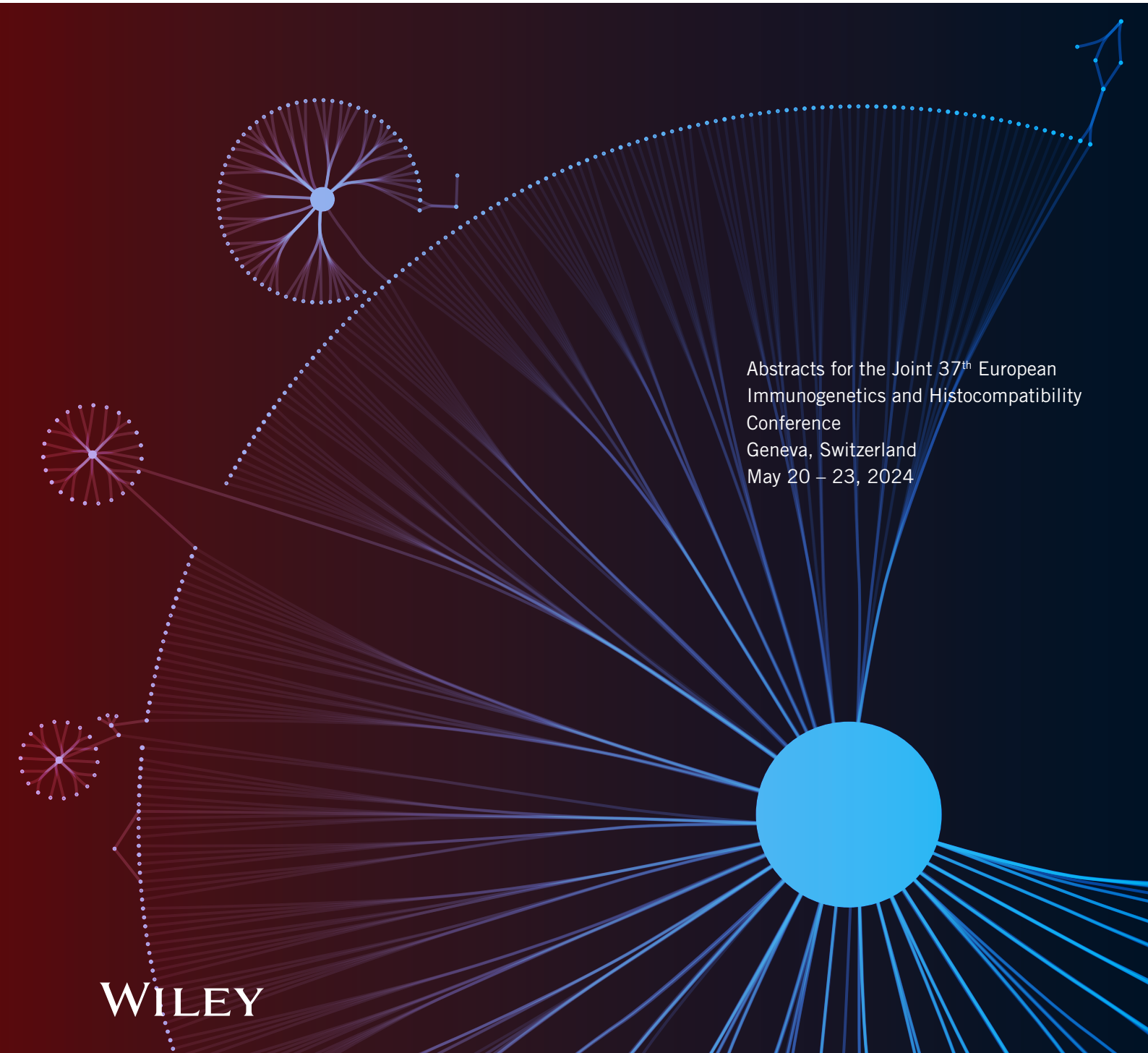
May 2024 Volume 103 Supplement 1

HLA

Immune Response Genetics

The official journal of the European Federation for Immunogenetics



Abstracts for the Joint 37th European Immunogenetics and Histocompatibility Conference
Geneva, Switzerland
May 20 – 23, 2024

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Accepted: 2 April 2024

DOI: 10.1111/tan.15477

ABSTRACT

HLA Immune Response Genetics WILEY

Abstract Book

Abstracts for the 37th European Immunogenetics and Histocompatibility Conference

Geneva, Switzerland, May 20–23, 2024
efi-conference.org

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José Manuel Nunes (Switzerland).

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Accepted: 2 April 2024

DOI: 10.1111/tan.15478

HLA Immune Response Genetics WILEY

Forward from the EFI President

Dear colleagues,

It is with pleasure that I welcome you to the 37th Annual European Immunogenetics and Histocompatibility Conference, taking place at Palexpo, Geneva.

The motto assigned to this conference is "Unveiling Diversity, Nurturing Transplant Bonds, Orchestrating Immunity". These themes are interrogated within the plenary sessions where population "diversity" will be the subject of the session held jointly by EFI and the Society for Immune Polymorphism. "Transplant bonds" will be strengthened by our appreciation of the role of living donors in kidney and stem cell transplantation. Finally, our ability to manipulate "immunity" to enable both transplantation of the most challenging highly sensitized patients, and the utilization of new therapies to treat acute leukemias, will enlighten all attendees.

During the opening session of the conference, we will be welcoming Professor Jamie Rossjohn from Melbourne, Australia who will deliver EFI's prestigious Ceppellini Award Lecture. Professor Rossjohn's research utilizes structural methods to enable detailed understanding of how T-cells function in the generation of immune responses against different antigen types. I congratulate Professor Rossjohn on this award and look forward to his lecture.

Also, during the opening session, we will receive presentations by the Julia Bodmer Prize awardee and the recipient of the HLA Journal award. Other awards including the oral abstract and poster prizes will be assessed during the meeting and awarded during the final session. Good luck to everyone that is participating.

This year I hope you will join us at a new and specific session to celebrate three EFI members that will be receiving the EFI medal, awarded to thank members for their services to our society.

My thanks are given to everyone contributing to this conference, especially to the local organizers Sylvie Ferrari-Lacraz and Jean Villard and their local team. I also am very appreciative of the input from our professional conference organizers Guarant, the chairs of the sessions, all the oral and poster presenters and the EFI Education and Scientific Committee members for their support in the organization of the scientific and teaching sessions.

We are also very grateful to the continued support from our corporate sponsors which is essential to enable us to host our wonderful EFI conferences.

This is our second visit to the beautiful city of Geneva. I wish everyone to have a stimulating experience at the conference and I hope you enjoy spending time and exchanging scientific dialogue with colleagues. I look forward to welcoming you all, to what promises to be an inspiring and successful conference.

Ann-Margaret Little
EFI President

Accepted: 2 April 2024

DOI: 10.1111/tan.15479

HLA Immune Response Genetics WILEY

ABSTRACT

Foreword from the Local Organizing Committee

Dear EFI members, dear participants, dear friends,

On behalf of the local organizing committee, we are very happy to welcome you to the 37th European Federation for Immunogenetics conference in the city of Geneva. It is with great pleasure and excitement that we gather once again to celebrate the remarkable achievements and ongoing advancements in the field of immunogenetics.

Under the theme "Unveiling Diversity, Nurturing Transplant Bonds, Orchestrating Immunity", this year's conference promises to be a dynamic forum for scientific exchange, collaboration, and inspiration. As we explore the intricate mechanisms of the immune system and delve into the genetic underpinnings of immunological responses, we uncover a wealth of knowledge that not only deepens our understanding but also opens new avenues for therapeutic interventions and personalized medicine.

The landscape of immunogenetics is constantly evolving, driven by breakthroughs in technology, innovative research methodologies, and the collective expertise of our diverse community. From unraveling the complexities of HLA typing, to pioneering novel approaches in transplantation medicine, our collective efforts continue to push the boundaries of what is possible, offering hope and healing to patients worldwide.

