database. Our algorithm, Poretyper, obviates this initial mapping by first grouping the raw reads based on the distributions of k-mers within each read. A multiple sequence alignment derived from the groups of raw reads results in a set of consensus sequences which represent potential alleles. These consensus sequences are then used to create a culled reference database dramatically reducing the search space, thus reducing artefactual raw read mappings. **Results:** HLA-A, HLA-B and HLA-C for a hundred samples drawn from the DKMS donor registry were sequenced using the MinION with R9.5 chemistry. All hundred samples were then genotyped using Poretyper and existing G-group pretypings could be recapitulated failing only for those alleles where no full length sequences were available. **Conclusions:** Nanopore sequencing presents a viable and accurate platform for cost-efficient full-length HLA Class I genotyping. For those alleles where full length reference sequences are not available, an in silico extension of such allele sequences using the full-length sequence of the next closest allele presents a viable approach for full-length genotyping.

P104

A REAL-TIME TRIPLEX PCR ASSAY FOR QUANTIFYING T-CELL RECEPTOR EXCISION CIRCLES, KAPPA-DELETING B-CELL EXCISION CIRCLES, AND BETA-ACTIN

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Aim: Here we describe an efficient multiplex real-time quantitative PCR (qPCR) assay that simultaneously quantifies T-cell receptor excision circles (TREC), Kappa- deleting recombination receptor excision circles (KREC) and beta-actin concentrations using a synthetic gene fragment that contains all three targets. TREC are episomal byproducts of T-cell rearrangement and development. Similarly, KREC form during B-cell maturation in the bone marrow. TREC and KREC concentrations can be used to assess and diagnose immune deficiencies, as well as monitor thymic and bone marrow immune reconstitution following hematopoietic stem cell transplant (HSCT) or in response to drug therapy. **Methods:** First, primers and probes were developed and optimized for each of the three individual targets: TREC, KREC and the reference gene, beta-actin. Each gene probe has spectrally unique 5' reporter dye as well as an internal and 3' quencher. After individual gene optimization, a triplex qPCR assay was then developed and optimized on the Roche LightCycler 480, using a standard curve prepared by serially diluting a synthetic genomic fragment containing all three targets. **Results:** TREC, KREC and beta-actin concentrations were determined from healthy individuals representing a wide range of ages for each of the three targets. After optimization of triplex assay, the same DNA samples were tested and TREC, KREC and beta-actin concentrations obtained from each individual assay were compared with those obtained from the triplex assay. Target concentrations obtained with the individual assays were comparable to those obtained with the triplex assay and as expected, TREC and KREC concentrations decreased with age. **Conclusions:** We developed a multiplex quantitative PCR assay that can quantify TREC and KREC concentrations as well as normalize to a reference gene simultaneously, allowing for rapid and accurate assessment of all three targets.

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AN ACCELERATED METHOD FOR REVERSE SEQUENCE-SPECIFIC OLIGONUCLEOTIDE PROBE-BASED HLA TYPING

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Aim: Turnaround time (TAT) for HLA typing is critical for transplantation. We sought to establish a faster protocol for low-resolution HLA typing using the rSSOP methodology. **Methods:** The LABType™ rSSOP method (One Lambda) was altered to reduce hybridization time. Modifications included centrifugation and incubation duration reductions, and reagent volume adjustments. Single parameters were first changed and tested. Successful modifications were used in the next round of tests. All modifications were tested with a set of PCR amplicons prepared from three samples, which contained previously HLA-typed high quality DNA. Following a series of rSSOP typings for these samples at HLA-A, B, C, DRB1, and DQB1/DQA1 loci, an accelerated protocol was established. Discovery phase concluded with protocol validation at all loci including HLA-DRB3/4/5 and DPB1/DPA1. Full validation confirmed intra-operator, inter- and intra-assay, as well as multiple DNA source reproducibility. DNA was extracted from different samples including ACD blood (n=4), EDTA blood (n=4), cord segment (n=5), buccal swab (n=5) and mouth-wash samples (n=4). **Results:** The original centrifugation of 3000 rpm for 2 min (total 2 min 22 sec) was changed to a quick spin (up to 3000rpm; 33 sec), reducing each centrifugation step by 1 min 50 sec. Additionally, one centrifugation and one transfer of plate step were eliminated. The duration of incubation time was changed for denaturation (from 10 min to 2.5 min), hybridization (from 15 min to 5 min), and streptavidin-PE (SAPE, from 5 min to 2.5 min). The reagent volume modifications includes the reduction of washing buffer (from 100 µl to 50 µl per well) and increase in SAPE (from 25 µl to 50 µl per well). Compared to the original protocol, the accelerated protocol reduced the hybridization time approximately 30 min. This method allowed us to obtain consistent and accurate typing results for all tested HLA loci among different operators, intra- and inter-assays, and a variety of DNA samples. **Conclusions:** Fulfilling requests for faster TAT may be achieved by adaptation of current methodologies, which may help improving patient management. The accelerated rSSOP protocol is an effective method for reducing testing time, while still giving robust results for HLA class I and class II typing. (RLU and JJX contributed equally)

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LINKSĒQ FOR ABO, A MOLECULAR BASED TYPING SOLUTION FOR THE ABO BLOOD GROUP

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Aim: ABO-incompatible (ABOi) organs have been successfully transplanted from A2 donors into B recipients, especially when the recipient's anti-A titers are reduced. Laboratories typically use serological methods to perform ABO typing. However, serology has significant limitations including (i) recently transfused patients may exhibit mixed field agglutination and (ii) both forward and reverse typing must be performed, but may yield discordant results. These limitations are most problematic with phenotypes involving weakly expressed antigens and ABO subgroups (i.e. A2). However, current ABO subgrouping methods using anti-A1 lectin have limitations: they only indirectly determine A2, and typically include a gray zone reactivity range for which subgroups cannot be reliably called. A combination of genotyping solutions (SSP, sequencing, etc) can be used for ABO subgrouping, but these methods are time- and labor-consuming, and require interpretation by subject matter experts. **Methods:** The Thermo Fisher Scientific solution is based on its LinkSēq real-time PCR technology, which was developed over 10 years ago for genotyping the complex Human Leukocyte Antigen (HLA) system. LinkSēq ABO analyzes 19 reactions that identify multiple relevant SNPs located within the ABO gene. We evaluated this solution by analyzing 40 archived DNA samples, including blood blank samples for which serotyping failed or produced discordant results, and samples from deceased solid organ donors for comparison with A1 lectin testing. **Results:** Genotyping results generated by LinkSēq were 100% concordant with typing obtained by traditional methodologies. In two cases, serology couldn't provide conclusive results and typing had to be reflexed to lectin tests or SBT. LinkSēq overcomes the major challenges of molecular typing by providing a robust, automated approach that increases laboratory productivity and reduces turn-around time. With less than 10 minutes of hands-on set-up, no further operator intervention with reagents, and SureTyper software fully automating all analysis, LinkSēq delivers

genotyping and predicted phenotyping results in approximately 90 minutes. **Conclusions:** We conclude that LinkSēq can provide a simple, effective and robust method for ABO typing including accurate A2 subgrouping.

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FROM THE SAMPLE TO DONOR/RECIPIENT COMPATIBILITY: A COMPREHENSIVE RANGE OF LABORATORY REAGENTS AND EQUIPMENT FOR NGS-BASED GENOTYPING OF CLASSICAL AND NON-CLASSICAL HLA GENES

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Aim: The French organization for blood transfusion (EFS), developed a national research and development program to provide a complete range of reagents and instrumentation leading to a fast, simple and robust NGS strategy for HLA genotyping. **Methods:** The specially-designed "**NG-Mix PCR reagent**" amplifies all classical Class I (HLA-A, B, C) and Class II (HLA-DRB1,DRB3, DRB5, DQA1, DQB1, DPA1, DPB1) genes as well as the non-classical HLA genes (HLA-E, F, G, DQA2, DQB2). Amplicon fragmentation is achieved with "**NG-Frag**", a novel instrumentation recently developed by EFS which, *in less than one minute*, allows ultra-sonication based fragmentation of 96 samples loaded in a PCR plate in a unique single step. This highly standardized sonication step generates fragments in a uniform range of optimal size (400-800 bp), *which eliminates the requirement to perform time-consuming fragment size selection and purification steps*. The library is subsequently generated by using "**NG-Lib**", which are specific reagents that produce a normalized library without the need to perform any extra PCR reactions for tagging *in less than one hour* for 96 samples. Library molarity is consistently and reliably quantified using "**NG-Quant**", a q-PCR method using a specific probe (*without presuming a hypothetical size range for library fragments, counter to other quantification technique*). The sequencing reactions are performed on the MiSeq system (Illumina) before analysis with "**NG-View**", a data compilation and interpretation software designed by the EFS. **Results:** This method was used to generate results on 41 homozygous reference cell lines and a panel of 190 random BMVD samples previously genotyped by another NGS commercial product (Omixon). These results will be presented along with three other important aspects of the EFS NGS product: the technical advantages of the EFS NGS approach at each step of the process, a description of DQA2/DQB2 allelic system, and the observed linkage disequilibrium between classical and non-classical HLA genes. Furthermore, during the development of the EFS NGS product, numerous new classical and non-classical alleles were identified and submitted to the IMGT/HLA allele database. **Conclusions:** At the time of preparation of this abstract, 52 alleles discovered by this method are already listed on the database.

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PRELIMINARY IDENTIFICATION OF IMMUNE CELLS IN THE PERFUSATE OF RENAL ALLOGRAFTS UNDERGOING HYPOTHERMIC MACHINE PERFUSION

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Aim: Renal allografts stored with hypothermic machine perfusion (HPM) have improved outcomes compared to static methods. Passenger immune cells from the allograft have been implicated in acute rejection; improved outcomes with HPM may be due to cell removal by the pump. This study aimed to identify passenger immune cells present in the perfusate of HPM-preserved allografts. Once characterized, future strategies to suppress these cells may prevent acute rejection and improve graft survival. **Methods:** All kidneys placed on HPM prior to transplant from May 2016 to August 2017 were eligible. Perfusate fluid was analyzed using flow cytometry for immune cells and information about the donors was collected. Wilcoxon Scores and Spearman Correlation Coefficient were used in statistical analysis. **Results:** Twenty-seven perfusate specimens were analyzed. Selected donor characteristics: 63.6% male, 36.4% female; 63.6% donation after cardiac death; 54.5% pre-procurement transfusion. The mean HPM and cold ischemia times (CIT) were 9.9 hours and 13.9 hours respectively, and mean KDPI 36%. Flow cytometry showed a heterogeneous mix of immune cell types in the perfusate, similar to blood. CD15+ granulocytes, CD14+ monocytes, and CD8+ T cells were predominant in the perfusate. Dendritic and natural killer

(NK) cells in perfusate were more abundant compared to blood. **Conclusions:** Passenger immune cells, specifically dendritic cells have been implicated in acute rejection. This study showed the perfusate of renal allografts contains an array of immune cells, with a significantly higher percentage of dendritic cells and NK cells, as compared to blood. Future studies would show if HPM removal of these immune cells accounts for improved outcomes.
**Frequencies of Cell Types in Human Peripheral Blood*, www.stemcell.com.

P110

CLINICAL PREDICTORS OF DONOR SPECIFIC ANTIBODY FOLLOWING KIDNEY TRANSPLANTATION

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Aim: Optimizing immunosuppressive drug therapy for individual patients has been one of the challenges of transplantation. In this study, we attempt to define the relationship between tacrolimus (TAC) and mycophenolate mofetil (MPA) in the development of donor specific antibody (DSA) in patients with kidney transplants. **Methods:** 253 patients underwent kidney transplantation from January 2011 to February 2017. All patients received induction therapy with 2-5 mg/kg of thymoglobulin. Standard immunotherapy after induction consisted of steroids, MPA and tacrolimus. All tacrolimus levels drawn after transplantation were included. A two-hour AUC for the active metabolite, MPA, and the glucuronide, MPAG, was determined 1-5 months following transplantation. **Results:** Patients that developed DSA were compared to those that did not using logistic regression. Variables in the logistic regression included age, mean tacrolimus levels with their standard deviation, MPA area under the curve (AUC) with its standard deviation, MPAG (MPA glucuronide) with its standard deviation (SD). For a given patient the mean of all TAC levels was calculated by using every level obtained following kidney transplantation. See Table 1. The coefficients associated with the variables above suggest that the most predictive variables for the development of DSA were mean TAC level, SD of the mean TAC level, mean MPAG level and age. See Table 2. **Conclusions:** Our data suggests: 1) Mean tacrolimus level and standard deviation of the mean has the highest predictive value for the development of DSA, 2) Mean MPAG AUC and not mean MPA AUC, is almost equally important in forecasting the development of DSA and 3) young age predisposes to the formation of DSA.

Table 1

	Age	DSA	MPA AUC	MPAG AUC
Coun t	232.000000	286.000000	232.000000	232.000000
Mean	55.387931	0.227273	55.089569	81.415474
Std	12.513384	0.419805	17.582329	22.501601

Table 2		
	Coefficient	Variable
0	-0.377901	Age
1	-0.057695	MPA AUC
2	0.421105	MPAG AUC
3	-0.493562	Mean TAC Level
4	0.442212	SD of TAC Level

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ANALYSIS OF HLA ANTIBODIES AFTER NATIVE NEPHRECTOMY IN RENAL ALLOGRAFT RECIPIENTS

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Aim: Many centers avoid nephrectomy of chronically rejected kidneys because of fear of sensitization to HLA antibodies. It is not clear if the rise in antibodies is from an overall immune sensitizing event or specific to the removal of the donor organ. We analyzed anti-HLA antibodies following native nephrectomy as a control event to the allograft nephrectomy. **Methods:** Twelve kidney allograft recipients who underwent native nephrectomy for cause had serum samples obtained before the nephrectomy and 1 - 32 weeks after nephrectomy. All patients remained on clinically indicated immunosuppression. The study was approved as an exempt study by the IRB. The serum samples were tested on a Luminex platform with Single Antigen Class I and Class II beads specific for HLA antibodies. Changes in calculated panel reactive antibody (cPRA) and peak mean fluorescence intensity (MFI) were measured by paired t-test. **Results:** Three of twelve patients (25%) showed an increase in HLA antibodies. One of the sensitized and one of the non-sensitized patients required a blood transfusion during the procedure. Nine patients had no class l or class ll antibody before or after native nephrectomy. The 3 patients who had sensitization to HLA prior to the native nephrectomy all had an increase in antibody post-nephrectomy: pre cPRA = 65 and post cPRA 74, p=0.02. Two of the 3 sensitized patients showed an increase of MFI in their highest antibody specificity class I MFI (7933 pre and 13733 post) and class II MFI (6200 pre and 8067 post), but this was not significant in this small group. One subject showed increased donor specific antibody, with no significant class I before native nephrectomy, but MFI of A3 of 14800 and B44 of 17600 after native nephrectomy; and class II DR1 MFI of 3300 before (no DR4) and DR1 MFI of 18,200 and DR4 MFI of 15500 after native nephrectomy. There was no incidence of allograft loss. **Conclusions:** HLA antibodies may rise following a native nephrectomy in sensitized patients. Monitoring of HLA antibodies after native nephrectomy may be prudent.

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CLEARANCE OF PRE-FORMED DONOR SPECIFIC ANTIBODIES AFTER LIVER TRANSPLANTATION IS SPECIFIC FOR DSA AND CROSS-REACTIVE ANTIGENS

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Aim: Liver transplant recipients with pre-formed HLA DSA are reported to “clear” antibodies post-transplant. We sought to determine if the reduction in HLA antibodies was specific for donor antigens or represented a global decline in alloantibodies. **Methods:** We retrospectively analyzed the post-transplant DSA course for 27 liver allograft recipients transplanted with pre-formed HLA DSA, measured by single antigen assay. **Results:** In 16 patients, DSA was persistently seen at last follow-up (although weaker than pre-transplant in all patients); another 2 patients also developed de novo DSA to other donor HLA. In the remaining 9 patients, DSA declined dramatically post-transplant and was not detected at last follow-up. We asked whether third party antibodies also declined (i.e.

global immunosuppression) or whether the patterns were specific for DSA and cross-reactive antigens. In 6 of 9 patients whose DSA declined, some non-DSA remained strong while the third party antibodies that reduced were clearly in cross-reactive groups with the donor specific antibodies. In one of the remaining patients, all of their antibodies disappeared post-transplant, in another the DSA did not fit into a CREG, and in the last patient DSA was to HLA-C locus.One example is given in the figure below, where strong antibodies persisted to third party A1 CREG; but DSA to A32 and antibodies to other A10/A19 CREG antigens declined post-transplant. In the same patient DSA to DQ6 disappeared during follow-up, as did non-DSA DQ5 antibodies; but antibodies to DQ2 persisted. **Conclusions:** Overall, liver allograft recipients experienced a global reduction in HLA antibodies, but the most profound effect was on DSA and CREG-related antigens. These cases suggest that liver clearance of HLA antibodies is specific for donor reactive antibodies and cross-reactive clones.