As we come together in Geneva, let us seize this opportunity to forge new collaborations, exchange ideas, and inspire one another to reach greater heights. Whether you are a seasoned researcher, a dedicated clinician, or a passionate advocate, your unique perspectives and contributions are integral to the success of our collective endeavors.

I would like to extend my heartfelt gratitude to the local team, the PCO, sponsors, speakers, and attendees whose dedication and enthusiasm have made this conference possible. Your commitment to advancing the field of immunogenetics and improving patient outcomes is truly commendable, and it is through our collaborative efforts that we will continue to make meaningful strides in the years to come.

I am confident that the 37th EFI Conference will be a memorable and enriching experience for all, filled with stimulating discussions, enlightening presentations, and lasting connections. May this gathering inspire us to embrace diversity, nurture bonds of collaboration, and orchestrate immunity in pursuit of a healthier and more equitable world.

Thank you for your participation and engagement, and I wish you a productive and rewarding conference.

Jean Villard.
On behalf of the Local Organizing Committee.

Accepted: 2 April 2024

DOI: 10.1111/tan.15480

HLA Immune Response Genetics WILEY

ABSTRACT

EFI Abstracts Oral and Posters

ORAL PRESENTATIONS

Best Abstracts

O1 | Do end-of-life events perturb the peptide landscape of transplant donor tissues

Akm Muraduzzaman[1], Ifrah Dini[1], Sanjay Krishna[2], Asolina Braun[1], Rochelle Ayala[1], Shanzou Chung[1], Patricia Illing[1], Anthony Purcell[1] and Nicole Mifsud[1]
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Brain death is accompanied by a catecholamine storm and a surge in proinflammatory cytokines and chemokines that provides a molecular switch for peptide generation via the immunoproteasome, whereas circulatory death exposes donor organs to extended warm ischemia and is associated with increased risk of delayed graft function. Peri-mortem events alter the tissue milieu in different ways, potentially perturbing the donor-derived self-peptide repertoire (i.e., total array of peptides known as the immunopeptidome) presented at the cell surface by HLA. We hypothesized that donation by brain death (DBD) or donation by circulatory death (DCD) exhibit differential immunopeptidomes that may influence allograft acceptance. Peptide/HLA class I complexes were isolated from tissues using immunoaffinity purification with HLA antibodies. Peptides were captured and identified using mass spectrometry. Data was analyzed for both the immunopeptidome and proteome (i.e., global proteins). Analysis of HLA-A*02:01 (A2) and pan HLA-I (A, B, C loci; excluding HLA-A2) immunopeptidomes identified both tissue-specific and ubiquitous peptides within the same donor, as well as peptides shared across all donors. A total of HLA-A2-restricted 19,639 heart-specific peptides (3 DBD, 1 DCD), 24,290 liver-specific peptides (2 DBD, 1 DCD), 63,516 spleen-specific peptides and 5439 PBMC peptides were identified. No differences were observed for peptide quantity, but distinct peptide landscapes were shared between DBD and DCD. Analyses for other HLA-I allotypes (e.g. A*01:01, B*08:01, B*57:01) mirrored the HLA-A2 data. Post-translational modifications revealed DBD had greater levels of oxidation, whereas DCD had increased acetylation. Proteomics analysis showed >100 differentially expressed proteins between DBD and DCD. This information provides a greater understanding of the influence of end-of-life events on the peptide repertoire across transplant donor categories.

O2 | High-resolution MHC sequencing reveals local genetic architecture of multiple sclerosis in a trans-ethnic cohort

Kristen Wade[1], Kerry Kizer[1], Rayo Suseno[1], Juliano Boquett[1], Stacy Caillier[1], Jorge Oksenberg[1], Paul Norman[2], Danillo G. Augusto[3] and Jill Hollenbach[1]
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Multiple sclerosis (MS) is a neurodegenerative disorder for which the primary risk allele, HLA-DRB1*15:01, was identified a half century ago. Nevertheless, multiple additional independent association signals have been mapped via single nucleotide polymorphism (SNP) studies to the extended major histocompatibility (MHC) region. However, the high levels of polymorphism, structural variation, and linkage disequilibrium characteristic of the MHC have confounded efforts to identify the true risk loci associated with these SNP signals. To address this knowledge gap, we applied targeted next-generation sequencing (NGS) to the entire extended 5 Mb MHC to comprehensively detail sequence variation across the region in two cohorts of MS cases and controls of Euro-American ($n = 382$) and African American ($n = 170$) ancestry. Using our novel pipeline, MHConstructor, we generated reference-free, de novo MHC sequence assemblies and performed association testing for disease across the entire region. We find five strong MS risk association signal peaks that occur in the same genomic locus in both the European and African

American cohorts, allowing definitive assignment of risk to these loci: SCAND3 downstream intergenic, OR2W1-AS1/OR2W1 upstream intergenic, HLA-G upstream intergenic, MUC21 downstream intergenic and SMIM40 upstream intergenic. We find evidence of an additional signal that occurs in a TSBP1 intron and TSBP1-AS1 in the African and European American cohorts, respectively. Of these, OR2W1, HLA-G and TSBP1 have previously been implicated as risk factors for multiple sclerosis and/or other autoimmune diseases. By examining all sequence variation across the region in this trans ancestral analysis, we provide the most high-resolution picture of MS risk variation within the MHC to-date, a critical step towards determining the full genetic underpinning of MS pathogenesis.

O3 | HLA class I mismatches associate with reduced survival in contemporary HCT with PTCy: A comprehensive study by the Cellular Therapy and Immunobiology Working Party of the EBMT

Esteban Arrieta-Bolaños[1], Edouard F. Bonneville[2], Pietro Crivello[1], Marie Robin[3], Tobias Gedde-Dahl[4], Urpu Salmenniemi[5], Nicolaus Kröger[6], Ibrahim Yakoub-Agha[7], Charles Crawley[8], Goda Choi[9], Annoek E.C. Broers[10], Edouard Forcade[11], Martin Carre[12], Xavier Poiré[13], Anne Huynh[14], Péter Reményi[15], Stig Lenhoff[16], Fabio Ciceri[17], Eleni Tholouli[18], Thomas Schröder[19], Eric Deconinck[20], Kristina Carlson[21], Liesbeth C. de Wreede[2], Jorinde D. Hoogenboom[22], Florent Malard[23], Annalisa Ruggeri[17] and Katharina Fleischhauer[24]

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HLA matching increases the chances of survival in allogeneic HCT with unrelated donors (UD). The introduction of post-transplant cyclophosphamide (PTCy) for GvHD prophylaxis is increasing the use of mismatched UD (i.e., <10/10), helping to improve access for patients lacking a fully matched donor. However, the role of HLA mismatches (mM) in current HCT practice using PTCy remains unclear. We studied outcomes in 17,292 adult patients with hematological malignancies reported to the EBMT Registry who underwent UD-HCT up to 2020. 23.5% of the transplants were performed across high-resolution HLA mM (9/10, $n = 3565$; 8/10, $n = 500$). PTCy was used in 7% and 15% of the 10/10 and <10/10 transplants, respectively. Overall survival (OS) was significantly lower in transplants across one (HR 1.24 [99% CI 1.15–1.34]; $p < 0.001$) or two (HR 1.29 [1.09–1.54]; $p < 0.001$) mM compared to 10/10 transplants. HLA class I (HR 1.31 [1.20–1.42]; $p < 0.001$) but not class II mM (HR 1.07 [0.93–1.22]; $p = 0.23$) were associated with significantly worse OS. mM at HLA-A (HR 1.37 [1.21–1.54]; $p < 0.001$) and HLA-B (HR 1.44 [1.23–1.69]; $p < 0.001$) conferred higher risks than HLA-C (HR 1.16 [1.01–1.33]; $p = 0.005$), and antigen-level mM associated with worse OS than allelic mM (HR 1.22 [1.01–1.46]; $p = 0.006$). PTCy significantly reduced the risks of GvHD and mortality compared to standard prophylaxis. However, the effects of HLA mM were similar, with a single mM conferring increased mortality risks with (HR 1.38 [1.09–1.75]; $p < 0.001$) and without (HR 1.23 [1.13–1.33]; $p < 0.001$) PTCy. Finally, immunopeptidomics-based class I peptide-binding motif (PBM) grouping identified PBM-GvH-matched pairs having better OS compared to PBM-GvH mM (HR 1.2 [1.02–1.4]; $p = 0.004$), both with and without PTCy. In conclusion, HLA class I mM are associated with increased risks of mortality in contemporary HCT, even under GvHD prophylaxis with PTCy. Better-tolerated PBM-matched mM could help provide the best possible outcome for all patients.

O4 | Megakaryocytes with downregulated HLA class I and II expression are protected from cellular and humoral allogeneic immune responses

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Platelet transfusion refractoriness (PTR) is a life-threatening condition which develops as a result of multiple transfusions. The main immunological cause is the formation of alloantibodies specific for HLA class I. As a consequence, the platelet (PLT) count does not increase upon transfusion in refractory patients. Currently, PTR caused by HLA antibodies is an unsolved problem. In our study, we aim to generate low immunogenic, universal Megakaryocytes (MKs) as an alternative to platelet preparations in thrombocytopenic patients. To generate HLA-universal MKs and PLTs, we reprogrammed PBMCs from selected donors with blood type O Rhesus negative into iPSCs. To further reduce the immunogenicity of the MKs, the iPSCs were genetically modified using lentiviral vectors encoding for shRNAs targeting HLA class I and/or class II transcripts. The HLA-silenced iPSCs fully maintained the capacity to differentiate towards functional MKs. The effect of silencing HLA class I and/or II expression on the strength of allogeneic immune responses was investigated in vitro and in vivo using a platelet refractoriness mouse model. In the in vitro setting, HLA-silenced MKs decreased MK-induced allogeneic CD4 and CD8 T cell proliferation ($p < 0.001$) and cytotoxicity ($p < 0.05$), compared to non-engineered MKs. Furthermore, Granzyme B secretion levels in T-cells were significantly ($p < 0.01$) reduced, even more evidently when HLA class I and II-silenced MKs were used (52%). Moreover, in the animal model, after transfusion of HLA-silenced MKs, the frequency of MK-produced PLTs in the mouse circulation remained stable compared to transfusion with control MKs. In parallel, to be able to produce MKs in a therapeutic scale, we scaled up our differentiation protocol in a GMP-compatible bioreactor system. In conclusion, the use of HLA-silenced MKs could open new frontiers in the management of highly sensitized thrombocytopenic patients.

O5 | Molecular diversity of HLA genes in Africa: Extensive analysis of long-range DNA sequences for 12 class I and class II loci in 32 populations living across the whole continent

Ndeye K. Faye[1], Thomas Goeury[1], Viktor Černý[2], Hiba Babiker[3], Hacene Brouk[4], Eric Crubézy[5], Youssef Idaghdour[6], Mohammed Melhaoui[7], Harilanto Razafindrazaka[8], Audrey Sabbagh[9], Carina Schlebusch[10], Paul Verdu[11], Amélie Chimènes[11], Pascale Gerbault[1], José Manuel Nunes[1] and Alicia Sanchez-Mazas[1]

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The characterization of HLA diversity in populations may bring fundamental knowledge on humans' demographic history and adaptation to environments, including resistance to diseases. Assessing HLA variation across populations is also needed in clinical studies such as histocompatibility testing and stem cell transplantation where the search of compatible donors is a priority. However, although hundreds of populations have been described for HLA so far, African samples remain largely under-represented in HLA studies and genetic databases. To address this, we fully sequenced the 12 HLA-A, -B, -C, -DRA, -DRB1, -DRB3, -DRB4, -DRB5, -DQA1, -DQB1, -DPA1 and -DPB1 genes using a PacBio SMRT technology in a large set of about 2000 individuals from 32 populations living in North, West, East, Central and South Africa. We then analyzed and compared their molecular diversity within and between populations through various biostatistical methods applied to both IPD-IMGT/HLA Database-defined alleles and nucleotide sequences. The whole set of nearly 45,000 extended sequences displays substantial variation along each HLA gene, showing peaks of molecular diversity in specific gene regions (e.g., HLA-DQB1 intron 2, exon 2, intron 3 and HLA-B exons 1, 2, 3) and either contrasted (for HLA-DR) or comparable (for HLA-DQ and -DP) variation between the A and B genes of HLA class II molecules. Allele and haplotype frequencies differ substantially across geographic regions and unique profiles are observed in some small populations like Dogon (Mali), Raashayda (Sudan), Bezan (Cameroon) and Fulani (several regions), which underlines their specific way of life. Among other remarkable results, we also found

some notable frequency differences between 2nd and 3rd field allelic profiles as well as a new DRA 2nd-field allele exceeding 10% in West Africa. Overall, this study represents a highly significant step forward in our understanding of HLA molecular variation in humans for both fundamental and clinical research.

O6 | Natural killer and T cell repertoire reconstitution is established early after allogeneic hematopoietic stem cell transp lantation and is profoundly imprinted by CMV reactivation

Antonia Schäfer[1], Zuleika Calderin Sollet[1], Marie-Priscille Hervé[1], Stéphane Buhler[1], Sylvie Ferrari-Lacraz[1], Paul Norman[2], Katherine Kichula[2], Ticiana Farias[3], Stavroula Maouridi-Levrat[1], Anne-Claire Mamez[1], Amandine Pradier[1], Federico Simonetta[1], Yves Chalandon[1] and Jean Villard[1]

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Besides genetic influences, non-genetic factors such as graft-versus-host disease (GvHD) and viral infections have been shown as important shapers of the immune reconstitution and diversification processes after hematopoietic stem cell transplantation (HSCT). However, the differential susceptibility to immune modulation by non-genetic factors is not fully understood. We determined to follow the reconstitution of the T cell receptor (TCR) repertoire through immune-sequencing, of natural killer (NK) cells using a 35-marker spectral flow cytometry panel, and in relation to clinical events. Longitudinal investigation was performed on samples derived from 54 HSCT recipients during the first-year post-HSCT. We confirmed a significant contraction in TCR repertoire diversity with a remarkable stability over time. CMV reactivation had the ability to significantly change TCR repertoire clonality and composition, with a long-lasting imprint. Our data further revealed skewing of NK cell reconstitution in CMV reactivated recipients, with an increased frequency of KIR2DL2L3S2+ adaptive, cytolytic and functional CD107a + NK cells concomitant with a reduced pool of NKG2A+ NK cells. We demonstrated that CMV behaves as a strong driver of peripheral homeostatic proliferation of circulating specific T and NK cells, which can be viewed as a compensatory mechanism to establish a new peripheral repertoire.

O7 | Peptide sharing between CMV and mismatched HLA class I peptides promotes T-cell-mediated rejection after kidney transplantation

Emma Peereboom[1], Jasvir Jairam[1], Frans Verduyn Lunel[1], Anna Blok[1], Arjan van Zuilen[1] and Eric Spierings[2]

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Cytomegalovirus (CMV) infection is related to acute rejection and graft loss after kidney transplantation, though the underlying mechanism remains largely unknown. Some CMV strains produce a peptide that is identical to a peptide sequence found in the signal peptide of specific HLA class I alleles. In this retrospective study of 486 kidney transplantations, we explored whether CMV-seropositive recipients without such an HLA class I peptide who received a transplant from a donor with this peptide, faced an increased risk of T-cell-mediated rejection (TCMR) in the first 90 days after transplantation. The combination of recipient CMV seropositivity with the VMAPRTLIL peptide mismatch was associated with TCMR with a hazard ratio of 3.01 ($p = 0.0001$) in a multivariate analysis. Similarly, the VMAPRTLLL peptide mismatch was associated with TCMR revealing a hazard ratio of 2.06 ($p = 0.03$). Transplantations featuring either a VMAPRTLIL or a VMAPRTLLL peptide mismatch had a significantly higher cumulative TCMR incidence ($p < 0.0001$), with the primary impact observed in the first 2 weeks post-transplantation. In summary, our data strongly suggest that CMV-positive recipients without an HLA class I peptide identical to a CMV peptide yet transplanted with a donor who do possess this peptide, have a significantly increased risk of early TCMR. Considering the prevention of such an HLA class I peptide mismatch in these patients or adjusting immunosuppression protocols accordingly may hold promise in reducing the incidence of early TCMR.