P113

DEVELOPING AN ASSAY TO DETECT AND QUANTIFY DONOR-DERIVED DNA IN THE BLOOD AND URINE OF SOLID ORGAN TRANSPLANT PATIENTS

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Aim: Detection of circulating donor-derived cell-free DNA (cfDNA) as a non-invasive biomarker of graft injury shows tremendous potential for transplant monitoring. Increases in the amount of cfDNA in blood or urine indicate injury to the transplanted organ, including rejection. Also known as a liquid biopsy, this method is less invasive and more sensitive than an actual biopsy to detect early rejection. We proposed to develop a clinical test based on the detection and quantification of donor single-nucleotide polymorphisms (SNPs) using cfDNA extracted from plasma and urine of solid organ transplant patients. **Methods:** Using genomic DNA, informative SNP markers from donor and recipient (ThermoFisher) will be identified using real-time PCR (Roche). cfDNA from transplant recipients will be extracted from plasma and urine (Qiagen), and informative SNPs will be quantified using digital-droplet PCR (ddPCR), (Bio-Rad). For development of the assay blood and urine samples were collected from 25 healthy individuals, genomic and cfDNA were extracted, and analytical parameters determined including limits of detection (LOD) and limits of quantification (LOQ) using ddPCR. **Results:** cfDNA extraction was successfully performed from blood and urine. The average cfDNA concentration was 1.36 ± 0.54 ng/ul from plasma, and 1.15 ± 2.1 ng/ul from urine. Determined from genomic DNA, an average of 4.9 informative markers was present between any two of the 25 individuals, although one pairing had no informative markers present, indicating the need for a larger panel using more than 36 SNPs. LOD studies determined the false positivity rate to be less than 0.0025%. Preliminary LOQ testing indicates that 0.5-0.75% donor-derived DNA can be detected. **Conclusions:** We have established a laboratory developed test to detect donor-derived cfDNA from multiple sample types such as blood and urine at a sensitivity that appears acceptable for clinical testing. In collaboration with the local solid organ transplant program, the clinical utility of this assay will be evaluated in kidney transplant recipients.

P114

DOES HLA-DR MATCHING PREVENT FORMATION OF DE NOVO ANTI-DONOR HLA-DQ ANTIBODIES?

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Aim: Donor specific antibodies (DSA) directed to mismatched HLA-DQB antigens are the most prevalent DSA after transplantation. Such DSA have been implicated in the pathogenesis of chronic antibody mediated rejection and graft loss. Currently, patients listed for deceased donor kidney transplantation receive priority points for each HLA-DR, but not DQ, matched with their donor. We aimed to determine whether HLA-DR matching impacts the formation of DQB DSA. **Methods:** Our study population included 567 patients without pre-transplant DSA who received kidney transplantation from Jan 1st 2009 to Dec 31st 2014. HLA typing of recipients and donors was performed by SSO/SSP. Pre- and post-transplant testing of anti-HLA antibodies was performed using solid phase assay (One Lambda). Prospective T and B cell crossmatches were performed in all cases by flow cytometry. **Results:** Out of 567 patients, 72 (13%) had zero, 215 (38%) had one and 280 (49%) had two DR mismatches (mm) with their respective donor. Overall, during the follow-up interval (median 4.6 years), 92 (16%) patients developed DSA. Twenty-four patients (4%) developed class I DSA, 44 (8%) had cl II DSA and 24 (4%) had cl I and II DSA. Among patients with zero HLA-DR mismatches, only 9% developed DSA. These DSA were directed to the mismatched HLA class I antigens and, in only one case, to a mismatched DQB antigen. The percent of patients who developed DSA was significantly higher in the groups with 1 or 2 DR mm, namely 19% and 14%, respectively (p=0.0002) The highest number of DQB DSA was observed in the group with 1 DR mm (Table 1).

Table 1. DSA distribution in patients with 0, 1 and 2 donor HLA-DR mismatches.

No. HLA-DR mm	N	De novo cl I DSA N(%)	De novo DR DSA N(%)	De novo DQB DSA N(%)
0	72	6(8)	0(0)	1(1)
1	215	8(4)	14(6)	25(12)
2	280	8(3)	18(6)	19(7)

Conclusions: Our results indicate that zero HLA-DR mm represents an acceptable surrogate for the absence of de novo DQB DSA. We suggest that the the number of priority points received by patients with zero HLA-DR mm should be increased. This may limit the number of patients who develop de novo DQB DSA, resulting in improved transplant outcomes. No additional points are warranted for a single DR match, which does not appear to have any protective effect.

P116

COMPLEMENT COMPONENT 1Q ASSAY LACKS SENSITIVITY AND TRENDS WITH DE NOVO DONOR-SPECIFIC ANTIBODY TITERS

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Aim: Despite major advancements in immunosuppression, long-term allograft survival rates from deceased and living donors remain poor following post-kidney transplantation. The presence of de novo donor-specific antibodies (dnDSA) and complement component 1q (C1q) following transplantation contributes to repetitive microvascular injury that correlates with worsening graft function and graft failure. Immune monitoring post-transplantation of dnDSA and C1q is commonly employed to assess patient graft failure risk and can guide clinical decision making regarding the patient's baseline immunosuppression. In this investigation, we sought to determine if the C1q assay provided added clinically relevant data as C1q positivity was often associated with dnDSA titer and the use of pooled patient serum for quality control varied greatly among different runs of the same lot number. **Methods:** Kidney transplant patients were monitored for the presence of human leukocyte antigen (HLA) class I and class II antibodies using the Labscreen Single Antigen Antibody Detection Tests and the C1qScreen (One Lambda, Inc., Canoga Park, CA). C1q risk scores were delineated as N = C1q negative, 2 = C1q weak positive, 3 = C1q moderate positive, 4 = C1q strong positive, 5-6 = very strong positive based on C1q mean fluorescent intensity (MFI) cutoff

ranges. **Results:** DSA titration (1:16, MFI = 3136) trended as N = C1q negative. DSA titration (1:256, MFI = 13136) trended as 5-6 = C1q very strong positive. Poor delineation of C1q risk at intermediate titrations may be due to confounding quality control variability in the daily pooled plasma serum measurements, which can alter baseline risk scoring of C1q MFI values. **Conclusions:** C1q and dnDSA are often tracked to make risk assessments regarding kidney allograft health post-transplantation; however, C1q MFI measurements may not be additionally informative for clinicians as it often tracked with dnDSA antibody titer and the C1q assay lacks sensitivity at lower concentrations and potentially makes C1q risk scores ambiguous or misleading.

P117

CLINICAL RELEVANCE OF SINGLE ANTIGEN BEAD IGG, IGM, AND C1Q ANTI-HLA ANTIBODY TESTING

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Aim: HLA single antigen bead testing for IgG antibodies has significantly improved the clinical outcome of transplantation. However, there are other single antigen bead assays, such as IgM and C1q that aid in identifying types antibodies. This study focuses on analyzing the clinical relevance of the IgG, IgM and C1q assays. **Methods:** 28 specimens were tested using the IgG, IgM, and C1q single antigen bead assays, according to the manufacturer's procedures. Using the raw data, percentage of positive reactivity, donor specific bead reactivity, and DSA reactivity in relation to creatinine levels were compared. **Results:** 1. It was observed that there is a higher percentage of reactivity with IgM versus IgG in HLA-A and HLA-C. Statistically, the difference in HLA-A is significant ($p < 0.01$) but the difference in HLA-C is not significant ($p = 0.124$). 2. When comparing donor specific reactivity: a. Very few samples had positive reactions with C1q and IgM but negative with IgG. b. Most positive C1q reactions were accompanied by positive IgG reactions, only. c. There was no direct link between the mean fluorescence intensity (MFI) of C1q and IgG or C1q and IgM. 3. Clinically, one case had elevated creatinine levels with no IgG, IgM or C1q DSA present. Later, that patient developed IgG, IgM and C1q DSA. In addition, there were several cases with low MFI IgG and IgM DSA, with normal creatinine levels. **Conclusions:** Due to the difference in percentage of positive IgM and IgG reactions to HLA-C not being statistically significant, more data needs to be collected to confirm the above results. For clinical interpretation, C1q reactivity may not be predicted based on IgG, IgM, or a combination of IgG/IgM reactivity. The immune binding capability and secondary antibody dilution factor for each assay should be strongly considered. Lastly, each patient's renal function, as well as anti-HLA antibody results, should be considered when diagnosing AMR and determining treatment.

P118

RELIABILITY OF VIRTUAL CROSSMATCH: REALITY OF PERCEPTION

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Aim: In this study we evaluated the concordance between vFXM and aFXM using SAB and a predetermined cut off point that has been established in our laboratory **Methods:** **AXM:**773 crossmatches performed by FCXM were retrospectively analyzed. The cut off for T &B are 64, 100 respectively. This includes 573 crossmatches performed using PB/ or spleen deceased donor cells with potential recipients & 200 consecutive final crossmatches performed for living transplantation. **VXM:** Donor HLA- antigens were compared against the potential recipient's antibody specificities. Prediction of vFXM was based on antibody identification using SAB. Normalized MFI values of 3000 were used to identify antibodies predicted to cause a positive FCXM for A, B, DR &5000 for HLA-C and DQ specificities when present as single antibody. A sum of multiple weak antibodies (MFI≤2000) that yield MFI of ≥ 3000 was used to predict positivity. **Results:** Only positive FCXM positive for B or B &T simultaneously are considered. The vast majority of aFXM were in concordance with vFXM with an overall concordance of 97**%**. Twenty-three predicted to be negative showed positive aFXM, 12 of them had 0% cPRA and 11 were found in patients with multiple non DSA- HLA- antibodies, dilution of these sera revealed weak DSA. Three predicted positive vFXM yield negative aFXM, two of them had allele specific antibodies. (Table1). Because sera of highly sensitized patients are diluted we rarely deceived by prozone phenomenon. Therefore the only cause of false negative VXM is auto or non HLA- antibodies. Due to the extreme sensitivity of SAB, reporting false positive VXM remains a possibility and will continue to be a limitation for VXM. **Conclusions:** vFXM based on precise characterization of antibody specificities detected by SAB using our cutoff point accurately predicted FXM in the majority of patients and can be used safely to allocate kidney offers without performing physical crossmatches in selected patients.

P119

CORRELATION BETWEEN THE STRENGTH OF DONOR SPECIFIC ANTIBODY (DSA) USING SINGLE ANTIGEN BEAD ASSAY (SAB) AND THE RESULTS OF CDC- CYTOTOXICITY AND FLOW CYTOMETRIC CROSSMATCH

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Aim: In this study we correlated flow and CDC- crossmatch outcomes with results of Luminex-single antigen bead (SAB). **Methods:** Between 2010- 2016 HLA-antibody Sera were tested by screening and SAB class I & II (One lambda) according to manufacturer recommendation. MFI of ≥1000 were considered positive. These sera were used to perform the following: 1. 80 crossmatches using isolated PB or spleen lymphocytes from healthy deceased or living donors respectively by CDC-crossmatch. Correlation was performed on sera with single antibody or multiple classes I and II antibodies. Titer of CDC was given as the inverse of the highest dilution yielding positive XM . **2.** 108 FCXM cross using isolated PB donor lymphocytes expressing one HLA- antigen (class I or II) and 78 crossmatches using cells expressing multiple antigens detected by the sera. Analysis performed on FACSCanto II & mean channel shift (MCS) was calculated. The cut off for T &B are 64, 100 respectively. T- Positive, B- negative results in the absence of HLA- antibody were excluded from the study. **Results:** Our results indicate good correlation of FCXM and CDC-XM with LUMINEX -SAB assay. SAB assay has good correlation with T-FCXM (r2=0.7) but not with B-FCXM. Fig.1 A cutoff MFI of 3000 for SAB assay (as single or sum of multiple weak antibodies) had a sensitivity & specificity of 100% in detecting a positive FCXM. The few number of sera with single anti HLA-Cw and DP antibodies did not give positive reaction up to 5000 and 10,000 MFI respectively but were not included in the study. Single anti HLA-A, B, DRB1of ≥ 10000MFI was associated with positive CDC-XM in most cases and multiple low levels antibody that yield this 10,000 would also give positive reaction. We noticed presence of A2 specificity among multiple anti class I or class II antibodies increased the chance of positive reaction even when sum of MFI is ≤ 10000. **Conclusions:** SAB correlates significantly with outcome of both cytotoxic and T-FCXM. Based on the threshold established it is possible to predict correctly results of crossmatch.

P120

PREVENTING FALSE POSITIVE CROSSMATCHES DUE TO RITUXIMAB BY USING ANTI RITUXIMAB ANTIBODY

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Aim: Rituximab (Rtx) is a humanized monoclonal antibody (Ab) to CD20 receptor expressed on B cells. Sera from patients who have received Rtx will have positive B-cell crossmatches (XMs) challenging the detection of clinically relevant Abs. There is a commercially available Ab advertised as blocking the binding of Rtx to CD20. We wanted to test if the addition of this anti-Rituximab (anti-Rtx) Ab to our XMs will prevent false positive (FP) results, allowing the detection of potential donor specific Abs (DSA). **Methods:** Negative control (NCS) and patient sera were spiked or not with Rit. Samples were incubated or not with anti-Rtx Ab (MB2A4, GeneTex) for 20 min. at known concentrations. These mixtures were used for XMs (flow cytometric (FC) and complement dependent cytotoxic (CDC)). Our FCXM uses a 256 channel scale. B-cell FCXM MCS above 9 is weak positive and above 20 is positive. **Results:** Addition of anti-Rtx blocked Rtx binding to B-cells: NCS spiked with Rtx causes positive B-cell XMs, which were negative in the presence of anti-Rtx (Table 1.A). Anti-Rtx prevented FP B-cell XMs in two samples from patients known to have received Rtx (Table 1.B). Anti-Rtx blocks Rtx in a dose-dependent manner. Anti-Rtx allowed the detection of DSA: A serum with anti-DQ2 Abs (4,000 MFI) was spiked with Rtx and incubated with anti-Rtx before crossmatching with cells expressing DQ2 antigen or not. The addition of anti-Rtx converted the XM with DQ2-negative cells to negative, but the XM with DQ2-positive cells was weakly positive, as was the XM performed with untreated serum (Table 2). **Conclusions:** Treatment of the sera of patients under Rtx immunotherapy with anti-Rtx before FC and CDC XMs prevents FP results and allow the detection of clinical relevant Abs.

P121

CCR5 DELTA 32 POLYMORPHISM IS NOT ASSOCIATED WITH NON-HLA ANTIBODY MEDIATED REJECTION IN HEART TRANSPLANT

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Aim: C-C chemokine receptor type 5 (CCR5) is predominantly expressed on T cells, macrophages, and dendritic cells. In addition to the wild type allele, a null allele (CCR5 Delta 32) has a 32-base pair (bp) deletion

(CCR5Delta32) in the coding region. Antibody mediated rejection (AMR) can be caused by non-HLA antibodies. In this study, we determined if the recipient diagnosed as non-HLA antibody mediated rejection is the CCR5 Delta 32 allele homozygous, hence could develop antibody against CCR5 which the donor carries. **Methods:** 11 heart transplant patients were diagnosed with AMR based on biopsy, but no donor specific HLA antibodies were detected. We performed CCR5 genotyping on these recipients and their donors by polymerase chain reaction (PCR). PCR primers (Forward (5'- AGGTACCTGGGCTGTC GTCCCCCA; Reverse 5'CTCACAGCCCTGTGCCTCTTC) were used to amplify the wild type alleles (329 bp) and the null allele CCR5Delta 32(297 bp). **Results:** Out of 11 patients, one patient was heterozygous at the CCR5 locus, carrying the wildtype allele and null allele CCR5Delta32. Out of 10 donors, 3 donors were heterozygous. PCR reaction failed for one donor sample due to the insufficient DNA. Neither the patients or donors were homozygous for a null allele CCR5Delta32. The frequency of the CCR5Delta 32 allele between the patient and donor were not significantly different.