O8 | Structural basis of public TCR recognition of the immunodominant NQK-Q8 spike epitope presented by the COVID-19 protective HLA-B15 molecule

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Although COVID-19 poses a significant threat to human health, around 20% of individuals infected with SARS-CoV-2 remain asymptomatic. While much attention has been given to identifying factors that contribute to severe COVID-19, studying asymptomatic cases offers a valuable opportunity to explore early disease and immunological features that facilitate rapid viral clearance. With our study on 29,947 individuals registered in the National Marrow Donor Program, part of the UCSF Citizen Science smartphone-based study aimed at tracking COVID-19 symptoms, we discovered a strong association between HLA-B*15:01 and asymptomatic infection. This suggests the potential presence of a pre-existing immune response, towards the NQK-Q8 epitope, that would protect HLA-B15+ individuals, given the role of HLA in presenting viral peptides to T cells. We identified the presence of high-affinity public TCRs able to cross-react with the seasonal coronavirus derived homologous peptide (NQK-A8). We have further studied those public TCRs to understand how they can engage with the homologous peptides, and with newly identified mutant peptide from the JLN1 variant. We discovered that the public TCRs were binding with an unusual docking mode onto the epitopes providing the basis of T cell cross-reactivity observed in HLA-B15+ individuals.

AUTOIMMUNITY, INFECTION, REPRODUCTION & CANCER

O9 | Potential imprinting of frequent HLA-DPB1 alleles on the genome and immunogenicity of cytomegalovirus

Luisa Betke[1], Fabienne Maaßen[2], Pietro Crivello[1], Esteban Arrieta-Bolaños[1], Corinna Schuler[3], Vu Thuy Khanh Le-Trilling[3], Sebastian Voigt[3], Thomas Schröder[4], Mirko Trilling[5] and Katharina Fleischhauer[6]

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HLA-DPB1 exhibits variability in allele frequencies. DPB1*02:01, *04:01, and *04:02 are present in approximately 70% of the European population. These abundant alleles share structural features leading to similar peptide motifs and overlapping immunopeptidomes. They comprise a core T-cell epitope group (TCE3-c) of DPB1 alleles which contrasts with the less frequent DPB1 alleles, characterized by distinct structural features, referred to as TCE-non-core (TCE-nc). In HCT, infection and/or reactivation of human cytomegalovirus (CMV) are frequent and clinically relevant events. CMV is known for its long-term coexistence in latently infected hosts and studies using the mouse cytomegalovirus model argue in favor of immune selection mediated by CD4+ T cells. This raises the hypothesis that CMV epitopes presented by frequent HLA allotypes such as those encoded by DPB1 TCE3-c alleles, may have been negatively selected during CMV evolution. To test this, we predicted CMV peptide binding to the 3 DPB1 TCE3-c and 5 TCE-nc DPB1 allotypes on the NetMHCIIpan4.3 platform. A significantly lower number of strong binding CMV peptides (340 vs 564; $p = 0.0003$) and respective binding scores (0.7 vs 0.74; $p < 0.0001$) were predicted for the TCE3-c compared to the TCE-nc DPB1 allotypes. Moreover, in 39 CMV seropositive healthy donors, we found significantly lower specific CD4+ T-cell responses against CMV lysates loaded onto artificial antigen-presenting cells expressing TCE3-c compared to TCE-nc DPB1 alleles (2.3 vs 6.4%; $p = 0.003$). Interestingly, in 2 patients after HCT from donors heterozygous for one TCE3-c and another TCE-nc allele, CMV responses against the former were consistently lower than against the latter. This effect was also observed in leukemia cells presenting CMV. Taken together, these findings are compatible with an imprinting of the CMV genome to evade from frequent DPB1 TCE3-c alleles, with potential implications for CMV-related related risk stratification in HCT.

O10 | HLA variation shapes the autoantibody repertoire in healthy individuals

Juliano Boquett[1], Ravi Dandekar[1], Rayo Suseno[1], Tom Ituarte[1], Bonny Alavarenga[1], Cynthia Vierra-Green[2], Stephen Spellman[2], Michael Wilson[1], Martin Maiers[2] and Jill Hollenbach[1]

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While autoantibodies are usually associated with autoimmune disease, they are also found in healthy individuals. We determined the specificity of antibodies in serum

samples from healthy subjects, stratified by HLA genotype, to understand how HLA variation shapes the autoantibody repertoire. We utilized Phage ImmunoPrecipitation Sequencing (PhIP-Seq), a programmable phage display for interrogation of antibody specificity. The assay encompasses 744,000 peptides tiled across the entire human proteome, including all known structural and splice variants. Serum samples from 100 healthy individuals homozygous for HLA-DRB1*03:01, HLA-DRB1*04:01, HLA-DRB1*07:01 or HLA-DRB1*15:01 alleles were analyzed for differences in autoantibody specificity. Comparing the peptide fold change from samples vs a control bead with the XGBoost machine learning tool, we were able to predict whether a sample is HLA-DRB1*15:01 positive or not with an accuracy of 82.5%; and HLA-DRB1*07:01, HLA-DRB1*04:01 and HLA-DRB1*03:01 positive or not with accuracies of 75%, 70% and 80% respectively. Using peptide average fold change, the ratio of enrichment in the allele of interest vs the three remaining alleles was also analyzed. The enriched peptides in the group of interest were used to sort and filter for genes, in some cases revealing specificity for antigens associated with autoimmune disease. For example, HLA-DRB1*03:01 is a known risk allele for systemic erythematous lupus (SLE). In the HLA-DRB1*03:01 group, antibodies against peptides from IKZF1, a gene associated with SLE, presented significant enrichment in comparison to the other three HLA-DRB1 groups. These results support the notion that autoantibody repertoires in healthy people vary according to HLA class II types, and suggest that individuals with specific HLA autoimmune risk alleles may harbor potentially pathogenic autoantibodies in the absence of, or prior to the establishment of, overt disease.

O11 | Heterogeneity of HLA-associated type 1 diabetes susceptibility among populations suggests that some risk may be conferred by novel mechanisms

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HLA association with type 1 diabetes was first reported more than 50 years ago. Although much is known about this association, some questions remain. The DR and DQ-encoding loci are well established to be the most strongly T1D-associated HLA loci, with risk for given alleles and haplotypes usually demonstrating consistent risk effects among populations. However, even the strong, widespread association of the DRB1*03:01 allele is not universal. For other classical HLA loci, B*39:06 and A*24:02 are often positively associated with T1D, and DPB1*04:02 is often negatively associated. Few other alleles at these loci show consistent risk among populations. We recently reported data from T1D patients and controls in Mali that suggest haplotypic context affects the apparent risk effect for some alleles. Further, we looked at the effect of individual amino acids within the loci and found that, while the T1D-associated amino acid positions for HLA-A and HLA-B were, as expected, primarily located in the exons that encode the peptide-binding groove, the T1D-associated amino acid positions in the HLA-C gene were more numerous and were spread throughout the coding sequence. Combined with the lack of consistency in HLA-C individual allele associations reported among populations, this leads to the hypothesis that T1D susceptibility conferred by HLA-C may rely, at least in part, on a mechanism other than traditional antigen presentation. In this report, we will address this hypothesis using amino acid analysis from five additional populations, including Azerbaijan, Bangladesh, Sudan, Haiti, and Pakistan. We aim to show

that the traditional method of simply assessing individual allele associations with T1D, while important, does not fully capture the entirety of HLA-associated T1D risk.