	CCR5 wt	CCR5 wt/del
Recipient	10	1
Donor	7	3

Conclusions: The frequencyof CCR5Delta32 in this cohort is about 20%, which is similar to the previous finding. No patients in this cohort are homozygous for the null allele CCR5Delta 2, suggesting the patient cannot develop antibody against CCR5, and AMR in these patients are not caused by CCR5 antibody.

P122

TO EDTA OR NOT TO EDTA - THAT SHOULD NOT BE THE QUESTION

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Aim: To systematically assess the effectiveness of EDTA in unmasking clinically relevant HLA alloantibodies **Methods:** Serum samples from transplant (txp) patients collected for clinical testing were pre-treated with various concentrations of EDTA for 15 min at room temperature and then tested with Luminex-based solid phase assays using HLA mixed beads (HMB) and Single antigen beads (SAB) for the presence and identification, respectively, of HLA alloantibodies. **Results:** Utility of pretreating sera with heat inactivation, dithiothreitol and/or EDTA to overcome prozone-like inhibition (PZLI) has been demonstrated. However, lack of details vis-à-vis time and concentration used, prompted us to systematically assess the effectiveness of EDTA in unmasking presence of clinically relevant HLA alloantibodies in serum samples with PZLI. Titration analysis using EDTA concentrations ranging from 0 - 50mM indicated that 1.25mM EDTA was ineffective, while 2.5mM EDTA was moderately effective, in removing PZLI. EDTA concentration =/> 5mM was effective in removing PZLI in all serum samples. Therefore, we selected 5mM EDTA for all subsequent studies. We observed that 5mM EDTA did not give rise to high background and/or false positives, *i.e.*, no unmasking of "new" specificities in serum samples with cPRA = 0% or in serum samples that demonstrated presence of limited HLA alloantibody specificities with MFI < 10,000. Most common HLA antibody specificities unmasked were anti-A1, -A2 (and associated CREGs), -DR53, and -DQ. EDTA treatment also removed non-specific reactivity observed in HMB and SAB. In the pre-txp setting, EDTA unmasked presence of DSAs that can cause positive flow cytometric crossmatch. This enabled a better correlation with virtual crossmatch calls. In the post-txp setting, EDTA aided in unmasking *de novo* DSA associated with episodes of rejection allowing a stronger clinical correlation with histological detection of rejection. **Conclusions:** The PZLI phenomenon observed due to presence of activated complement components in sera, challenges the ability of HLA labs to accurately identify HLA alloantibodies. Our study underscores the utility of treating serum with EDTA in identifying pre-formed and *de novo* DSAs that would be deleterious to the allograft in txp recipients.

P123

ANTI-A TITER METHOD COMPARISON

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Aim: To determine an appropriate testing method to evaluate the anti-A titer in blood group B renal transplant waitlist patients to qualify for consideration of blood group A_2 (A subgroup) donors. UNOS has established a maximum anti-A titer of 8 to qualify for an A to B kidney. **Methods:** A review of methods in use at transplant centers performing ABO incompatible kidney transplants identified four categories of method variation. The four categories were:

- Dithiothreitol (DTT) treatment of serum vs. no DTT treatment
- Direct agglutination vs. Indirect agglutination (anti-human globulin enhancement)
    - Direct = Room Temperature Test Tube or MTS-Gel buffer card
    - Indirect = Anti-IgG Test Tube or MTS-Gel anti-IgG card
- Test Tube method vs. MTS-Gel microcolumn method
- A_1 target cells vs. A_2 target cells

Standard commercial blood bank reagents were used for all testing. The same technologist performed all testing. Ten blood group B patients from the kidney transplant waitlist were tested using 8 different methods. After choosing the preferred variable for each of the first three categories, 15 group B potential recipients were tested comparing A_1 vs. A_2 target cells. **Results:**

Method (all methods used A1 target cells)	Percent anti-A Titers ≤ 8
DTT - Direct - Test Tube	90%
DTT - Indirect - Test Tube	90%
DTT - Direct - MTS Gel	90%
DTT - Indirect - MTS Gel	60%
No DTT - Direct - Test Tube	30%
No DTT - Indirect - Test Tube	22% (1 sample QNS)
No DTT - Direct - MTS Gel	10%
No DTT - Indirect - MTS Gel	20%

The categories of Test Tube vs. MTS-Gel and Direct vs. Indirect agglutination had little impact on titers. Pretreatment of serum with DTT provided qualifying titers for three times as many patients as using serum not treated with DTT. DTT pretreatment of serum was chosen along with the indirect agglutination method to optimize detection of IgG anti-A. Using the DTT - Indirect - MTS Gel titer method, comparing A_1 vs. A_2 target cells with 15 samples showed the choice of target cells also impacted titers. 8 of 15 patients had a 3-fold or greater titer increase using A_1 cells compared to A_2 cells. 11 of 15 patients (73%) had a qualifying titer with A_1 cells, compared to 100% with A_2 cells. **Conclusions:** Use of DTT treated serum and A_2 cells were associated with the lowest anti-A titers. Efforts to establish acceptable method specific titer limits are encouraged.

P124

BEST TARGET FOR ANTI-A TITER DETERMINATION: A1 OR A2 CELLS

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Aim: To determine the appropriate target cell for assessment of anti-A titer in blood group B kidney transplant patients waiting for a deceased donor and qualify for blood group A_2 (A subgroup) donors according to OPTN/UNOS guidelines. **Methods:** 248 sera samples collected from 57 blood group B candidates (56% male, 79% African American) and tested after DTT treatment. Titers were done using A_1 and A_2 reagent target cells using the

MTS Gel anti-IgG card method. Standard commercial blood bank reagents were used for all testing (2 donors per lot of A_1 or A_2 cells). The same technologist performed all titers. The titer for eligibility is ≤ 8. **Results:** 37 patients were eligible (titer ≤ 8) with either A_1 or A_2 cells. 20 patients (35%) shown below had titers from 16 up to 128 with A_1 cells. 2(4%) patients had a titer >8 with A_2 cells. Of these 20 patients, the cPRA varied from 0-100% and was not correlated with anti-A titer. Sex and race was also not correlated with an anti-A titer ≥8.

Blood Group B Candidates with an Anti-A Titer ≥8

Patient	# Sera tested with A_1 cells	Titer with A_1 cells	# Sera tested with A_2 cells	Titer with A_2 Cells
A	**5**	**≥16,8,4,≥16,16**	**4**	**<2,<2,<2,<2**
B	**4**	**≥16,≥16,≥16,128**	**5**	**8,8,8,16,4**
C	**7**	**8,8,4,4,4,8,32**	**4**	**≥16,8,<2,4**
D	**4**	**≥16,≥16,≥16,128**	**1**	**4**
E	**3**	**≥16,≥16,64**	**3**	**2,<2,2**
F	**3**	**≥16,≥16,≥16**	**3**	**2,2,<2,**
G	**2**	**≥16,64**	**3**	**8,8,4**
H	**4**	**≥16,≥16,≥16,64**	**4**	**<2,2,<2,4**
I	**4**	**8,≥16,≥16,8**	**1**	**<2**
J	**3**	**≥16,8,≥16**	**4**	**<2,<2,<2,<2**
K	**3**	**≥16,≥16,128**	**1**	**8**
L	**1**	**32**	**2**	**8,2**
M	**2**	**≥16,128**	**1**	**8**
N	**3**	**≥16,≥16,32**	**3**	**2,<2,<2**
O	**4**	**≥16,8,≥16,≥16**	**3**	**<2,<2,<2**
P	**3**	**≥16,≥16,≥16**	**2**	**<2,<2**
Q	**1**	**16**	**4**	**2,<2,2,<2**
R	**2**	**≥16,≥16**	**1**	**2**
S	**3**	**≥16,≥16,32**	**4**	**<2,<2,<2,<2**
T	**6**	**8,8,≥16,≥16,≥16,32**	**1**	**4**

Conclusions: Patient eligibility may vary considerably depending on the target cell used. Additional clinical studies will be required to determine the optimum approach.

P125

HLA ALLELE SPECIFIC ANTIBODIES: WHAT SHOULD BE ENTERED IN UNET, ANTIGEN, ALLELE OR EPITOPE (case report)?

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Aim: Identification of donor-specific antibodies (DSA) represents one of the major functions of the HLA laboratory. Pre-existing DSAs are known to be important in organ allocation, while *de novo* developed DSA contribute to antibody (Ab) mediated rejection. Advances in solid-phase antibody analysis (SAA) now allow for the detection of HLA allele-specific Ab. The aim of this study was to examine HLA-A locus specific Ab in a kidney transplant candidate (TC). **Methods:** A 64-yo male patient (typed A*02:xx, 26:xx) was evaluated for a deceased donor kidney transplant. Two previous heart transplant (in 1986 and 2005) donors typed A1, A31 and A3, A31. HLA-A locus low

and high resolution typing was performed using PCR-SSOP method (One Lambda Inc.), and sequence based typing (SBT) (GeneDX), respectively. Ab analysis was performed using SAA (One Lambda Inc.). Epitope analysis of HLA to which TC had Ab was conducted using HLAMatchmaker computer algorithm. **Results:** Ab reactivity and antigen epitope analysis are presented in Table 1. Notably, TC had strongly reactive Ab against A*26:01. Subsequent SBT of TC at A locus revealed presence of A*02:01 and A*26:09 alleles. The latter has a very low frequency in the US population. An epitope analysis of HLA-A locus Ab revealed that all positively-reactive HLA-A locus alleles share epitope 163R. Thus, we faced two questions. First, how should the TC's typing at A locus be entered in Unet? Second, what antigens/alleles should be listed as unacceptable? **Conclusions:** The TC's A locus typing was entered in Unet as A*02:01/02:01 (considering changing A locus typing of the recipient to A2/A26 after kidney transplantation), and the list of unacceptable antigens included A1, A43, A25, A*66:01, A*11:01, A*11:02, A26 (considering that all A26 donors will most likely have A*26:01 allele). The results of this study present a new insight in organ allocation and post-transplant Ab monitoring, i.e. entering epitopes/eplets shared between unacceptable alleles (in this case 163R) instead of unacceptable UNOS antigens.

Epitope analysis of HLA-A locus alleles to which TC developed antibodies

Allele	MFI	Ab verified eplets
A*01:01 (immunogen)	20691	163R
A*43:01	19251	163R
A*25:01	17191	163R
A*66:01	14952	163R
A*11:02	13821	163R
A*26:01	12482	163R
A*11:01	11632	163R
A66:02	363	Negative, MFI=400 was used as a cut off

P126

COMPARISON STUDY OF CDC AND FLOW CROSSMATCH VS. DONOR SPECIFIC ANTIBODIES IN TRANSPLANTATION

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Aim: Our approach to transplant highly sensitized patients has been to perform CDC crossmatch (XM) when the results of flow xm are above 200 mean channel shifts(MCS) with donor specific antibodies (DSA). The aim of our study was to determine if the flow crossmatch and solid phase antibody testing can be used as the only measures for decisions to transplant **Methods:** Results of flow xm, CDC xm and solid phase DSA testing were analyzed for 44 donor and recipient pairs. In all cases, the flow xm were positive with greater than 200 MCS values. Undiluted patient sera were tested by Luminex single antigen (LSA) test for HLA antibodies (ABS). The ABS detected by this method were grouped into weak (MFI 2500-5000) moderate (MFI 5000-7500), and strong (MFI 7500-greater than 17000). The CDC xm was performed with and without DTT treatment using the same sera for flow xm. **Results:** In 70% of this group with positive T and/or B CDC xm at least one DSA detected was in the strong binding range above 17,000 MFI. In 30% of cases multiple DSA detected in weak to moderate range below 7500 MFI values. In 50% of neg T cell and 96% of neg B cell CDC crossmatches, the MFI of DSA detected was in moderate to strong range. In 90% of negative CDC xms the MCS values for flow T was below 300 and flow B below 400 MCS. The range of flow MFI values for positive CDC B cells xm was 156-596 and 238-671 for T cells. **Conclusions:** While the CDC xm test is the classical standard to exclude transplant, it is not always a reliable test. This assay is quite subjective and dependent on quality and strength of complement, technical skills of the laboratory technician and

donor cell viability. This assay is also not reliable when patient is under desensitization therapy. There is a need to develop this assay using flow cytometer platform. Use of solid phase testing methods and determination of standards for decision to transplant based on number, type and strength of antibodies can be a better method

Summary of results					
		POS	FLOW	T	
	N	AVE MCS	MCS RANGE	DSA RANGE MFI	NO OF DSA
NEG CDC	26	178	70-292	2000-12500	1-2
POS CDC	19	347	156-596	2000-17500	1-4
		POS	FLOW	B	
NEG CDC	24	296	160-482	2500-17000	1-2
POS CDC	29	378	238-671	2500-17000	1-4

P127

TRANSFER OF FLOW CYTOMETRIC CROSSMATCH DATA FROM THE BD FACSCANTO II INTO THE HISTOTRAC DATABASE

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Aim: Flow Cytometric Crossmatch (XM) is critical to the work-up of renal transplant patients. The complexity of the data acquired in XM testing and the amount of manual data entry into reporting software is a challenge. Testing personnel are under pressure to provide timely, accurate, and reliable results. The development of the HistoTrac Canto Utility has enabled fast and accurate calculation and the automated transfer of data. **Methods:** The HistoTrac Canto Utility was developed by the TriCore IT team to reduce data entry errors when transferring data from the BD FACSCanto II to HistoTrac. The utility was developed using the computer language C#, and built on a Windows Presentation Foundation app foundation. The Utility imports a CSV file from the FACSCanto and generates an XLS file that contains all laboratory established control ranges and standard deviations (SD) which can be updated as needed. The Utility performs the Calculations by picking the lowest MDCF value of the Negative controls, places it in the “Neg used for Calculation” cell, and generates the MCS value and shift results for each patient serum and controls. If all Negative controls are below the lab defined negative range, the lowest patient sample is used for calculations, and the XM is given an assessment of “**ERROR**” instead of “**VALID.**” Assessment of XM validity is dependent on the Positive control MCS value, which must be higher than 3 SD for both T and B cell in order for the XM to be “**VALID**”. The Utility will use the higher MDCF values (T and B cell) of the patient duplicates for calculations. Currently T cell duplicates must be within 6% and B cell within 10% of each other. If duplicates are not within these ranges, an assessment of “**BAD**” is made. The XLS is then imported into Histotrac using the "Histotrac Interface Canto Utility". **Results:** The HistoTrac Canto Utility aids in assessing XM results by selecting the lowest negative control and using that data to calculate the shifts. Using the established Control Ranges and SDs, the Utility is able to assign a result and assess quality assurance metrics such as sample duplication, negative control evaluation and XM validity. **Conclusions:** The HistoTrac Canto Utility eliminates manual data entry errors in the shift calculations, and reduces testing turnaround times.

P128

IDENTIFICATION OF NOVEL A24 ALLELE WITHOUT SUBCLONING

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Aim: To identify the mutation in one of the alleles at HLA-A locus in African American kidney transplant recipient. **Methods:** Initial evaluation was done using OneLambda's PCR-SSOP. Sequencing kits from GenDx's AlleleSEQR (HLA-A) and Protrans' Domino Stones 2.0 (HLA-A4) were used to sequence the DNA. GenDx SBT Engine Software was used for sequencing data analysis. BLAST search was performed using IMGT database. Allele specific re-sequencing was done using primers tailed with M13 sequence. The 5` primer was located in the second exon (240-257) and 3` primer (540-557) was located in the 2nd intron. **Results:** PCR-SSOP listed A*23:01 allele in the possible genotypes, but final typing was inconclusive ("No Match"). Exclusion of false positive reacting beads resulted in A*23:01 homozygous typing. Olerup's PCR-SSP confirmed presence of A*23:01 allele. However, SBT Engine indicated presence of potentially new allele. Knowledge of A*23:01 sequence helped in discriminating the known (A*23:01) from the unknown A*New heterozygote at exons 1-4. A*23:01 and A* New were identical at exons 1, 3 and 4. BLAST search of A*New allele gave lowest score/highest similarity with A*24:24. Data from direct sequencing of A*24:New allele (M13 primers) identified a base substitution at 282 position (codon 70), G->C. This mutation leads to amino acid change from Glutamine (CAG) to Histidine (CAC). The sequence of novel HLA-A*24 allele was submitted to IMGT/HLA database and designated HLA-A*24:392 by the WHO Nomenclature Committee in December, 2017. GenBank Database assigned Accession Number BankIt 2058784 RBHLA MG463105. Thus, specimen's HLA typing is HLA-A*23:01, A*24:392; B*07:02, 50:01 and HLA-DRB1*08:06, 09:01. **Conclusions:** Three-dimensional structure analysis of the HLA protein (Fig.1) encoded by A*24:392 showed that it is likely that mutation at codon 70 (α-helical coil) is involved in peptide binding, and mismatched 70HT epitope can elicit antibody production.

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FLEXMAP 3D® GENERATES A COMPARABLE RESULT FOR LIFECODES® LSA SINGLE ANTIGEN PRODUCTS WITH RESPECT TO LUMINEX® 200 PLATFORM

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Aim: The objective was to demonstrate equivalency of qualitative results among the LIFECODES LSA Single Antigen products performed on two different Luminex Instrument Platforms: The Luminex 200 and the FLEXMAP 3D. **Methods:** For each LIFECODES LSA Single Antigen Assay (LSA 1 and LSA 2), a panel of 32 samples was selected. Samples were chosen based on previously characterized reactivity to either HLA Class I or HLA Class II antigens. For the first incubation of the assay, replicate samples were pooled together (sera + beads) in sufficient volume to be subsequently divided and distributed to the designated wells to be run on the different instrument platforms. This was done to minimize any variation between the replicates that may occur during the initial incubation. For each LSA assay, samples were tested side by side on both the Luminex 200 Instrument and on the FLEXMAP 3D Instrument using the "Standard" reporter gain setting. Because the FLEXMAP 3D produces higher MFI values, an MFI divider was applied to obtain MFI equivalent to the Luminex 200. To identify the appropriate divider, a data simulation was performed by applying a variety of MFI Dividers with values in the range of 1.00 to 2.00. For each MFI Divider simulated, the sum of the MFI values for each sample was compared to the sum of the MFIs for the same sample run on the Luminex 200. A two-tailed T-test was then used to determine which simulated MFI divisor provided the most equivalent data points between the FLEXMAP 3D and LMX200 platforms. In a

follow-up experiment, an assay was performed on FLEXMAP 3D by incorporating the most equivalent divider value. **Results:** Over the evaluated MFI divider range of 1.0 to 2.00, a value of 1.67 gave the maximum p-value when compared to data generated by the Luminex 200 Instrument. A 2x2 analysis of antigen assignments for LSA1 & LSA2 show an overall concordance of > 98% between FLEXMAP 3D (with a divider value of 1.67) and Luminex 200. **Conclusions:** This study with 32 samples suggests that comparable results can be obtained between the Luminex 200 and FLEXMAP 3D instrument platforms when a divider value of 1.67 is applied to the MFIs generated by the FLEXMAP 3D.
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RAPID VIRTUAL CROSSMATCH ASSESSMENT USING HISTOTRAC DATABASE

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Aim: The aim of this study was to develop a Virtual Crossmatch (VXM) tool utilizing our HLA database program Histotrac. The goal was to quickly visualize a patient's current and historical anti-HLA antibody history, PRA, HLA match, sensitizing events and most recent serum. Following a director's interpretation the program will order the test and generate a clinical report for distribution to the requesting transplant professional. **Methods:** For this study we used our labs database system Histotrac. A customized program was generated based on the several factors we requested needed to be visualized in order to make a virtual crossmatch assessment. Flow PRA and single antigen bead testing were performed using One Lambda reagents and analyzed using Fusion software. All current and historical anti-HLA antibody data from Kidney, Heart, and Lung patients were sent from Fusion to Histotrac database thru a direct interface. Local donors were HLA typed by RTPCR using SABR kits. For imports, donor HLA typing was reviewed in DonorNet including the attached HLA typing report. All donor HLA data was entered into Histotrac and required to run the VXM tool. **Results:** Our lab receives on average 250 VXM per month requiring an hour to complete each one from initial request to report. The VXM tool displays the current DSA MFI and searches all historical samples to identity the peak MFI for each DSA >1000 MFI. In validation, the VXM tool was refined to be 100% accurate compared to manual review. The VXM tool was able to decrease this time to 30 mins per VXM and 15 mins if the donor HLA typing was added in advance. With our transplant centers running VXM on 5 patients per donor this could be completed in 30 mins saving an average of 2hrs 30 min. **Conclusions:** Virtual Crossmatch has become a widely utilized method for assessing donor-recipient compatibility in the absence of a physical crossmatch. We have developed a VXM tool that is capable of rapidly assessing a patient's current and historic anti-HLA antibody history compared to a donor HLA typing. This procedure is able to significantly decrease the on call time required by the lab from initial request to virtual crossmatch report sent out. Furthermore, this shortens the time to where transplant coordinators and physicians receiving this information can assess donor organ offers and living donor paired exchanges.

P131

USING DTT AS A TOOL TO REMOVE BACKGROUND IN HLA ANTIBODY IDENTIFICATION

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Aim: The goal of this study is to investigate the effectiveness of DTT as a tool to remove excessive IgM, in Single Antigen Bead assays, which can lead to high negative control values. **Methods:** In the present study, nine sera (class I) and seven sera (class II), with NC MFIs ≥1,000 MFI, were tested using One Lambda SAB Assay, neat vs. DTT, Adsorb-out, Adsorb-out x 2 and DTT & Adsorb-out. Sera were tested using the manufacturer's protocol. Sera were DTT treated by combining 5µL 50mM DTT to 195µL sera. This mixture incubated at 37°C for 15 min. All sera were pretreated with EDTA. For this study, an elevated NC is defined as an MFI ≥1,000 (two sera demonstrated borderline positivity (953 & 816)). **Results:** With class I, all but one DTT treated serum, demonstrated a decrease in NC MFI. The outlier serum NC MFI demonstrated a decrease but was slightly above acceptable level (1,790 MFI → 1,083 MFI). The sera were also treated with Adsorb-out, with all resulting NC MFIs >1,000. Additionally, eight NC

MFIs either did not decrease or increased following Adsorb-out. These sera were treated either with Adsorb-out x2 or DTT & Adsorb-out. Four sera continued to have NC MFIs >1,000. Regarding class II DTT treated sera, all but two showed a NC MFI decrease. Of those two, both demonstrated a decrease vs. neat but not <1,000 MFI. Following treatment with Adsorb-out, all NC MFIs were >1,000 and three showed an increase. The sera were treated with Adsorb-out x 2 or DTT & Adsorb-out. Of these sera, two achieved acceptable levels. **Conclusions:** DTT is a commonly used reducing reagent which is efficient at cleaving exposed disulfide bonds, thus eliminating IgM-based interference. This treatment, in regards to SAB testing, is performed to remove interfering IgM molecules. DTT is also able to obviate C1 interference but does so poorly. EDTA prevents complement interference far more efficient through Ca^{2+} chelation. Based on our results, we will further investigate the use of DTT as an effective treatment on sera with a high NC MFI which are not amendable to a single treatment of Adsorb-out.

NC MFIs											
class I						class II					
NC neat	NC Trt (1)	Trt (1)	NC Trt (2)	Trt (2)	NC DTT	NC neat	NC Trt (1)	Trt (1)	NC Trt (2)	Trt (2)	NC DTT
1,856	4,406	adsorb			106	4,092	6,095	adsorb			167
2,919	3,883	adsorb	621	DTT & adsorb	925	6,440	3,929	adsorb	1,256	DTT & adsorb	2,499
1,555	1,663	adsorb	1,604	adsorb	498	2,503	1,794	adsorb			696
953	1,225	adsorb	1,204	adsorbx2	130	1,012	1,160	adsorb	1,167	adsorbx2	181
1,790	1,091	adsorb			1,083	1,810	1,142	adsorb	5,246	adsorbx2	1,209
2,167	3,509	adsorb	1,152	adsorb	561	140					481
816	2,480	adsorb			529	60					426
3,414			22	adsorbx2	656	5,968			16	adsorbx2	660
24,714	25,529	adsorb	24	DTT & adsorb	16	24,895	28,962	adsorb	61	DTT & adsorb	21

P132

IMPROVING PRE-KIDNEY TRANSPLANTATION LABORATORY UTILIZATION IN AN ERA OF INCREASING WAITLIST SIZE

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Aim: Beginning on March 25, 2015, our institution's kidney transplant program has implemented a "readiness" protocol, whereby patients are listed after limited workup and comprehensive testing is deferred until the anticipated time to transplantation is approximately 6-12 months. Prior to implementation of this protocol, our histocompatibility laboratory would receive monthly sera for anti-HLA antibody screening immediately upon listing. However, as part of the readiness strategy, the lab now requests monthly samples only when a candidate is predicted to have 6-12 months of waitlist time remaining. The monthly samples are stored and screened in the lab per protocol (every 3 months for most patients). All candidates are also screened once prior to listing and again immediately after listing. **Methods:** Data from our laboratory database and UNOS were analyzed to determine the number of samples received from waitlisted kidney transplant candidates and the number tested in the three-year period before and after implementation of the readiness protocol. **Results:** Our kidney transplant waitlist has grown from 379 candidates on 7/1/2012 to 948 as of 4/5/2018. Despite this rapid growth, the number of samples received from kidney transplant candidates has decreased from 9171 during the three-year period prior to the implementation of readiness (~3057/year) to 7924 during the subsequent three-year period (~2641/year). Based on a sample

submission rate of ~7 samples/patient/year, an estimated 4961 samples per year would have been received without the readiness protocol, with an estimated 6669 samples expected for 2018. From 3/25/12-3/24/15, 4730 antibody screening tests on kidney waitlist candidates were performed by Luminex (~1577 tests/year), compared to a slight increase to 5082 from 3/25/15-3/24/18 (~1694 tests/year), despite a 2.5-fold increase in patients on the waitlist from 2012-2018. **Conclusions:** Implementation of the readiness protocol has streamlined the pre-transplant testing process and reduced both the number of samples received and the number tested. This has allowed us to manage a greatly increased waitlist with the same level of resources and has resulted in reduced cost per patient.

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A REAPPRAISAL OF THE IMPACT OF HEAT INACTIVATION OF KIDNEY GRAFT RECIPIENT'S SERUM ON DONOR T CELL AND B CELL CDC CROSSMATCHES: AN ANALYSIS OF 22175 CONSECUTIVE CROSSMATCHES PERFORMED BY A SINGLE HISTOCOMPATIBILITY LABORATORY

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Aim: Interfering factors in sera may confound crossmatch (XM) results. Heat inactivation (HI), DTT and dialysis treatment of sera have been utilized to minimize these confounders. We investigated the impact of HI on donor T cell complement dependent cytotoxicity (CDC) XM (T Cell AHG XM) and donor B cell CDC XM (B Cell NIH XM) **Methods:** We queried our electronic database for all donor CDC T cell and B cell XM performed during 2011-2017 in which potential kidney transplant recipient's serum was tested in parallel with or without HI and using potential donor's T cells and B cells as target cells. **Results:** A total of 6295 living donor XMs and 15880 deceased donor XMs performed in our laboratory were reviewed. Table 1 shows the impact of HI on the 22175 consecutive XM results. 394 of the 6295 living donor T Cell AHG XMs were positive using neat sera and 303 were positive following HI at 63C for 6 min, a 23% conversion from positive to negative results. 698 of the 15880 deceased donor T Cell AHG XMs were positive using neat sera and 540 XMs were positive following HI, a 23% reduction. 1372 of the 6295 living donor B Cell NIH XMs were positive using neat sera and 539 were positive following HI, a 61% reduction. 3015 of the 15880 deceased donor B Cell NIH XMs were positive using neat sera and 842 XMs were positive following HI, a 72% reduction from a positive result to negative result.

Conclusions: A substantial percentage of XMs are converted from a positive CDC result to negative result and such conversion is more frequent with B cell XM compared to T cell XM. Transplant centers seldom transplant across a positive T cell CDC XM even when HI converts a positive result to a negative result. On the other hand, it is not uncommon to proceed with a kidney transplant when HI converts a positive B cell CDC to a negative CDC provided there are no other immune contraindications. There is an urgent need to establish the clinical significance of XMs converted from a positive to a negative result by heat inactivation.

P134

ANTIBODIES TO INTER-LOCUS SHARED DR11 AND DP EPITOPE: CHARACTERIZATION AND ANALYSIS CONSIDERATION

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Aim: Antibodies (AB) to shared HLA epitopes can result in a single sensitizing event conferring incompatibility with a large percentage of donors. In addition, apparent AB strength on Luminex single antigen bead (SAB) assays can be artificially lowered, impacting virtual crossmatch (XM) assessment. "DEE" and "DED" epitopes at position 55-57 on DPB1 are known to be immunogenic. Our goal was to further characterize these AB in our patient population, with an interest in the "DEE" epitope present at position 57-59 on DRB1. **Methods:** Patients with DP AB to either DPB1*04:02 (DEE) or DP14 (DED) were queried, and SAB results reviewed for reactivity to known immunogenic DP epitopes (DED - DP3, 6, 9, 14, 17, 20; DEE - DP2, 04:02, 10, 18, 28). AB to DR antigens were also noted, as well as patient HLA typing. To confirm AB cross-reactivity to shared sequence, a serum with AB to multiple DP epitopes (DPA1*02/04, DED/DEE, and DEAV) was adsorbed using lymphocytes containing just the DEE epitope. **Results:** Patients who lacked DED and DEE on their cells formed an AB of combined specificity: 35 to DED/DEE, and another 25 to DED/DEE plus other DP antigens. Of 8 patients with isolated DEE DP AB, typing identified DED epitope in 5 (no typing available for 2); of 7 patients with isolated DED DP AB, typing identified DEE epitope in 3 (no typing available for remaining 4). DR11 AB were co-present in 60 of 68 patients containing DEE DP AB (with or without other specificities); of the patients without DR11 AB for whom typing was available, all 6 typed as DR11. None of the 7 patients with isolated DED AB had DR11 AB, only 2 of whom typed as DR11. Eluate from the adsorption study reacted exclusively with DED and DEE DP antigens as well as DR11. **Conclusions:** DED and DEE are immunogenic epitopes on HLA-DP, although specificity can be to either or both depending on self-antigens. DEE and combined DED/DEE antibodies (but not DED) cross-react with DR11 due to a shared epitope. However, if DR11 is a self-antigen it's possible to form DP-exclusive DEE AB, confirming that DR-specific sequences outside the "DEE" epitope are important. AB to shared epitopes are important to recognize because they can cause positive flow XM despite low MFI on SAB.

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LIVING DONOR KIDNEY PAIRED DONATION TRANSPLANTATION: FIRST REPORT FROM KINGDOM OF SAUDI ARABIA

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Aim: Kidney paired donation (KPD) is an effective way to transplant patients with willing but incompatible donors. We report our experience with KPD through development of local Registry for incompatible pairs. **Methods:** 62 recipients & their incompatible donors plus 3 non-directed donors were entered into KPD registry. Incompatibility was due to ABO-incompatibility (ABOi) in 25% & HLA-incompatibility (HLAi) in 75%. Patients with HLAi had cPRA between 60-99%. Antibodies with MFI of ≥ 3000 by SAB assay were listed as unacceptable antigens. We used balanced, unbalanced & non-directed anonymous donors (NDAD). ABOi donors were accepted if patients had low titer (≤ 16) of Isohemagglutinins. Amenable DSA for desensitization are those weak, single or multiple that yielded negative or weakly positive flow cross match (< 125 MCS for T&B- FXM). 5 patients had positive B-FXM due to weak DSA. Alliance for Paired Donation (APD) software was the platform used for virtual cross match. **Results:** 23 candidates (37%) were transplanted, enabling pre-emptive transplant in 1 patient. The longest chain was seven -ways domino chain initiated by a NDAD & ended in transplant of deceased donor listed patient with multiple access failure. Additional desensitization was used for 9 patients due to DSA and/ or positive FXM. 1 patient received TPE due to anti ABO antibody titer of 16. We had one case of DGF, AMR occurred in 2 patients & 1 patient had ACR. Graft function was good through follow-up time of 6 -18 months but one patient died 8 months post-transplant due to refractory sepsis. **Conclusions:** Although our series is early with 62 patients, transplant rate is excellent compared to other old programs(Netherlands, UK, & Canada) where the transplant rate were 37%, 29%, 44% respectively. This might be attributed to involving NDAD, accepting ABOi & combining desensitization for low level DSA in selected patients. Considering that 75% of the patients were highly sensitized yet received living transplants with excellent results, our experience demonstrates promising short-term outcomes. However, longer follow-up is needed to assess the impact of KPD on organ shortage & the outcome of highly sensitized patients transplanted through KPD program.