O12 | Single-cell transcriptomics of immune cells from zymosan-treated mice clarifies the anti-inflammatory effect of β-NMN in sepsis treatment

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Sepsis is a syndrome of shock and dysfunction of multiple vital organs associated with a high mortality rate that is caused by an uncontrolled inflammatory immune response to infection. No specific therapy or drugs are available for sepsis. Nicotinamide adenine dinucleotide (NAD+) is an important regulator of immunoinflammatory responses. We previously showed in a mouse model that the administration of NMN, a natural biosynthetic precursor of NAD, attenuates the dysregulated inflammatory response during sepsis and thus prevents clinical deterioration and improves survival. To better understand this effect of NMN, we collected immune cells that accumulate in the peritoneal cavity 24 h following intraperitoneal (i.p.) administration of the fungal cell wall extract zymosan (a trigger of sepsis shock), with or without pretreatment by NMN. Mice were administered either 185 mg/kg of NMN (NMN group; $n = 3$) or saline (control group; $n = 3$) delivered by the intraperitoneal route. The immune cells collected on the site of injection were subjected to single-cell transcriptomic sequencing (scRNA-seq). UMAP clustering analysis of scRNA-seq data followed by differential gene expression between the two conditions showed that two cell populations were strongly affected by the NMN treatment. First, we observed that neutrophils from NMN group migrate to the injection site in greater abundance and keep their oxidative phosphorylation metabolism. In contrast, neutrophils from the control group switch to a glycolysis metabolism and express genes characteristic of high ROS production. Second, we identified two antagonistic sub-populations of macrophages in NMN-treated versus untreated mice. The population predominantly present in the control group is dependent on the NF-κB transcription factor and expresses many inflammatory genes (TNF-α, Il1, and Il6). The sub-population over-represented in the NMN group relies on the Irf7 transcription factor and expresses genes from the IFN-I pathway.

O13 | Paternal HLA-derived epitopes and live birth in secondary recurrent pregnancy loss: New insights from a clinical trial

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Recurrent pregnancy loss (RPL), defined as two or more pregnancy losses before the 24th week of gestation, affects 1%–3% of women worldwide. Approximately 40% of RPL cases are secondary RPL (sRPL), where women have given birth before facing pregnancy losses. The underlying causes of RPL remain unclear, but immune-related factors may play a role. Previously, a randomized controlled trial using immunoglobulin (IVIG) in sRPL women with a history of four pregnancy losses performed in our RPL unit did not show significant effects of IVIG treatment overall. Yet, some evidence suggests potential benefits for a subset of sRPL patients. In the cohort used for the randomized controlled trial, we examined the role of maternal HLA class II-presented fetal HLA-derived epitopes in sRPL using the Predicted Indirectly Recognizable HLA Epitopes (PIRCHE-II) algorithm. In the placebo group, sRPL mothers with an anti-HLA antibody response had higher PIRCHE-II scores when having a live birth compared with sRPL women who experienced another pregnancy loss. This difference was not observed in the IVIG-treated group. Furthermore, as a proxy for T-cell memory, the number of overlapping peptides between the two paternal haplotypes in couples having live births without treatment displayed a larger number of overlapping peptides. This effect was primarily driven by class II-derived peptides. These results suggest that specific combinations of sRPL mothers and fathers, particularly those with an anti-HLA antibody response, may generate higher PIRCHE-II scores which could contribute to successful live births. Understanding these immune interactions may provide insights for personalized diagnostic and therapeutic strategies in sRPL.

O14 | Characterization of LAIR-1 polymorphism, an inhibitory collagen-binding receptor, present in various primate species

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The leukocyte-associated immunoglobulin-like receptor-1 (LAIR-1) gene is situated within the leukocyte receptor complex (LRC) on chromosome 19, where the KIR and LILR genes are also located. LAIR-1 serves as an immune inhibitory receptor and is present on T cells, B cells, NK cells and monocytes. The expression of LAIR-1 is regulated by the differentiation and activation status of these cells. In addition to collagen, complement component 1q (C1q) has been identified as a ligand for LAIR-1. Extensive studies have been conducted on LAIR-1 in humans. The potential role of LAIR-1 in the development of autoimmune diseases, tumors, and malaria makes it an interesting receptor to investigate as a therapeutic target. Nevertheless, information regarding LAIR-1 in non-human primates is currently lacking. In the present study, we characterized full-length LAIR-1 transcripts in a panel of chimpanzees ($n = 18$), gorillas ($n = 3$), rhesus macaques ($n = 23$), cynomolgus macaques ($n = 40$), hamadryas baboons ($n = 15$), and common marmosets ($n = 22$). The results revealed polymorphism in LAIR-1 in the two macaque species, with significant differences among alleles. Phylogenetic analysis substantiated this, demonstrating clustering of various macaque LAIR-1 alleles into at least five distinct lineages. In contrast, the LAIR-1 gene appears to be conserved in chimpanzees, gorillas, hamadryas baboons and common marmosets, aligning with the human equivalent. Additionally, different alternative splice events were identified, including a 51 bp deletion in exon 4 (resulting in a 17 amino acid deletion). This deletion is frequently observed in humans and results in a shorter stalk region. The possible implications of these findings will be discussed.

O15 | HLA combination analysis reveals differentially multiple sclerosis-associated combinations, suggesting differences in the underlying pathogenesis mechanism

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HLA associations in Multiple Sclerosis (MS) involve 13 MS-alleles (7 susceptible: LTA-H51P, HLA-DRB1*03:01, 08:01, 13:03, 15:01, HLA-DQB1*03:02, HLA-DPB1*03:01 and 6 protective: HLA-A*02:01, HLA-B*38:01, 44:02, 55:01, HLA-DQA1*01:01, HLA-DQB1*03:01). Our objective was to identify HLA-wide combinations of those 13 alleles, differentially associated with MS. We analyzed WTCCC HLA data for 11,376 MS-cases and 18,872 controls. We used principal component analysis (PCA) for genetic homogeneity selection. HLA alleles, imputed using the HIBAG R package or inferred with proxy SNPs (rs2229092, rs9273912 and rs9277565), were recoded to consider only the 13 MS-associated alleles. We searched for MS-associated genotype combinations in a 20% subsample, nominal combinations being to be tested for replication in the remaining 80%. Confirmed combinations were investigated in the whole dataset. Haplomat provided putative diplotypes. Following PCA analysis, we retained 9024 MS and 13,923 controls. In the 20% sub-sample, we observed 776 different HLA-wide recoded genotype combinations, 41 were nominally MS-associated ($P < 0.05$) and 22 of those were replicated (P corrected <0.05), 14 susceptible combinations (23.61% MS) (odds ratios (OR) 1.83–6.75), within which 9 had another one with non-overlapping OR 95% confidence intervals, 8 protective combinations (4.29% MS) (OR 0.30–0.57). Within those HLA-combinations, using unrecorded HLA typing, alleles and putative diplotypes frequencies were similar between MS and controls, suggesting that no other alleles than the 13 alleles provide MS genetic risk. Such differentially associated HLA-combinations suggest a direct contribution to different HLA-associated pathogenesis pathways, with potential relevance for personalized medicine in MS and other HLA-associated diseases. Supported by LabEx IGO project (ANR-11-LABX-0016) funded by Investissements d'Avenir program and the French National Research Agency.