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SUCCESSFUL ADEPTNESS OF DONOR EXCHANGE PROGRAM IN RENAL TRANSPLANTATION AT KING FAISAL SPECIALIST HOSPITAL JEDDAH

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Aim: Different methodologies were established to overcome HLA immunological barriers to match the highly increasing number of patients with end-stage renal disease in Saudi Arabia, which is currently ranged between 120 to 150 patients per million per annum. At our hospital, King Faisal Specialist Hospital Jeddah, we therefore successfully established the desensitization and ABOi renal transplantation. Recently we have started to use a donor exchange program. We hereby report about our experience to overcome the immunological incompatibilities by using this program. **Methods:** We used an IT program with high grade on flexibilities by performing different options including simple two-pair exchanges, more complicated domino exchanges and chain donations. All recipients with willing donors and with high titer HLA antibodies and/or strong positive cross match were included in the program. HLA typing was performed by using One Lambda SSOr and SSP. HLA Antibody Identification, Single Antigen Class I/II and C1q test, was performed by One Lambda. T and B cell IgG XM is performed by FACSCanto II flow cytometer, where the cut-off for positive XM is determined based on normal human studies. **Results:** 20 patients were transplanted successfully by using the donor exchange program within a period of 12 months. Considering a total number of 220 transplanted kidneys during this time, which makes our hospital as the largest center for renal transplantation in Saudi Arabia, we were able to increase the number of transplanted organs significantly by 9% and we continue to extend and optimize our histocompatibility services at the hospital. **Conclusions:** Our experience demonstrates the high efficiency of the donor exchange program in overcoming immunological barriers in renal transplantation. This program provides in some cases also an alternative to the invasive and costly desensitization protocols. Further efforts and thoughts are going on to include deseased donors as well in this successful donor exchange program.

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GO PRO(NASE)! THE IMPORTANCE OF DONOR LYMPHOCYTE TREATMENT WITH PRONASE IN FLOW CYTOMETRIC CROSSMATCH TESTING.

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Aim: Flow cytometric crossmatch (FCXM) is performed to confirm donor/recipient histocompatibility. Previous multicenter study (Liwski et al. ASHI 2012) suggested that protocol differences contribute to FCXM result variability and that pronase treatment improves B cell FCXM. In this study we assess the impact of pronase on FCXM results. **Methods:** Donor lymphocytes, isolated from spleens/lymph nodes, were treated with pronase (4.7 U/ml) or saline. FCXM were performed at Santa Casa Lab in Porto Alegre using the Halifax protocol, acquired on BD Canto II and analyzed using a median channel fluorescence (MCF; 1024 channel) scale. 3SD cutoffs were determined by testing neg ctrl (NC) sera against 63 donor cells. Pos ctrl (PC) sera were used in each FCXM. A total of 240 FCXM (122 predicted neg; 118 predicted pos) were performed in parallel using pronase treated vs untreated cells. Pos/Neg FCXMs were predicted based on LABScreen single antigen bead testing with a 1,000 MFI cutoff. **Results:** NC serum testing shows that pronase significantly reduced B cell background reactivity (pronase MCF = 195+/-32 vs untreated MCF = 363+/-89, $p < 0.001$; Fig.1). PC reactivity was not affected by pronase treatment for T and B cell FCXM (Fig.1). Thus, pronase improved B cell FCXM signal to noise ratio. The false positive FCXM rate was similar with pronase vs untreated cells (1.3 vs 0.7% for T cell and 5.7 vs 4.9% for B cells; Fig.2). Pronase treatment had no effect on FCXM specificity (98.7 vs 99.2% for T cell and 94.3 vs 95.1% for B cell; Fig.3) However, B cell FCXM sensitivity was greatly improved by pronase (74.6 vs 26.3%; Fig.4). False neg B cell FCXM rate (T+/B-) was reduced by pronase from 60% to 0%. A slight improvement in sensitivity was also seen with pronase in T cell FCXM (61.5 vs 70.3). **Conclusions:** B cell FCXM sensitivity is unacceptably low (26.3%) when untreated cell are used. Pronase treatment greatly improves B cell FCXM sensitivity (74.6%) and decreases the rate of false negative B cell reactions by reducing the background and improving signal to noise ratio.

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CLINICAL CHARACTERISTICS OF PATIENTS WHO MANIFEST UNEXPLAINED CROSSMATCH RESULTS FOR SOLID ORGAN TRANSPLANTATION

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Aim: The flow cytometric crossmatch (FCXM) and the virtual crossmatch (VCXM) assess compatibility between solid organ donors and recipients. Theoretically, both methods should give the same results. Realistically, however, they can be discrepant due to limitations of assays used to detect alloantibodies, non-HLA alloantibodies, autoantibodies, and non-specific reactivity. We sought to identify clinical features of patients who manifest such discrepancies, hypothesizing an association with female gender, autoimmune disease, and potential heart recipients. **Methods:** We retrospectively evaluated all living and deceased donor FCXMs (performed using BD FacsCanto flow cytometer, BD Biosciences, San Jose, CA) for solid organ transplant offers for patients at University of California San Diego from 2015-2017. Based on previous validation studies, a positive FCXM was coded as "expected" when a single donor-specific HLA antibody (detected using LABScreen single antigen assay, One Lambda, Canoga Park, CA) demonstrated a mean fluorescent intensity of 3,000 or higher, or when the sum of multiple antibodies reached that threshold. Deceased donor typing was performed using LinkSeq real-time polymerase chain reaction kit (Linkage Biosciences, South San Francisco, CA). Living donor HLA genotyping was performed by SSO or NGS-based methods. FCXMs with unexpected positive results were investigated. **Results:** 1594 FCXMs (1369 for kidney, 139 for heart, 47 for lung, and 39 for multiple organs) for 967 potential recipients (621 males, 346 females) were analyzed. Unexpected positive results occurred in 109 T cell FCXMs in 81 patients (8%) and 40 B cell FCXMs in 23 patients (2%). Analysis by both crossmatch and patient demonstrated correlations with female gender (T cell $p<0.01$; B cell $p<0.02$) but not transplant organ. The diagnoses most commonly associated with unexpected positive FCXMs were diabetes mellitus (N=18), systemic lupus erythematosus (N=15) and IgA nephropathy (N=10) in potential kidney recipients, and non-ischemic cardiomyopathy (N=11) in potential heart recipients. **Conclusions:** Unexpected positive FCXMs correlate with female gender, but not with transplant organ. In 5 cases with persistent unexpected positive FCXMs, we have successfully transplanted patients using the VCXM.

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FLOW CROSSMATCH THRESHOLDS: WHERE (AND HOW) DO WE DRAW THE LINE?

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Aim: Flow cytometry crossmatch (FCXM) is used to assess pre-transplant immunologic risk. Each laboratory must establish FCXM positive thresholds but the method to do so is not standardized. Many factors affect FCXM and its interpretation. Our aim was to compare methods of calculating FCXM thresholds and evaluate the impact of cell source and pronase. **Methods:** Threshold study (TS) data were collected by FCXM with healthy control sera negative for HLA antibodies (n=24) *vs* pronased, negatively selected T & B cells from either peripheral blood (PBL), fresh or frozen spleen (n=264, 215, and 96, respectively). Non-pronased FCXM were tested in parallel (n=72). Data normality tests were performed on this and previous TS data. Outlier values were assessed by 1.5 interquartile range (IQR) and standard deviation (SD). Thresholds were calculated by mean or median + varying SD as well as 97.5th percentile. Thresholds were assessed in expected positive FCXM (PBL & spleen) with/without pronase. **Results:** Median channel values (MCV) were not normally distributed in either TS dataset (Fig 1). Thresholds vary by cell source: fresh spleen<PBL<frozen spleen. The best test for removing outliers was 1.5IQR: low numbers of FCXM with high MCVs excluded. Non-pronased T cell thresholds were higher than in pronased cells; T cell FCXM was more sensitive with pronased cells. T cells were better able to detect low levels of class I antibody in PBL FCXM but sensitivity is similar between T & B cells from spleen. **Conclusions:** Non-parametric means of assessing FCXM thresholds may be required. Cell source affects FXCM sensitivity for class I antibodies, perhaps due to number of T *vs* B cells. We have previously shown that pronase increases class I detection on T cells; these FCXM data support this finding; preliminary thresholds calculated from non-pronased cells do not correct this reduced sensitivity. A thorough understanding of factors that affect FCXM will optimize determination of relevant thresholds and assist in the interpretation of this commonly used assay.

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PROPER SETTING OF CUT-OFF VALUE FOR THE LYMPHOCYTE CROSSMATCH BY FLOW CYTOMETRY IN KIDNEY TRANSPLANTATION

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Aim: The flow cytometric lymphocyte crossmatch (FCXM) has become a routine test performed prior to kidney transplantation. Many studies have reported that a proportion of transplant recipients experienced early graft loss despite a negative complement-dependent cytotoxicity (CDC) crossmatch test. The FCXM is able to detect Human Leukocyte Antigen (HLA) antibodies of low titer or of a non-complement binding nature that may be responsible for graft loss. It is important to report positive and negative results using appropriate cut-off values. We reviewed the results of FCXM with CDC and panel reactive antibody (PRA) and attempted to set clinically useful cut-off values for FCXM. **Methods:** From January 2015 to October 2017, 518 kidney recipients' results of FCXM, CDC, and PRA (Screening test, Panel kit, and single antigen bead assay, LABScreen Luminex kits, One Lambda, Canoga Park, CA, USA) were analyzed. The PRA test was performed simultaneously from 403 patients, and 159 patients who were negative for HLA antibodies in the all PRA tests. Fifty-seven patients were positive for CDC test results and 37 patients were both positive in T cells and B cells. In 20 patients, only B cells CDC results were positive, and 13 patients were negative for FCXM test results. **Results:** The final interpretation of FCXM result was decided through CDC, PRA result and existence of donor specific antibody (DSA). T cell FCXM result was interpreted as negative in 451 patients and positive in 67 patients according to DSA. The appropriate cut-off value of the Mean fluorescent intensity (MFI) ratio using the ROC curve for 518 patients was 1.86 (sensitivity 100%, specificity 98.2%, Youden's index 0.982). However, patients who underwent desensitization treatment due to DSA or ABO blood type incompatibility were included. The cut-off value obtained using the ROC curve for 446 patients who have not experienced desensitization treatment was 2.45 (sensitivity 100%, specificity 99.3%, Youden's index 0.993). Generally used, Median + 2 SD value of MFI ratio of patients judged as negative FCXM was 1.60. **Conclusions:** Based on the statistical method used, various cut-off values could be obtained. It is important to determine the clinically significant cut-off value based on the data accumulated in each laboratory, not the simple median value from negative patients.

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KAS THREE YEARS LATER: LIFE AFTER THE BOLUS EFFECT

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Aim: The new Kidney Allocation System (KAS) resulted in a significant bolus in the transplantation of 99-100 CPRA candidates due to increased priority. We examined the impact of an individualized approach to maintain a high transplant rate for these candidates three years after KAS implementation. **Methods:** The early post-KAS period (EPKAS) was defined as deceased donor transplants occurring in 2015 and 2 year post-KAS period (2YPKAS) as 2016 and 2017. Donor specific antibody positivity (DSA+) was defined by single antigen bead assays performed pre-transplant (+/- 7 days) and 90 days post-transplant (+/- 14 days). Flow cytometric crossmatches (FCXM) were performed using the pre-transplant serum, donor lymphocytes, and acquisition on a BD Canto instrument. Data from SRTR was used to assess outcome at 1 year for our center and nationally. **Results:** Nationally, transplantation of 99-100 CPRA candidates rose sharply (17.7%) in the EPKAS period and has since tapered (10%) in the 2YPKAS period. At our center, we observed a similar increase in the EPKAS period (16%, n=30) but this trend increased further during the 2YPKAS period (28%, n=75). In the EPKAS period, 60% (n=18) of 99-100 CPRA recipients were DSA+ and 39% (n=7) of DSA+ recipients were also FCXM positive. 61% (n=11) of DSA+ recipients during this period had persistent DSA at 90+ days post-transplant. In the 2YPKAS period, a similar percentage (57%, n=43) of 99-100 CPRA recipients were DSA+ at time of transplant; however, fewer (8%, n=6) DSA+ recipients were also FCXM+. Correspondingly, fewer (34%, n=26) DSA+ recipients had persistent DSA at 90+ days post-transplant. A total of five (5%) candidates in the post-KAS era experienced a rise in DSA post-transplant that required intervention. The hazard ratio for 1 year graft survival was 1.05 for our center and 1.00 nationally. **Conclusions:** Our center continues to successfully transplant 99-100 CPRA candidates at a rate higher than the national average. This has been achieved through a personalized approach for defining unacceptable antigens and assessing donor offers, transplanting across DSA barriers, and a stringent post-transplant monitoring protocol. Refinement in our approach in the 2 year post-KAS has resulted in more compatible transplants, less DSA persistence, and the maintenance of good clinical outcomes.

A.M. Jackson: *3. Speaker's Bureau; Company/Organization; Thermo-Fisher.*

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PRONASE TREATMENT CAN CAUSE FALSE POSITIVE T-CELL FLOW CYTOMETRY CROSSMATCHES IN PATIENTS WITH AUTOIMMUNE DISEASES: TWO CLINICAL CASES

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Aim: Pronase treatment of lymphocytes for flow cytometry crossmatching is used to reduce non-specific B-cell reactivity due to the binding of immune complexes to Fc receptors. However, pronase has been shown to produce false-positive T-cell crossmatches in patients with HIV. We have also encountered this phenomenon with patients suffering from autoimmune diseases. In two cases, the observed T-cell reactivity decreased significantly when sera were crossmatched against unpronased cells. **Methods:** Crossmatches were performed according to standard flow cytometry protocols. Antibodies were assessed with One Lambda Luminex single antigen with a positive cutoff of 2000 MFI. **Results:** Case 1 is a 15 year old African-American male listed for kidney transplant. He has a primary diagnosis of SLE and no sensitization history. Pre-transplant medication history is notable for prednisone and cyclosporine. Sera were crossmatched against pronased deceased donor cells, with T-cell positive results in the absence of DSA. This reactivity disappeared when the crossmatch was repeated with unpronased cells. Case 2 is a 40 year old African-American female listed for kidney/pancreas transplant. She has a primary diagnosis of type 1 diabetes and a sensitization history of 2 pregnancies and moderate transfusions, none recent. Pre-transplant medication history does not include any immunoactive regimens. She is mildly sensitized to Class I and not sensitized to Class II. Sera across several years exhibited positive T-cell crossmatches against multiple cells of type A2. She has A2 antibodies with low titers (2600 MFI). No other DSA was involved. T-cell reactivity was reduced to negative ranges when unpronased cells from A2 donors were used. **Conclusions:** In both cases, auto flow cytometry crossmatches were negative. Therefore, autoreactive antibodies recognizing cryptic epitopes that might be exposed after pronase treatment does not fully explain these results. Neither patient received rituximab at any time. Patients suffering autoimmune diseases may exhibit false positive T-cell crossmatches against pronased cells, in the same manner that has been documented for HIV patients. This reactivity is not attributable to DSA and disappears or significantly decreases when crossmatching is performed with unpronased cells.

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DEVELOPMENT AND VALIDATION OF LSAB MFI CUTPOINTS FOR USE IN THE VIRTUAL CROSSMATCH TEST TO PREDICT FLOW CYTOMETRY CROSSMATCH (FCXM) AND CDC CROSSMATCH (CDC XM) RESULTS

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Aim: Luminex Single Antigen bead assay (LSAB) derived mean fluorescence intensity (MFI) values of anti-HLA antibodies directed at the potential donor's HLA are the primary parameter used in the virtual crossmatch to predict physical crossmatch (XM) outcome. We aimed to develop statistically validated LSAB MFI cutpoints for predicting CDC XM and FCXM results. **Methods:** We leveraged the ASHI proficiency testing (PT) 80% consensus results of 7156 T FCXM, 6758 B FCXM, 2917 T CDC XM and 2233 B CDC XM as the reference results to investigate whether LSAB MFI of IgG anti-HLA antibodies predict validated physical XM results. LSAB MFI was determined in our laboratory using One Lambda Single Antigen HLA Class I and Class II beads. The 80% consensus results are from 8 consecutive challenges during 2013 to 2016, and 107 laboratories across USA tested sera and HLA typed cells distributed by ASHI, and reported the results to ASHI for assessment of the laboratory's proficiency. Data analysis included: summing of MFI of IgG antibodies directed at HLA-A, B and C for investigating their association with T FCXM and T CDC XM results; summing of MFI of antibodies directed at HLA-A, B, C, DR, DQ and DP for investigating their association with B FCXM and B CDC XM results; assigning alternate ASHI challenges to the Discovery set and the Validation set; investigating the association between LSAB MFI and physical XM results by logistic regression analysis corrected for overdispersion; and identification of LSAB MFI cutpoint for maximizing the sum of sensitivity and specificity. LSAB MFI cutpoints derived from the Discovery set to predict FCXM and CDC XM results were investigated in an independent validation set. **Results:** Table 1 demonstrates that LSAB MFI

cutpoints from the Discovery set predicts XM outcomes in the Validation set (ROC AUC range from 0.974 to 0.999).

Conclusions: We have developed and validated LSAB MFI cutpoints for use in the virtual crossmatch to accurately predict physical T FCXM, B FCXM, T CDC XM and B CDC XM results.

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IMPACT OF LOW-LEVEL (MFI) HLA-ANTIBODIES ON LIVING DONOR KIDNEY TRANSPLANT REJECTION RISK

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Aim: Histocompatibility testing is used to predict the risk of hyperacute and accelerated-acute transplant rejection. The presence of HLA-specific antibodies, as detected by single antigen bead testing (SAB), are used in organ allocation through the calculated panel reactive antibody (cPRA) and by assessing the presence of donor HLA-specific antibodies (DSA). The presence of DSA and cPRA are considered in the algorithm for assigning patients to immunosuppression protocols. However, the interpretation of low level antibodies in SAB and DSA testing is challenging. Our aim was to look at antibodies below our current threshold of 2000 MFI but above our analytical threshold of 1000 MFI to determine if there is residual risk related to these antibodies. **Methods:** Patient antibody screens were evaluated with 2000 MFI and 1000 MFI cutoff values. 181 patients were assessed for pre-transplant patient risk category and donor compatibility by determining: the change in cPRA, number of identified HLA specific antibodies between the thresholds, the presence of DSA identified between thresholds and the predicted change in immunosuppression protocols. Living donor kidney transplants with an average of 2 years of follow-up were reviewed. 81 recipients were scored for the presence of DSA and examined for the development of de novo DSA (dnDSA) post-transplant. Biopsy data was also reviewed on 40 living donor kidney transplant recipients to determine the incidence of biopsy-proven rejection. **Results:** The reduction in antibody threshold was associated with an average increase in cPRA of 15% for HLA-Class I and 18% for HLA-Class II which corresponded to detection of 10 additional HLA specificities. We determined that using the 1000 MFI threshold, 15% of patients would have been considered higher risk based on transplant center protocols and would have necessitated increased immunosuppression. Changes in risk category were weakly associated with higher rates of dnDSA post-transplant

and biopsy-proven rejection. **Conclusions:** In this study, the development of biopsy-proven rejection was not associated with pre-transplant DSA or post-transplant dnDSA that were between 1000 and 2000 MFI.

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FURTHER EVIDENCE OF THE RELEVANCE OF DP EPITOPE ANALYSIS IN THE VIRTUAL AND ACTUAL CROSSMATCH IN SOLID ORGAN TRANSPLANTATION. A CASE REPORT

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Aim: Epitope-based HLA matching has been developed as a better matching method in solid organ transplantation. Here we present a highly sensitized patient with 100% cPRA but low donor specific antibody (DSA) level at the DP locus. Virtual crossmatch (vXM) with a cadaveric donor predicted a negative result but the actual crossmatch gave an unexpected positive result in B cells maybe due to the presence of a cross-reactive epitope. The clinical relevance of antibodies directed against HLA-DP antigens is discussed in the literature with conflicting reports. **Methods:** Single antigen bead assay (SAB) was used to detect the levels of alloantibodies in the patient serum. Flow crossmatches were performed with 3 conjugated antibodies: FITC-anti-Human IgG, CD3-RD1 and CD19-PC5. Serum-incubated cells were stained with fluorochrome conjugated antibodies prior to running on Navios instruments. Test was done using current and historical sera from the patient. **Results:** SAB analysis showed high levels of alloantibodies in both Class I and Class II but only a DSA for DP6. In our lab, DSA are considered "Positive" for HLA-DP when MFI values are ≥ 15000. MFI values for current and historical sera were 7536 and 10520 respectively suggesting a negative crossmatch. FCXM results were negative in both T and B cells for the last serum but unexpected positive crossmatch only in B cells was observed in the historical serum. Retrospective analysis of previous sera showed a well-defined antibody to the DP epitope, 56E, with gradual decreasing levels of MFI. The apparent discrepancies between the virtual and actual crossmatch results can be probably due to the "peanut butter" effect of this cross-reactive epitope. The patient was transplanted based on the negative crossmatch from current serum followed by immunosuppression protocol. **Conclusions:** Our results provide evidence to the impact of epitope analysis in the vXM and its effect in the actual crossmatch. Implementation of epitope-based HLA matching in our lab will help to better select compatible donors. Characterization and matching DP epitopes using computational methods seems to be more relevant than common matching alleles. Transplant in the presence of DSA at the epitope level needs to be closely monitored to determine their true clinical relevance in solid organ transplantation.

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VARIATIONS IN THE EXPRESSION OF ANTIBODY EPITOPES ASSOCIATED WITH DQB1*03:01 ALLELES IN B CELLS

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Aim: To evaluate variations of the expression of antibody epitopes associated with DQB1*03:01 alleles in B cells. **Methods:** Antibody tests were performed using Luminex Single Antigen Bead (SAB) assay on serum samples treated with DTT. XMs were performed by flow cytometry, using lymphocytes from peripheral blood of healthy volunteers, which were treated with pronase. **Results:** Four sera reacted strongly with beads carrying the following alleles: DQB1*03:01-DQA1*06:01, DQB1*03:01-DQA1*05:05, and DQB1*03:01-DQA1*05:03 (Figure 1). Sera JM and RH reacted strongly with B cells from all 6 donors who were positive for DQB1*03:01 indicating that these cells express adequate amount of DQ molecules (Figure 2A). However, XMs of sera CG and SR with the 4 heterozygous DQB1*03:01 B cells showed weak or no reactivity respectively (Figure 2B). In comparison to XM reactivity from sera JM and RH the XMs with sera SR and CG exhibited a 25-50 fold lower ΔMESF values (Figure 2A versus 2B). This unexpected low reactivity of sera SR and CG with B cells is striking since their reactivity with SAB is similar to that of serum JM except with the DQB1*03:01-DQA1*03:01 allele (Figure 1). Notably, SR and CG reacted with B cells that were homozygous for DQB1*03:01 (Figure 2B, Donor 1 and 2) indicating that the reactivity of these sera with the SAB was not due to denatured epitopes. **Conclusions:** These results suggest that the observed variable expression of DQB1*03:01 epitope on B cells could be due to variation in the conformation of this epitope on the same DQ molecules with a potential impact on antibody affinity to this epitope. The discrepancy

between SAB MFI values and XM reactivity is important to consider in assessing HLA compatibility and lymphocyte crossmatching.

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DECREASING MEDICARE COST BY TAILORING SINGLE ANTIGEN TEST UTILIZATION ON RENAL ALLOCATION SCORE

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Aim: There are currently 95,121 patients awaiting a renal transplant in the US (UNOS data). The average cost of combined testing for HLA class I and II by single antigen (SA) is around $900. If all renal patients were screened monthly this would translate in over $1 billion in annual Medicare costs. We investigated whether cost saving could be achieved by devising a test utilization strategy based on patients' UNOS status and allocation score. **Methods:** First, we performed a query in UNET to obtain the renal allocation score and status (1 active or 7 inactive) of all renal transplant patients listed at our center. Second, we performed a search of all kidneys allocated by our OPO in the last 3 months to determine what allocation category and patient renal allocation score received the kidneys. **Results:** Of the 588 patients on our waitlist, 65.3% were listed active and 34.7% were listed inactive. The average allocation score for kidneys placed by our OPO in the last 3 months was 7.27 for the first allocated kidney and 5.64 for the second kidney (scores above 15 were excluded). 8.4% of first allocated kidneys were transplanted in patients with scores below 4. These included ABO incompatible (A2 into B), 0-ABDR mismatch, and pediatric patients. Only one kidney with a KDPI of 35-85% was allocated to a patient with less than 4 points. A greater proportion of second kidneys were transplanted in patients with lower scores particularly 86-100 KDPI kidneys, kidney with long ischemic times, and high risk donors. Based on these results we developed a testing strategy whereby patients which are listed status 7 do not get tested by SA. Status 1 patients with less than 4 points are tested annually and those with more than 4 points are tested quarterly. Utilization of this testing strategy has resulted in a saving of 87.9% vs monthly testing. **Conclusions:** It is possible to devise an SA test utilization strategy based on the listing status and allocation score of renal patients. We recognize that this approach may need to be tailored to each center depending on local allocation data and each center's transplant practice. However, using such strategy can significantly decrease Medicare cost. When compare to monthly screens, we calculated that if used nationwide our approach could yield annual savings to Medicare of up to $902,810,700.

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PRECISE DEFINITION OF HLA-ANTIBODY BY RULING OUT FALSE POSITIVE REACTIVITY FROM LUMINEX SINGLE ANTIGEN ASSAY IN SUPPORTING SUCCESFUL THORACIC ORGAN TRANSPLANTATION

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Aim: False positive antigen specificities (FPAS) from the Luminex Single Antigen (LSA) Assay are difficult to identify due to multiple interfering factors and various corresponding resolving approaches. Over calling antibodies limits a patient's access to deceased donor organs and can result in erroneous immune therapy. Here we report how to properly identify and resolve FPAS from LSA assays to precisely provide HLA-antibody results in supporting Thoracic Organ Transplantation (TOT). **Methods:** We retrospectively studied 66 TOT cases that had suspected FPAS reactions with LSA assays. The initial antibody work up was tested by LSA and FlowPRA Screening Beads. Discordance between the two assays and/or ambiguity were verified by Luminex Phenotype Beads. LSA was used for all antibody follow up. If an obvious antibody increase was observed, further investigation for sensitizing events and /or possible interfering medication (such as IvIg), warranted additional testing. Detailed approaches for FPAS identification and resolution are listed in Table 1a. **Results:** Of the 66 FPAS cases, 56 (85%) were caused by denatured antigen (dAg) and 10 (15%) were due to IvIg infusion. The distribution of complete FPAS and partial FPAS (the mixture of true positive and FPAS) from dAg and IvIg were almost equal, 54% vs 46% and 50% vs 50%, respectively. The FPAS dAg patterns with highest occurring frequencies were Cw1, Cw12, Cw15 (27%) from class I and DP1, DP11, DP13, DP6, DQ4 (34%) from class II. The elimination of FPAS reduced the average PRA by 67% from dAg and 78% from IvIg interference. 22 cases received TOT after FPAS were ruled out (Table 1b). **Conclusions:** FPAS is the prevalent issue of LSA when false negativity is eliminated by serum EDTA treatment. Alternative assays by multiple platforms, supplementary LSA data analysis, and referencing clinical records are warranted for ruling out FPAS. This provides precise antibody testing results for successful access to TOT.

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ANTI-ANGIOTENSIN II TYPE 1 RECEPTOR ANTIBODIES ARE ASSOCIATED WITH POSITIVE FLOW CROSSMATCHES IN HEART Transplant Candidates

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Aim: Heart transplant (HTX) candidates with pre-formed anti-HLA antibody (HLA-Ab) have lower transplantation rates and increased waiting time due to positive crossmatches. Anti-angiotensin II type 1 receptor antibody (AT1R-Ab) has recently been associated with allograft loss and antibody-mediated rejection. The aim of the present study was to evaluate: 1) the prevalence of anti-angiotensin receptor 1 antibody (AT1R-Ab) in HTX candidates and 2) the impact of AT1R-Ab on flow cytometry crossmatch (FXM). **Methods:** 115 HTX candidates were tested by ELISA for the presence of AT1R-Ab (U/mL after 1:50 dilution, positive cut-off = 10 U/mL, strong-positive cut-off = 20 U/mL. 43/115 cases were identified with either positive (N=15) or negative (N=28) flow crossmatches in the absence of any donor-specific anti-HLA alloantibody (virtual negative crossmatches for HLA antibody). Mean channel shift (MCS) for flow cytometry crossmatches was measured on a 1024 scale. Finally, two index HTX cases with pre-formed AT1R-Ab and positive flow crossmatches are discussed. **Results:** A high proportion of HTX candidates with HLA-Ab exhibited AT1R-Ab (Strongly reactive AT1R-Ab 46/115, 40%; weakly reactive AT1R-Ab 25/115, 21.7%; no AT1R-Ab 44/115, 38.2%). Furthermore, AT1R-Ab levels were significantly higher in patients with a positive versus a negative flow crossmatch (54.81±54.92 versus 9.04±7.36, p=0.00001, figure 1). In addition, MCS was significantly higher in patients with AT1R-Ab versus patients without antibodies (73.95±94.21 versus -51.45±52.07, p=000003, figure 2). In the 15 cases with positive flow crossmatch without HLA-specific antibody, all 15 patients exhibited AT1R-Ab, 11/15 had strong AT1R-Ab, while only 5/25 patients with negative FXM exhibited AT1R-Ab, Chi-square =24, p=0.00001). **Conclusions:** More than half of HTX candidates exhibit AT1R-Ab, which can interfere with flow crossmatch interpretation even in the absence of HLA-specific antibodies.

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HLA TYPING AMBIGUITIES ARE INCREASING AT ANTIGEN LEVEL

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Aim: During the last years, DNA typing with commercial kits based on PCR-SSO methods were most commonly used for clinical purposes for low resolution HLA typing. However, the increase of alleles that are being described suggests that SSO methods might not be enough even for HLA-typing at antigen level because new ambiguities are continually being found. Some of the ambiguities involve alleles that were described at low frequencies and need to be resolved for clinical purpose. Our Aim is to describe the proportion of samples in our laboratory with any ambiguous result at antigen level by SSO. **Methods:** We have analyzed retrospectively the samples typed by SSO Luminex HLA-A, HLA-B, HLA-DRB1 using One lambda reagents (LABType® SSO) from 2017 until now. **Results:** During 2017 we typed 735 samples for the three named genes; 633 family-related samples and 102 unrelated samples. Ninety out of 633 samples (14 %) showed at least one locus with ambiguous result at antigen level (27 for HLA-A, 43 for HLA-B and 18 for DRB1 and two samples showed two loci with ambiguities). From unrelated samples 9,8 % showed ambiguities for any of the studied genes (5 samples for HLA-A, 4 for HLA-B and 1 for DRB1). During the first trimester of 2018 we typed 158 samples (138 family-related and 20 unrelated). We found ambiguous results at 32/138 samples (23 %) from family-related samples (4 for locus A, 22 for HLA-B, 5 for HLA-DRB1 and one sample for HLA-B and DRB). For unrelated samples 4/20 (20%) showed ambiguities (1, 2 and 1 for HLA-A, HLA-B and HLA-DRB1 respectively). The most frequently found ambiguity was: A*02:ANCHT, A*24:ANCBX or A*02:19, A*02:571. The allele A*02:19 was first described in our country, it is considered WD (CWD alleles catalogue version 2.0.0), the allele A*02:571 is not included at the named catalogue. **Conclusions:** Our results show that LABType® SSO technique is not enough for HLA- typing with clinical purposes, even as a screening method to look for suitable donors because many samples need to be further typed with other method

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ELIMINATING FALSE POSITIVE FLOW CYTOMETRIC CROSSMATCHES DUE TO MONOCLONAL ANTIBODY THERAPY

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Aim: Biologics are increasingly used in autoimmune, cancer, and transplant patients. Unfortunately they can also interfere in laboratory tests. Monoclonal antibodies, such as Rituximab, are well known to cause positive flow cytometry crossmatches (FCM) independent of donor specific HLA antibody (DSA). We assessed the ability of blocking antibodies to Rituximab (RTX) and Alemtuzumab (ALM) to negate interference by these biologics in the FCM. **Methods:** FCM was performed with serum from patients receiving RTX and/or ALM. Some sera were pretreated with anti-Rituximab (αRTX) and/or anti-Alemtuzumab (αALM) blocking antibodies (Bio-Rad, Inc). HLA DSA was determined using multiplex bead arrays (LabScreen Single Antigen, Thermofisher, Inc.). FCM channel shifts (CS) were compared between treated sera and sera treated with PBS as a control. **Results:** Blocking antibodies were titrated in serum to determine the working dilution to use in subsequent studies. Sera from patients receiving RTX and/or ALM were treated with the predetermined dilution of blocking antibody. Blocking antibody pre-treatment reduced FCM CS with surrogate donors to levels comparable to PBS controls: RTX blocking: pre-treatment CS = 23 T and 302 B; post-treatment CS= 35 T and 29 B; ALM blocking: pre-treatment CS = 262 T and 236 B; post-treatment CS = 34 T and 2 B. A highly sensitized (cPRA = 99%; DSA = B35, Cw4, DR17, DR52, DQ5) pediatric renal transplant candidate (RTX and ALM treated) was crossmatched with their living donor using pre-transplant and 4 day post-transplant (post-RTX and ALM treatment) sera. Post-treatment CS were increased without blocking but reduced with blocking antibody treatment. Blocked FCM CS results correlated better with reduced DSA MFI summed values in contrast to the unblocked serum (Table 1).