O16 | Gene expression of TLRs, MYD88 and NFκB associated with risk for severe autism

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Autism is a neurodevelopmental disorder with an unknown etiology but a genetic and immune component. More than 1000 genes involved in the development of autism have been discovered, and researchers do not have a consensus. More often, autoimmune and inflammatory reactions are found in autistic people, which may contribute to the pathogenesis of the disease. The key mediator of inflammatory processes is NFκB, whose classical method of activation in cells of the immune system is released by TLRs through MyD88, the inhibitor is IκBα. Aberrations in the expression of genes in the inflammatory signaling pathway in cells of the immune system may act as a risk factor for the development of autism and its severity. The aim of the study was to assess the expression of genes of signaling pathways regulating cytokine production: TLR2, TLR4, MyD88, NFκB (p50 and p65) and IκBα in leukocytes of children with varying severity of autism. The study involved 81 children with autism spectrum disorder (ASD) and 45 typically development children. All children with ASD were divided into 2 groups using the Childhood Autism Rating Scale (CARS): 51 with mild to moderate autism and 30 with severe autism. Messenger RNA (mRNA) of TLR2, TLR4, MyD88, NFκB (p50 and p65) and IκBα was measured in peripheral blood leukocytes by qRT-PCR real-time using specific primers. The statistics was calculated using one-way ANOVA and Tukey's test. It was found that in the leukocytes of children with severe ASD the expression of MyD88 ($P = 0.034$) and the p65 subunit of NFκB ($P = 0.013$) was reduced and expression of IκBα ($P = 0.006$) was increased compared to healthy controls. Thus, we have shown that low levels of MyD88 and p65 subunit of NFκB and high levels of IκBα may be associated with the risk of developing severe autism in children. It can be assumed, that the decrease in the functional activity of leukocytes may be an adaptation to chronic systemic low-grade inflammation, which we indicated in these children.

BIOINFORMATICS, DATA ANALYSIS IN IMMUNOGENETICS

O17 | Assessment of HLA-DQ genetic variation with an HLA-DQ heterodimers tool and implications in clinical transplantation across diverse populations

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HLA-DQ is an αβ heterodimer formed by the β chain product of HLA-DQB1 and an α chain product of HLA-DQA1. In addition to molecules defined by the parentally-inherited cis haplotypes, α-β trans-dimerization is possible between certain alleles, leading to up to four unique HLA-DQ molecules and a potential source of mismatched molecules. Recently, researchers uncovered that clinical outcome after HLA-DQB1-mismatched unrelated donor HCT depends on the total number of HLA-DQ molecule mismatches and the specific αβ heterodimer mismatch, where HLA-DQ molecules in Group 2 (G2) were associated with worse outcomes when compared to Group 1 (G1) HLA-DQ molecules. We developed a tool called QLASSy (HLA-DQA1 and HLA-DQB1 Heterodimers Assessment) to automate these complex rules to optimize the selection of candidate stem cell sources. To enhance the model, our tool also imputes missing HLA-DQA1, provides information on the quality of annotation for trans-dimerized αβ heterodimers, and highlights HLA-DQ-optimal sources. In our study, we leverage 668,496 high-confidence, statistically-phased (via a modified expectation–maximization algorithm) HLA-DRB1∼DQA1∼DQB1 haplotypes, 1052 pedigree-phased HLA-DQA1∼DQB1 haplotypes, and 62,756 historical transplants to characterize HLA-DQ heterodimers data across multiple race and ethnicity populations. Here, we uncovered more high-risk HLA-DQ molecules in Asian/Pacific Islander and Black/African American populations, demonstrated the effect of HLA-DQA1 and HLA-DQB1 mismatching on HLA-DQ molecular mismatches, and highlighted where source selections could be improved at the time of the search for historical transplants with this new HLA-DQ information (where 65% of G2-mismatched transplants had lower-risk, G2-matched alternatives) across diverse populations. Altogether, this tool and these analyses serve as actionable resources for enhancing source selection and improving outcomes for all patients.

O18 | Kidney graft survival is predicted by allele-specific solvent-accessible surface-protruding amino acid mismatches and indirect T-Cell epitopes

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Antibody epitope and indirect T-cell epitope compatibility have shown complementary roles in supporting HLA antigen matching in predicting organ transplantation outcomes. An optimal combination and configuration of the specialized predictors of histocompatibility has however not been established. XGBoost models considering death-censored graft survival were applied to the SRTR kidney transplant dataset based on numbers of amino acid mismatches, Eplet mismatches (HLA Matchmaker), HLA mismatches per locus, the recently suggested Snow algorithm and an updated version of PIRCHE (v4.2). Ethnicity-guided multiple imputation based on National Marrow Donor Program haplotype frequencies was applied to serological HLA typing. A sensitivity analysis was carried out using repeated random sampling. The analysis considered 400,935 complete cases. All tested molecular compatibility metrics were found to correlate with graft survival using XGBoost models. Optimal concordance index and integrated area under curve (c = 0.54, auc = 0.56) for histocompatibility models was assessed by a model of HLA-A, -B, -DR matching, PIRCHE-II- and Snow-scores per locus, respectively. Performance of a baseline prediction model of donor/recipient ethnicity and age, maintenance immunosuppression, CMV mismatch and number of previous transplantations could be improved by adding molecular matching scores (c = 0.66, auc = 0.68).The evaluated molecular compatibility metrics are predictive of kidney graft survival considering the SRTR dataset. Antibody epitope and indirect T cell epitope predictors were shown to enrich HLA antigen matching and improve clinical prediction models. This confirms the necessity of joint models for optimal assessment of histocompatibility in transplantation.

O19 | HLA amino acid residues determining epitope defining HLA serotypes under the spotlight

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HLA antigens were historically defined according to the unique reactivity pattern of cells expressing HLA molecules with distinctive clusters of allo-antisera and/or monoclonal antibodies. Subsequently, amino acid (AA) residue patterns determining epitope (DEP) in the HLA molecule were correlated with the reactivity patterns. In the current clinical practice, the presence of the allo-antibodies is assessed using the solid phase single antigen bead (SAB) system for transplantation. Recently, novel serotypes were proposed for the HLA molecules those DEP do not fit with the known antigens. To validate the serotypes, mean fluorescence intensity (MFI) values of SABs tested with >13,000 patients' sera were extracted from the clinical database and analyzed by scatter plots and linear regression model. High correlations ($R^2 > 0.95$) were observed when two proteins were considered as the same serotype, for example, A*02:01 and A*02:06. In contrast, discrete asymmetric outliers were observed when they were different serotypes, for example, A*66:01 and A*66:02, confirming a total of 14 novel serotypes. The outliers according to the residue positions with lower correlation were confirmed to be false positive reactions in the SAB assay when tested by the flow cytometric cross-matches (FXM). In addition to the previously defined residues for serotype assignments, findings in the validation process indicate further distinction between common antigens by evaluating the substitutions at residue 67 of HLA-B, 67 of DRB1 and 74 of DRB3. Our analyses validated all the previously selected AA positions for assessing serotypes and allowed to exclude many eplets that were thought initially to play a relevant role defining serologic epitopes. Taken together improvements in molecular typing to assess donor's serotypes and the optimized development SAB panels will improve virtual DSA assessment. The novel serotype classification will allow for optimal donor allocation of organs according to HLA mismatches.

O20 | Imputing HLA class II alleles in admixed populations with high accuracy

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HLA class II genes are essential in immune response modulation and are highly polymorphic, influencing disease susceptibility and transplantation outcomes. The SNP-HLA Reference Consortium (SHLARC) aims to improve HLA imputation from SNP array data to accelerate immunogenetics studies. Current reference panels mainly represent homogenous populations, limiting accuracy for diverse ancestry backgrounds. We obtained SNP genotypes and HLA alleles from 30X WGS for all unrelated individuals from the 1000 Genomes (1KG, $n = 2504$) and SABE (Brazilians, $n = 1170$) cohorts using the hla-mapper workflow. We evaluated HLA imputation for HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQB1, and HLA-DRB1 genes. Three imputation models were created for each gene using HIBAG: 1KG, SABE, and full (1KG + SABE) reference. We applied these models to predict HLA alleles in an independent Brazilian population ($n = 192$) with SNP array data and HLA calls from commercial kits. Furthermore, we compared our results with those obtained from the Michigan Imputation Server ($n \approx 20{,}000$). The full model achieved the highest overall accuracy (94.8%), as determined by the percentage of correctly predicted class II alleles, surpassing the Michigan Server (87.0%); 1KG or SABE models showed accuracies of 94.0% and 94.7%, respectively. Moreover, the full model outperformed other models in terms of F1 score. As an example, the Michigan Server displayed the lower mean F1 score for HLA-DPA1 (0.31 vs. 0.80 for the full model) possibly due to its inability to predict less frequent alleles, not represented in its reference panel. In conclusion, our findings emphasize the critical need to improve and adapt reference panels to capture the genetic diversity inherent in diverse target populations comprehensively. This approach is essential for improving HLA imputation accuracy and advancing our understanding of immunogenetics across diverse populations.

O21 | IPD-IMGT/HLA sequence feature annotation tool

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The IPD-IMGT/HLA Database is a centralized repository for the allelic variants of HLA named by the WHO Nomenclature Committee for factors of the HLA system. It receives submissions of data from all over the world which are expertly curated and assigned official designations. To submit to IPD-IMGT/HLA sequences must be annotated for their exons and introns. This allows the coding sequence to be extracted and translated allowing the correct allele designation to be assigned. Annotating the sequence can be a time consuming and laborious process, particularly for submitters with limited bioinformatics support. To facilitate the submission of data to the IPD-IMGT/HLA Database we have developed the Sequence Feature Annotation Tool. This tool allows users to provide a genomic or coding sequence and have the exon and intron features automatically annotated. The tool uses an alignment-based approach to annotate homologous feature positions using the IPD-IMGT/HLA Database reference sequences. The tool can quickly and accurately annotate homologous features, however more complex sequences such as splice site variants may show variation from the expected annotation and accuracy cannot be guaranteed; cDNA sequencing remains the most accurate way of determining the coding sequence in these cases. The tool has an intuitive web-based UI which allows users to download results in a variety of formats for submission to the EMBL-European Nucleotide Archive or GenBank as well as a JSON format which can also be obtained directly from an Application Programming Interface. This work helps facilitate submission of data to IPD, providing support for bioinformatic users through an API and clinical scientists and small laboratories through a web form, streamlining the process improving the accessibility of submitting to the database.

O22 | When HLA individual data is no longer personal health information: Developing digital genetic twins under the balance of utility and privacy

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In the era of Big Data, anonymization using synthetic data simulation is the backbone of privacy reinforcement of sensitive patient information and a holy grail to unleash post-hoc analyses. While the HLA field has pioneered data sharing, General Data Protection Regulation underlined vigilance to privacy risks with legal consequences. Thus, there is a need to expand anonymized digital twin creation to individual HLA genotypes, while preserving the utility of the data. From the US National Marrow Donor Program, restricted to European Caucasians ($N = 1{,}242{,}890$), we estimated haplotype frequencies by the expectation–maximization algorithm, implemented in Hapl-o-mat, as a basis to subsequently sample in silico HLA digital twins mimicking the representability of real patients' HLA genotype data. Hence, we developed 4 methods with increasing complexity: (1) a naive weighted sampling of 2 haplotypes; (2) a prior statistical phasing of haplotypes to sample phased diplotypes; (3) a hybrid of both methods to fortify reidentification metrics; (4) similar as (3), save for a correction algorithm to maintain a more precise haplotypes distribution in the synthetic HLA population. For each of the 4 methods, we ran simulations with 10,000 synthetic patients, checked the consistency of the data and calculated for each method the fraction of re-identified patients. The haplotype frequency distributions between the real and the synthetic HLA population match well (modified Hellinger distance <0.2). The 1st, 2nd, 3rd and 4th methods have a re-identification percentage of 12%, 7%, 10% and 5%, respectively. We showed that both utility and privacy of the synthetic HLA population are well conserved, especially for the 2nd method and even more for the 4th method. This work paves the way for in silico data to be reutilized without breaching privacy in patients' genetic data. It showcases facilitated data-sharing of HLA data under the umbrella of open research.

O23 | AlloPipe provides new insights in alloreactivity and related immune processes

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Recipients transplanted with a genoidentical donor can develop Graft-versus-Host disease after allogeneic hematopoietic stem cell transplantation (aHSCT), highlighting the contribution of polymorphisms outside the MHC region in triggering alloreactivity. Here, we report a bioinformatics tool called AlloPipe, which retrieves the non-synonymous polymorphisms (nsSNP) within a donor/recipient (D/R) pair from exome sequencing data and then infer their immunopeptidome differences. AlloPipe is a standalone tool divided into two distinct modules: Allo-Count and Allo-Affinity. Allo-Count compares the exomes within the D/R pair and compiles the nsSNP into a unique file. If the HLA typing is known, this can be processed by Allo-Affinity to output the differences between the immunopeptidomes within the pair. Allo-Count and Allo-Affinity outputs can be approached either quantitatively or qualitatively as they report information of interest on the nsSNP or peptides respectively. Allo-Count, first developed in the context of kidney transplantation, has highlighted the correlation between the amount of nsSNP within the D/R pair and the occurrence of chronic graft rejection ($n = 53$ pairs, previously published). We are currently testing whether this correlation can be extrapolated to acute or chronic GvHD in the context of aHSCT by analyzing genoidentical ($n = 71$) and haploidentical ($n = 40$) pairs. As Allo-Affinity outputs the list of candidate peptides for priming the alloreactive response with relevant information (i.e. related protein, gene and transcript), it offers the potential to customize the AlloPipe tool by shortlisting genes of interest. AlloPipe is therefore a powerful and flexible tool that provides insights to better understand alloreactive processes. It could be used to better match donors and recipients or to personalize immunosuppressive therapies after transplantation. AlloPipe is open source and available at https://github.com/huguesrichard/Allopipe.

O24 | Gnomic typer: Uncovering homopolymer and repeat region sequencing errors via k-mer based HLA typing

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HLA class II introns are abundant in homopolymer and repeat regions (HRRs), which, despite advances in sequencing technology, are challenging to sequence. Potential errors, both in sequencing and further analysis are well documented, resulting in variable length HRRs, which can lead to downstream problems with alignment-based typing methods and can require intensive manual inspection of outputs. Difficulties in determining HRR length are compounded by the use of alternative reference sequences and thus the number and location of possible differences that are reported. By identifying HRR length mismatches, any potential sequencing error is highlighted to the user. We have developed the alignment-free Gnomic Typer that performs HLA typing using k-mer profile similarity under L2 distance via FAISS (Facebook AI Similarity Search). HRR length errors result in fewer k-mer changes than other mismatches, thus incurring a lower k-mer profile distance score than the equivalent number of mismatches in other positions. This can be used to help resolve the obfuscation described above, as sequences with HRR variants are preferentially selected for reporting over unrelated alleles. We compared Gnomic Typer to our in-house alignment-based typer (ANTS), both validated for typing sequences of known HLA alleles. Using a panel of 131 unique HLA class II sequences, Gnomic Typer identifies 16% of the potentially novel sequences as having only HRR length mismatches compared to a known allele, these were reported against an alternative allele in ANTs. Previously, manually checking each novel allele averaged 10 min per sequence to rule out HRR differences. The Gnomic Typer provides this information in ~30 min for the entire panel, substantially reducing the post typing analysis requirements. This typing methodology is both sequencer and gene agnostic and is therefore applicable to any other context in which repeat regions are prevalent and the search space of known alleles is dense.