Table 1. FCM CS (Blocked and Control) and HLA DSA MFI Values Pre- and Post-Transplant

Serum Treatment	T CS (Cl I MFI sum)	B CS (Cl I and II MFI sum)
Pre-transpl.	239 (16181)	344 (39740)
Post-transpl. (Unblocked)	378 (8590)	488 (25729)

Post-transpl. (PBS Treated)	304	418
Post-transpl. (αRTX Blocked)	311	278
Post-transpl. (αALM Blocked)	111	414
Post-transpl. (αRTX αALM Blocked)	106 (8590)	234 (25729)

Conclusions: Antibodies to RTX and ALM effectively block interference by these biologics in FCM resulting in the ability to provide useful FCM data for patient management.

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EVALUTION OF ELECTRONIC DP EPITYPE PROFILING TO DETERMINE DP DONOR-SPECIFIC ANTIBODIES DURING VIRTUAL CROSSMATCH

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Aim: Our aim is to evaluate the utility of incorporating epitype analysis into the computerized virtual crossmatch (vXM) workflow for donors with DP alleles that are not covered by a recipient's HLA antibody screening and identification assay. Our lab has used vXM to select deceased donors for transplantations for more than ten years. If the donor DP antigens are covered by the assay, the DSA can be determined directly by vXM. If the donor antigen is not included in the assay but is covered by an epitype (EDP) group which includes a DP antigen covered by the assay, the DSA can be determined based on the positivity of the EDP antigen using manual epitype profiling. A new epitype profiling system was deployed by informatics vendor HLA Data Systems, developers of the mTilda Lab Management System and VxMatch, to ensure that DP alleles not covered by HLA antibody tests are incorporated into an electronic vXM workflow. **Methods:** The epitype profiling system contains a data table of the highly polymorphic DP hypervariable regions (HVR) for DPB1 alleles. There are a total of six HVR regions (HVR-A, B, C, D, E, F) containing the unique amino acid profile per allele and 291 epitype groups determined so far which correspond to alleles belonging to the same HVR motif. The system returns the HVR motifs for donor and alleles of the recipient antibodies and then sorts alleles of recipient antibodies by increasing reactivity. Total negative and positive HVR matches are based on a configurable positivity cutoff value. Matches are color-coded for investigation of possible DSA when there is a positive match that is accompanied by zero negative matches. If a DP antigen bead is negative, presence of antibodies to polymorphic motifs carried by the DP antigen can be excluded. **Results:** The computerized epitype profiling system was validated against our manual process for donors with alleles not contained within the assay, DPB1*16:01 and DPB1*85:01, and HVR motif profiling performed without error. With the increasing discovery of DP alleles, the system can cross-reference G-groups to determine HVR motifs for new alleles and can be configured to profile other HLA genes. **Conclusions:** Our evaluation suggests that extending vXM to include computerized epitype profiling is an efficient and reliable means to evaluate DP alleles not covered by antibody assays.

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COULD PBLS PROVIDE WITH ENOUGH B CELLS FOR A SUCCESSFUL FLOW CROSSMATCH?

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Aim: Although lymph nodes are preferred resources for isolation of lymphocytes for crossmatches, the nodes may not be available at the time of crossmatching. An unusual case led us to revisit 39 deceased donor's crossmatch results to see if enough B cell events were acquired in flow crossmatching. **Methods:** Cells were isolated using Density Gradient of Ficoll-Hypaque from pronase-treated PBL, and/or from lymph nodes. Flow crossmatches (FCXM) were performed on a BD FACSCalibur. In FCXM, anti-CD3 was used to mark T-cells, anti-CD19 was used to mark B-cells and goat anti-human IgG was used as a secondary antibody for detection of donor specific antibody. **Results:** A 38 years old Caucasian female suffered a severe injury from a car accident was evaluated as the potential deceased donor for three kidney recipients. Three tubes of PBL were received for initial FCXM. The crossmatch failed due to lower B cell events (<400) acquired. Crossmatches were repeated using cells of lymph

nodes. All crossmatches were negative and one of the three potential recipients was transplanted. We further analyzed 39 deceased donors. Out of the 39 donors, 23 had nodes at the time of crossmatch, 10 only had PBL, and 6 donors had both PBLs and nodes available. We found that T and B cell ratio was between 1:1 and 32:1 in PBL. 90% of donors fall in the range of 1:1 to 10:1. We further investigated the cause of donor death, WBC counts and medical histories but found no association between these factors with the T/B cell ratio or total lymphocyte number. All crossmatches with cells of lymph nodes were successful. Out of 16 crossmatches with cells of PBLs, only one (6%) did not have enough B cells. **Conclusions:** Our findings suggest the PBLs, as the resources of cells for crossmatch, most of the times (94%) could result in successful crossmatches.

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VARIABILITY IN THE SENSITIVITY OF THE FLOW CYTOMETRIC CROSSMATCH

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Aim: The sensitivity of the flow cytometric crossmatch (FXM) is often variable across labs in the presence of weak donor specific antibodies (DSA). In addition, inter-laboratory variation to the interpretation of Mean Fluorescence Intensity (MFI) values escalates the potential for discordance. The aim of this study is to compare FXM results among laboratories and correlate the results with DSA strength. **Methods:** The study collected and analyzed data reported by participants in the Flow and Virtual Crossmatch Exchange over a period of 4 years. Data collected included antibody specificities, MFI values, T- and B-cell positive cutoffs, mean channel shifts (MCS), and negative control MC. In addition, the coefficient of variations (%CV) were calculated using the center-specific MFI values to analyze the variability in the reporting of antibody strength among labs. **Results:** A total of 24 cells were tested against 48 sera over 12 Exchanges for a total of 96 T/B flow crossmatches. Among the 96 FXM pairs, 84% of T-cell and 88% of B-cell crossmatches were reported with good agreement (>80%). Positive concordant T-cell crossmatches were observed in 92% (57/62) of cases when class I DSA were reported with MFI > 3000. Positive concordant B-cell crossmatches were observed in 89% (49/55) of cases when class I and class II DSA were reported with MFI > 5000. **Conclusions:** The data shows there is good consistency in T/B Flow crossmatch outcomes among labs in the presence of moderate or strong DSA. However, when DSA strength is weak, the sensitivity of the flow crossmatch is more widely variable. In addition, center practice, such as cutoff values and the reporting of MFI values may influence FXM outcomes. Thus, educational activities should continue to provide standards for quality control for the FXM.

P155

DEFINING THE HIERARCHIES OF SEROGENIC HLA ALLOTYPES DISCOVERS KEY HLA TYPES THAT WARRANT MATCHING FOR KIDNEY TRANSPLANTATION

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Aim: To study the ability of 121 core HLA allotypes in provoking antibody (Ab) production upon distinct exposures to identify the vigorous serogenic HLA allotypes that deserve matching in kidney transplantation. **Methods:** HLA-Abs were analyzed in 277 patients (44% females) waiting for 2nd-kidney transplantation (Ktx), who had no HLA-Ab at pre-1st Ktx. The frequency and strength (mean of composite MFIs) of 1st Ktx-induced HLA-Abs were compared with those from patients who had >1 Ktxs (n=148), and patients waiting for the 1st-Ktx (1477 females - pregnancy-induced Abs and 2274 males - naturally occurring Abs). Single antigen beads were used to assay HLA-Abs (One Lambda). **Results:** The most prominent Abs (most frequent and strongest) produced by 1st-Ktx are DQ-specific (Fig.1). The hierarchy of 1st-Ktx-induced Abs based on prominence is DQ3>B76>DQ4>DQ6>DQ2>B45>B57>DR9>A1>DQ5>DR7>B58>A24>DR53>B49>B82>A11>B44>A69>A2>A23>A68>A25>A32>B7C>A10C>B5C>DR52C>DR51C>Cw/DP. No gender-specific deviation was noted implying that prior pregnancies do not aggravate HLA-Ab production post-Ktx. The constellation of HLA-Ab in patients who had >1 Ktx is comparable to those had a single Ktx (Fig.2), indicating that multiple Ktx do not exacerbate the serogenicity of HLA types. The most abundant pregnancy-induced Abs are HLA-B and A2 CREG-specific (Fig.3). Over 60% of males waiting for 1st-Ktx have no HLA-Ab. The following Abs are detected at a frequency of 2-5% in these unsensitized males, and hierarchy built on prominence is B76>B45>Cw15>B82>Cw1>B44>Cw12>DQ7>A80>B57>B8>B81>A25>Cw17>B7>A66>A31>B37>A26

(Fig.4). Most of these Abs were <4000 MFI, do not belong to specific CREG, and negative with crossmatch and other Ab assays. **Conclusions:** HLA-DQ, DR53 CREG, and A2 CREG antigens are most aggressive Ab inducers following Ktx and thus to be considered for matching. Priority for HLA-B and A2 matching will benefit females as they are strong serogenic during pregnancy. HLA-Abs detected in unsensitized males require additional scrutiny.

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OPTIMIZATION OF A RAPID PLATE FLOW CYTOMETRY CROSSMATCH ASSAY ON THE CYTOFLEX PLATFORM: THE BAYLOR PROTOCOL

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Aim: The Flow Cytometric Crossmatch (FCXM) assay has become the standard method for immunologic risk assesment of donor/recipient pairs. There is growing interest in streamlining the assay to expedite results and reduce performance costs without compromising assay accuracy or sensitivity. The introduction of powerful benchtop instruments, such as the Beckman Coulter CytoFLEX system, combined with the previously published Halifaster FCXM by Liwski et al, allowed our group to optimize and simplify the crossmatch procedure. **Methods:** Instrument analytical performance was defined to validate use of the CytoFLEX, a research use only platform, for high complexity clinical testing. The instrument was evaluated for precision, stability, sensitivity, carryover, and accuracy. Performance characteristics were defined by Median Fluorescence Intensity (MFI), counts, and percentages, and compared to the current cytometer platform. The FCXM assay was optimized for time reduction (centrifugation cycles, incubation times, plating and washing), reduction of sample and reagent volumes, and minimization of cell loss during processing (plate type, pronase treatment). **Results:** *Instrument Performance:* The instrument analytical performance is summarized in the following table. The CytoFLEX showed increased MFI precision and sensitivity when compared with current instrumentation. *Assay Optimization:* Each optimized component of the Baylor Protocol FCXM is summarized in the following table. The resulting process reduces assay completion time by 2 ½ hrs with optimal cell recovery. **Conclusions:** In this report we describe a model for step-by-step performance characterization of a RUO insturment and optimization of every component of a clinical test. We provide a method for CytoFLEX standardization, which may be reproducible with minimal variation across labs. We also demonstrate that the Baylor Protocol FCXM is a sensitive, fast, and cost effective method for use in clinical transplant labs.

	MFI	**FC500**	**CytoFLEX**
Precision	Repeatability (30 x 1 day)	5%	1.5%

	Reproducibility (1 x 30 days)	10%	2%
	Stability - Long Term Reproducibility (6 months)	15%	3%
Sensitivity and Linearity	MEFL (Molecules Equivalent FITC)	150	60
	MEPE (Molecules Equivalent PE)	90	30
	MEPE (Molecules Equivalent PC5)	478	60
Carryover	Carryover (10 second backflush)	Not tested	<1.2%
Accuracy	Correlation (n=35)	>95%	
	Steps	**Current Protocol**	**Baylor Protocol**
Method Optimization	Cytometer Startup	45 min	5 min
	Cytometer Setup	30 min	5 min
	Well Type	5 ml tubes	96 well plate
	Well Shape	Round	Round
	Well Material	Polystyrene	Polypropylene
	Cell Isolation Time	45 min	45 min
	Pronase Concentration	0.5 mg/mL	0.5 mg/mL
	Pronase/DNAse Treatment Time	15 min	17 min
	Number of Cells/Well	$5.0x10^5$	$2.5x10^5$
	Serum Volume	40 uL	30 uL
	1st Incubation	30 min	20 min
	Antibody Cocktail Volume	80 uL	40 uL
	2nd Incubation	20 min	5 min
	Additional Assay Time (e.g. washing, plating)	30 min	5 min
	Total Assay Completion Time	4 hrs	1 1/2 hrs

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VIRTUAL CROSSMATCH CAN BE USED SUCCESSFULLY AS PRE-TRANSPLANT CROSSMATCH FOR MOST DECEASED DONOR TRANSPLANTS AT A HIGH VOLUME CENTER

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Aim: We developed a reliable virtual crossmatch (VXM) method for use as pre-transplant XM for deceased donor (DD) kidney transplantation (Ktx). Here we report the outcomes of 248 Ktx performed based on VXM. **Methods:** Our VXM method includes 3 integral processes: accurate identification of HLA antibodies (Ab) using right tools and principles, assessment of correlation between the strength of donor specific Ab (DSA) and T and B cell bindings

by flow XM (FXM), and listing of clinically relevant unacceptable antigens that will not impact DD offers or graft survival. From the start of the new KAS through Dec. 2016, 434 DD-Ktx were performed at UCSF, of which 375 (86%) were performed based on VXM (350 were VXM^{-ve} with $DSA^{+/-}$ and 25 were VXM^{+ve} with DP/DQα DSA^{+}). 59/434 (14%) of the Ktxs were performed based on pre-tx FXM (54 were FXM^{-ve} with $DSA^{+/-}$ and 5 were FXM^{+ve} with DP-DSA^{+}). Six month protocol biopsy (Bx) and cause Bx were evaluated for C4d rejection. **Results:** All 350 pre-tx VXM^{-ve} assessments were negative by retrospective FXM. There were no hyper acute rejections in these 350 Ktx. Biopsies of 273 negative VXM-based Ktx revealed no significant difference in the rate of antibody-mediated rejection (ABMR) or in acute cellular rejection (ACR) between 3 distinct CPRA groups (Table). Females, re-tx, well-matched tx, and de nova DSA producers were more frequent in both high CPRA groups compared to 0-79% CPRA group. Pre-tx DSA was more common in 99-100% CPRA group compared to other groups. **Conclusions:** Our VXM protocol is reliable, has excellent graft survival, and works even for those with 100% CPRA. Accurate VXM method improves organ allocation for broadly sensitized patients, increases the efficiency of regional/national sharing, and avoids reallocation into unintended recipients.

P158

SELECTIVE ANTIBODY REACTIVITY AGAINST HLA-DQA1 IN TRANSPLANT CANDIDATES IS RARE

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Aim: Single antigen bead (SAB) assay is used to test the presence of antibodies against HLA antigens including HLA-DQ. Each SAB DQ bead is comprised of an alpha and a beta chain representative of an HLA-DQ antigen. A positive result represents reactivity to either or both chains. The aim of this report is to evaluate the frequency of HLA-DQ alpha antibodies in transplant candidates known to have Class II antibody. **Methods:** Class II SAB data (One Lambda Inc.) was collected from 2346 transplant candidates at Brigham and Women's Hospital from 6/9/2012 to 1/15/2014. The reactivity to HLA-DQ alpha was considered positive (MFI>3000) if all the beads containing a specific HLA-DQ alpha were reactive but the beads with corresponding HLA-DQ beta chain were negative. For example, the sera which were considered positive for HLA DQA1*03:02, had positive reaction with two beads containing DQA1*03:02, [DQA1*03:02, DQB1*03:02] and [DQA1*03:02, DQB1*03:03], but negative reaction with five beads positive for either DQB1*03:02 or DQB1*03:03 combined with DQA1 chains other than DQA1*03:02. **Results:** Of the 29 HLA-DQ assay beads, 12 different HLA-DQ alpha chains were combined with different HLA-DQ beta chains. Sixteen (16/2346, 0.69%) sera reacted with beads specific for DQA1*03:02. These sera reacted with the alpha chain in combination with either DQB1*03:02 or DQB1*03:03. A similar pattern could be observed with sera (17/2346, 0.72%) that had specific reactivity to DQA1*02:01. Interestingly, none of the sera appeared to react selectively with DQA1*01:01, DQA1*01:02, DQA1*03:01, or DQA1*05:01. Although reactions with beads coated with DQA1*01:03, DQA1*03:03, DQA1*04:01, DQA1*05:03, DQA1*05:05, or DQA1*06:01 were present, they could not be attributed to DQA1 as the number of reactions or beads were not sufficient to rule out reactivity to DQB1. Out of the 33 patients who showed reactivity to either DQA1*03:02 or DQA1*02:01, only three (9.1%) had reactivity exclusively to the HLA-DQ alpha chain with no reactivity to any other HLA class II antigen. **Conclusions:** The frequency of antibodies which appear to have HLA-DQ alpha specificity in transplant

candidates is low (1.4%). Our results imply that a large number of sensitized patients will have to be studied in order to determine the clinical significance of antibodies to DQAlpha.

P159

EVALUATION OF DIFFERING METHODS FOR CALCULATING CPRA FOR KIDNEY ALLOCATION

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Aim: Currently, there are two different approaches to calculating cPRA. The first, used in USA since 2009, is the expectation maximization (EM) algorithm. The second, utilized in Europe/Canada, is to simply observe the percentage of donors with a particular antigen ("count method"). Recently, the UNOS/OPTN Histocompatibility Committee has been re-evaluating the optimal method for determining cPRA. This has been driven by the need to consider incorporating DP (and other) locus antibodies. The benefit of the EM method, based on haplotype frequencies, is to estimate population frequencies that would include unobserved, rare phenotypes. However, because DP is not in linkage disequilibrium, it may no longer be feasible to use. Here, we evaluate the use of the count method in comparison with the EM approach for determining HLA antigen frequencies in the U.S. donor population. **Methods:** Using the UNOS/OPTN data, we evaluated HLA typings from 39,568 deceased donors from 1/2015-1/2018. We then compared the observed antigen frequencies (count method) with those derived from the UNet cPRA calculator (EM method). **Results:** See table **Conclusions:** There was high concordance between the two methods of calculating cPRA in antigen frequencies within the A, B, and DR loci. Instances where there were large discrepancies were primarily due to parent antigens that were subsequently split; the UNet cPRA was based upon a pool of donors from 2007-9. This suggests, at the very least, that updates to the cPRA calculator are needed. Since DPB is not in linkage disequilibrium with the other loci, thereby negating our ability to use the EM algorithm, we suggest a new updated cPRA calculator be based upon the count method of calculating antigen frequencies.

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ANALYSIS OF REPEAT TYPINGS DONE ON DPB, DPA, AND DQA LOCI; THE 2018 UNOS STAR FILES.

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Aim: Mandatory HLA-DPB and DQA typing has recently been instituted for deceased organ donors. While these loci are not routinely used for organ allocation or listed as unacceptable antigens, if they are in the future, consistent typings will be important. We sought to determine the current state of compliance with the mandatory typing as well as to analyze repeat typings for discrepant results **Methods:** We examined 162,598 HLA typings submitted to UNOS on deceased donors from 10/1/87 through 12/31/17. Data was obtained with permission from UNOS as a SAS "STAR" file and analyzed with JMP® (SAS Institute). **Results:** Prior to January, 2016, just fewer than ten percent of the 146,896 donors were typed for DPB, DPA, DQA while typing results were submitted for DPB and DQA for virtually all donors subsequently and in 76% DPA was also submitted. In the era prior to 1/1/16, less than 3% of typings were repeated. This increased to 30-36% in the current era (Table 1). The total number of discrepancies for DPB typings was 152 out of 5,495 typings that were repeated (2.8%). The most common discrepancies in DPB typing were between DPB*104:01 and 03:01 (N=47), DPB*04:02 and 105:01(N=29), and DPB*04:02 with 04:01 (N=13). A number of other discrepancies were observed involving DPB*03:01, 04:02, 104:01, 105:01 and 04:01, which accounted for 71% of the DPB discrepancies.

TABLE 1

	Typings before 1/1/16	Repeats before 1/1/16	Typings After 1/1/16	Repeats After 1/1/16
HLA-DPB	8.4%	2.3%	99.0%	35.0%
HLA-DPA	2.3%	0.9%	76.0%	29.0%
HLA-DQA	3.2%	1.3%	99.0%	36.0%
(N)	146,896		15,702	

Conclusions: HLA-DPB and DQA typing of deceased donors is now mandatory. There has been excellent compliance with submission of typing results. Over three quarters of typings also include DPA results. The large proportion of repeat typings has permitted an analysis of both the rate of discrepancies and which antigens account for most of them. If these loci are to be considered for allocation, further analysis of the nature and reasons for these discrepancies will be needed to reduce the error rate.

P161

AUTOANTIBODIES: ANGIOTENSINOGEN VS AT1R AS A TARGET, IS THERE A CORRELATION?

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Aim: Autoantibody directed at the Angiotensin II Type 1 Receptor (AT1R) has been reported as detrimental to solid organ transplants. An ELISA based AT1R antibody test is available, but presents challenges including different methodology, lot-to-lot variability, and cost effectiveness among them. A Luminex bead based assay autoantibody panel is available, currently detecting 33 targets, with more targets in development. One of the targets on the assay is Angiotensinogen (AGT), the precursor to Angiotensin II. This assay overcomes many of the challenges of the AT1R ELISA test. The aim of this study is to compare results of AGT autoantibody detection using the bead panel to the results of the AT1R autoantibody detection using the ELISA based testing. **Methods:** OneLambda AT1R ELISA kits were used to test 30 sera from transplant patients for the presence of AT1R antibody. The trays were read on the VictorX and results were calculated using either the OneLambda 4PL curve calculation software, or the Workout Plus software on the VictorX, (validated as equivalent to the OneLambda software). OneLamda Autoantibody kits

were used to test for the presence of antibody to angiotensinogen (AGT). The kit is a Luminex bead-based assay. Results were read on the Luminex 3D and results were analyzed using Fusion Research 6 software. **Results:** The Reference values for the AGT target were determined by the manufacturer in a non-transplant population of 139 people. 75% had MFI of 777 or less, 85% had MFI of 1045 or less, and 95% had MFI of 1739 or less.

Conclusions: These results show that ranges are overlapping and MFI mean values of AGN as a continuous variable are not consistent with the level of AT1R We conclude that there is no suggestion of a correlation between the results of both assays.

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PROGRESS TOWARDS GENE EDITING OF HLA-DRB1*04:01 BY CRISPR/CAS9

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Aim: The HLA-DRB1 locus represents the greatest genetic risk factor for susceptibility for rheumatoid arthritis (RA). We have refined this genetic association to a single amino acid polymorphism at position 71 that is associated with both susceptibility or resistance, depending on the presence of a basic or acidic residue. Mutating DRβ1*04:01 at position 71 from lysine to glutamic acid (K71E) completely abrogates the preferential binding of citrullinated vimentin$^{66\text{-}78}$ and citrullinated α-enolase$^{11\text{-}25}$ peptides compared to the native forms of these peptides, and eliminates binding of collagen$^{258\text{-}272}$. This single amino acid substitution renders the arthritogenic peptide-binding profile of DRβ1*04:01 nearly identical to that of the resistant allele, DRβ1*04:02. The strong correlation between peptide binding and genetic susceptibility to RA suggests that CRISPR/Cas9 could be used to specifically edit position 71 of DRB1*04:01 and eliminate the binding preference for citrullinated peptides, thus attenuating the disease. **Methods:** The CRISPR/Cas9 system was utilized to specifically edit the DRB1*04:01 gene. We created two guide sequences (208/fwd and 185/rev) and prepared Lentivirus constructs to test their specificity in T2 cell lines expressing different human HLA molecules. Since indels are often formed by Cas9 editing, the loss of HLA-DR expression was used as an indication that the guides were specific. **Results:** Since HLA-DRB1*04:01^{K71E} has not been seen in nature, we produced a DRB1*04:01^{K71E} transgenic mouse to demonstrate that this modified allele is not conditionally lethal. This mouse will also be used to demonstrate that stem cells from DRB1*04:01^{K71E} mice transplanted into HLA-DRB1*04:01 mice do not cause graft-vs-host disease and can render mice resistant to RA. Next, DRβ1*04:01 T2 cells were transduced with lentiCRISPR 208/fwd or 185/rev. HLA-DR expression decreased from 95% to 20% or 12%, respectively. No loss of HLA-DR expression was seen on DRβ1*04:02 or DRβ1*08:01 control cells, demonstrating specificity of the guide RNAs. Experiments using homologous directed repair to introduce the K71E gene edit are in process. **Conclusions:** The CRISPR/Cas9 system can be used to specifically edit DRB1*04:01 suggesting that gene editing could be a potential treatment for RA.

P163

ACCOMMODATION IN TRANSPLANTATION - A NEW MODEL

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Aim: First defined as the survival and function of an organ graft in the face of otherwise lethal immunity and inflammation, accommodation might be the most common outcome of ABO-incompatible and a frequent outcome of ABO-compatible but HLA-disparate organ grafts. Accommodation was postulated to reflect an acquired resistance to injury; however, more recent work reveals a far more complex condition reflecting predisposing factors of donor and recipient origin and dynamic processes, extending over time and beyond survival and death of cells assaulted by the immune system. The overall goal of the research was to identify and test genetic determinants of accommodation. **Methods:** We investigated donor and recipient genes and processes potentially contributing to accommodation, as it develops over periods of days, by analysis of clinical kidney transplants, murine heterotopic cardiac transplants and cell cultures. **Results:** Human kidney transplant recipients were found commonly to exhibit B cell responses specific for the donor. The nature of these responses anticipated rejection or absence of rejection, suggesting that qualitative aspects of immunity such clonal persistence and affinity maturation, as much as presence or absence of immunity determines graft fate. Genetic loci impacting on the character of adaptive immune responses were sought and two highly polymorphic (non-MHC) loci, TNFRSF13B and TNFRSF17, were identified. Sequencing revealed distinct regions in which variants correlated with rejection versus graft well being. Mice with gene variants introduced in the locus corresponding to the human "rejection-associated" locus predicted the quality of immune responses to model antigens and the severity of rejection of heterotopic allografts. **Conclusions:** These findings contribute to a larger and more complex picture of biological interactions between graft and recipients that

determine whether immunity eventuates in accommodation or rejection of transplants. The interactions have implications that extend beyond organ transplantation.

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REPETITIVE IN-VITRO EXPOSURE TO DFP AFFECTS MHC CLASS I AND CLASS II ANTIGEN EXPRESSION

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Aim: *O,O-Diisopropyl phosphofluoridate also known as* Diisopropylfluorophosphate (DFP) a surrogate compound of nerve agents (Cyclosarin, VX, Sarin), is an organophosphate, used in this study to understand the mechanisms of toxicity on T cells, B cells and monocytes. Our aim was to study MHC class I & II antigen expression on T cells, B cells and monocytes after repetitive exposure to sub-lethal doses of DFP *in-vitro*. This study was conducted as a proof of concept since this compound was used in rodent models to study Gulf War Illness (GWI), which is a chronic multisymptom disorder affecting military veterans and civilian worker that served during 1990-91 Gulf War. **Methods:** We isolated T cells, B cells, and macrophages from 10 healthy donors. Freshly isolated cells were maintained in culture and were exposed to 0.1-100μM DFP for 3 days at a 24hr interval. HLA typing was performed both by using the PCR-sequence specific primer (SSP) and by serological methods before exposure to DFP. After exposure, the cells were screened for the presence of the specific HLA-B and HLA-DR antigen. We isolated the culture supernatant to study the soluble HLA antigens **Results:** HLA typing was done for both A and B allele for T cells and DR for B cells using serology and by PCR-SSP. Once the antigens were delineated, after exposure they were tested for the specific antigen. Doses above 50μM of DFP showed weak antigen expression. The cell surface antigen appeared weak by serology. The cell supernatant tested positive for HLA antigens, although they were combined with other proteins, it will be interesting to see how these HLA cell surface markers detach from the cell. We have isolated the supernatant for LC-MS analysis. Control cells that were not treated with DFP gave a consistently positive result and the HLA A, B, and DR were same as pre-exposure. There was no detectable sHLA in the supernatant. **Conclusions:** Exposure to organophosphate-based pesticides can cause immunotoxicity. Although we suspected and were trying to understand the GWI, these results offer a valid clue that there is relevance to HLA antigen expression and to look for soluble HLA antigen in the serum/plasma, as this sHLA, may very well initiate an autoimmune response by triggering inflammatory signals.

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NEW RELEVANT ASSOCIATIONS WITH VOGT-KOYANAGI-DISEASE (VKH) WERE FOUND IN MEXICANS, USING NGS TECHNOLOGY. A NEW ALLELE AT DPA1 LOCUS WAS DETECTED IN A VKH PATIENT.

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Aim: VKH is a systemic autoimmune disorder affecting tissues containing melanin, mainly the eyes. It is characterized by severe bilateral granulomatous uveitis. VKH is a T cell mediated disease vs. specific auto-antigens relevant in its pathogenesis. We and others showed that a shared epitope in the first DRB1 domain is clearly associated with VKH. DRB1*04:05 and 04:04 are strongly involved in Mexicans and other contributing DRB1 alleles, were also published by us: DRB1*04:05> *04:04> *04:07> *01:01>*01:02. We now explored the whole MHC region, to unravel the complete MHC genetics of VKH. **Methods:** We selected 77 unrelated donors from Mexico City and 49 VKH patients from the APEC. DNA samples were extracted in the Maxwell 16 instrument. For HLA typing, the MIA FORA *NGS* FLEX HLA Kit (Immucor, Inc) and The Illumina MiSeq, were used. Sequence analysis was performed with the Mia Fora software. Genotypes were assigned at all loci unambiguously, except for DPB1. Allele and haplotype frequencies (AF/HF) were done with the Arlequin, PyPop and Hapl-o-Mat v.1.1 software. **Results:** A hierarchy of Class I/class II associations were found with deep sequencing: DQB1*03:02:01 (p=0.000007); DQA1*03:01:01(p=0.0002); C*03:04:01 (p=0.007); DRB1*04:04:01(p=0.005); DRB1 *04:07:01 (p=0.01); DRB1*04:05:01; (p=0.02); B*48:01:01 (p=0.04); A*68:01:01(p=0.02); DPB1 *03:01:01(p=0.02). The OR was between 2.9 and 7.3. Amerindian (AMI), Hispanic and Asian alleles are evident at all loci. The most frequent haplotype in VKH is of Mexican AMI descent**: A*02:01:01 B*39:05:01 C*07:02:01 DRB1*04:07:01**

DRB4*01:03:01 DQA1*03:01:01 DQB1 *03:02:01 DPA1*01:03:01 DPB1*04:02:01 (HF=0.03). A new DPA1*02:07:01_5407G>A was found. **Conclusions:** As we published previously, the DRB1*04-DQA1*03-DQB1*03 association is strong but it is shown for the first time, that A*, B*, C* DQA1* and DPB1* alleles of Mexican AMI ancestry are strongly associated. The genetics of VKH is undoubtedly of Oriental origin, and present therefore in AMI, admixed groups such as Hispanics, Mediterranean and Asians. The shared epitope at S57-LLEQRRAA 67-74 in DRβ1 chain and the new class I associations are the basis to look for immunological intervention to block the T-cell response Vs. the antigens that trigger the autoimmune response in VKH.