HEMATOPOIETIC STEM-CELL TRANSPLANTATION (HSCT)

O25 | Barcoded peptide–MHC multimers to measure T cell frequencies against a large repertoire of HLA class I-restricted minor histocompatibility antigens after allogeneic stem cell transplantation

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Allogeneic stem cell transplantation (alloSCT) is a curative treatment for hematological malignancies. After HLA-matched alloSCT, donor T cells recognize minor histocompatibility antigens (MiHAs), which are polymorphic peptides due to genetic differences between patient and donor and presented by HLA on patient cells. Dependent on broad or hematopoietic-restricted tissue expression of antigens, MiHA-specific T cells induce Graft-versus-Host Disease (GvHD) or Graft-versus-Leukemia (GvL) reactivity. Besides tissue expression, frequencies of these T cells determine the strength of clinical response. To systematically identify new MiHAs in seven common HLAs by genome-wide association screening, T-cell clones isolated from 39 patients with GvHD or GvL reactivity were tested against 191 EBV-B cell lines of the 1000 Genomes Project. SNPs strongly associating with T-cell recognition patterns were validated to encode MiHAs. DNA barcode-labeled peptide MHC-multimers (MULTs) were used to track MiHA-specific T cells. We identified 89 new MiHAs, thereby more than doubling the repertoire to 159 HLA-I restricted MiHAs. T-cell clones from different patients often recognized the same MiHAs, and with each new patient included in the study, discovery of new MiHAs declined. This suggests that the dominant repertoire of MiHAs is mostly discovered for common HLAs. We next used MULTs to measure and follow MiHA-specific T cell responses after alloSCT. Of our cohort of 39 patients, 16 patients were screened and high T-cell frequencies were measured for 12 MiHAs in 13 patients, including all patients with severe GvHD ($n = 10$), two patients with limited GvHD ($n = 3$) and one patient without GvHD ($n = 3$). In conclusion, we used the identified MiHA repertoire to measure and follow T-cell responses after alloSCT by MULTs. We demonstrate its use for screening large patient cohorts to

investigate association of MiHAs with GvL and GvHD and compare clinical outcome after alloSCT treatment modalities.

O26 | HLA-DPB1 allele and rs9277534 expression SNP mismatch number drive increased aGvHD and TRM, in a UK cohort of 10/10 matched HCT patients

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Matching or permissively mismatching HLA-DPB1 in unrelated donor hematopoietic cell transplant (UD-HCT) is well understood. Studies have also shown that 11/12 HLA matched patients with one DPB1 high expression rs9277534G SNP have higher acute graft vs host disease (aGvHD) risk. To further investigate the role of rs9277534, we performed a multicenter, retrospective outcome study in a cohort of UK patients with hematological malignancies, receiving UD-HCTs from 1997 to 2020. Samples were full-gene HLA typed on a PacBio Sequel using in-house laboratory and bioinformatic workflows. Multivariate analyses with Cox proportion hazard models and logistic regression were applied. Unambiguous CDS, 10/10 matched pairs ($n = 1063$) were grouped by number of DPB1 allele and rs9277534 expression SNP mismatches (XMs). Patients were considered 12/12 matched ($n = 181$), 11/12 + 0 XMs ($n = 245$), 11/12 + 1 XM ($n = 334$), 10/12 + 0 XMs ($n = 91$), 10/12 + 1 XM ($n = 150$), or 10/12 + 2 XMs ($n = 56$). Five-year overall survival (OS) was significantly reduced in two 10/12 patient groups (1 XM, HR = 1.41, $p = 0.035$, and 2 XMs, HR = 1.88, $p = 0.003$) compared to 12/12 HLA matched patients. All groups had higher rates of grades 2–4 aGvHD than the 13.4% seen in the 12/12 group. Risk of aGvHD rose with increasing numbers of both mismatches (11/12s + 0 XMs, HR = 2.05, $p = 0.011$ to 10/12s + 2 XMs, HR = 2.97, $p = 0.005$). The 10/12 + 2 XM group also had higher transplant related mortality (TRM) risk compared to 12/12 matched patients ($p = 0.007$, HR 2.14), although specific cause of death was unclear. In all the <12/12 matched patients, non-permissive T-cell epitope (TCE) DPB1 mismatches reduced OS vs permissive mismatches ($p = 0.035$, HR = 1.22), but risk of aGvHD ($p = 0.904$) and TRM ($p = 0.173$) were unaffected. No effect on relapse risk was seen in this study. We propose a novel DPB1 risk

model, which could augment the TCE model, where avoiding allele and XMs reduces aGvHD risk when a 12/12 matched donor is unavailable.

O27 | High-resolution KIR genotyping and its implication in allogeneic hematopoietic stem cell transplantation

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The success of allogeneic hematopoietic stem cell transplantation (alloHSCT) partly relies on the beneficial graft-versus-leukemia effect, mediated by donor-derived alloreactive NK cells through their killer-cell Immunoglobulin-like receptor (KIR). Conflicting results have been reported with a scarcity of data interrogating the effect of KIR allelic polymorphism. With the aim to fill this gap, donor KIR genes derived from a national cohort of 1292 donor/recipient alloHSCT pairs were genotyped at a high-resolution and combined with the recipient HLA genotype to test their association with various transplant outcomes. In univariate analysis, we observed a lower progression-free survival (PFS) ($p = 0.01$) and higher transplant-related mortality (TRM) ($p = 0.012$) in donor/recipient pairs bearing KIR2DS4*00101D—HLA-C2/A11R interactions, which was confirmed by multivariate analysis (PFS: hazard ratio [HR], 1.38, $p = 0.0025$; TRM: HR = 1.53, $p = 0.018$). PFS was also significantly influenced by the number of KIR2DL3D–HLA-C1R interactions (HR = 1.08, $p = 0.02$). In addition, these recipients showed a higher risk of developing chronic graft-versus-host disease with KIR2DS4*00101D–HLA-C2/A11R interactions (HR = 1.31, $p = 0.021$) or by the strength of KIR2DL2/L3D–HLA-C1R interactions (HR = 1.21, $p = 0.009$). Recipients lacking a KIR2DS2D–HLA-C16R interaction had a lower rate of relapse ($p = 0.0023$), although the significance was lost in multivariate analysis. Our study indicates the potential detrimental effect of KIR activating interactions, especially KIR2DS4, potentially due to its sustained expression in an overactivated environment.

O28 | Cryopreservation of peripheral blood stem cell grafts for hematopoietic stem cell transplantation changes the frequencies of CD56+ MAIT cells

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In a collaboration between DKMS and NMDP, we are comprehensively characterizing the cellular composition of 2000 Peripheral Blood Stem Cell (PBSC) products using a 34-color flow cytometry panel to define the optimal graft composition with respect to specialized immune effector cells. We aim to relate the cellular composition of PBSC grafts to the outcome of allogeneic hematopoietic cell transplantation (alloHCT). As part of this project, we investigated the effect of cryopreservation on the cellular composition of PBSC products. Cryopreservation is thought to influence cellular composition of the graft, but comprehensive data are lacking. We analyzed the cellular composition of 20 fresh grafts and compared them to their frozen and thawed counterparts. Cryopreservation of product samples followed routine

clinical procedures using a 10% DMSO containing cryomedium and a controlled rate freezing. After thawing at 37°C, cells were washed, stained, fixed and analyzed immediately by flow cytometry. Our panel is designed to capture conventional and unconventional T cell subsets, as well as myeloid and stem cell populations. Proportions of immune cells were assessed using a novel unbiased computational clustering method. Specifically, within the CD8 T cell compartment, a cluster enriched for CD161+ and Valpha7.2+ (which define mucosal-associated invariant T cells; MAIT) was significantly reduced ($p < 0.05$) in frozen and thawed samples. A fraction of cells in this cluster expresses CD56. We confirmed these results using traditional gating strategies and observed a marked reduction of CD56+ MAIT cells following cryopreservation (55.5% vs 44.3%, $p < 0.0001$). CD56 expression on MAIT cells has been reported, but their potential role in alloHCT is unknown. The results provide further insight into the influence of cryopreservation on PBSC graft composition and warrant further efforts to explore mechanisms behind differential outcomes observed using fresh vs cryopreserved PBSCs.

O29 | Study of HLA-DP mismatch effects in a Swiss hematopoietic stem cell transplantation cohort

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Allogeneic hematopoietic stem cell transplantation (alloHSCT) is the standard of care for hematological malignancies such as leukemia. A main goal of such treatment is to re-establish the immune system of a patient whose diseased immune cells have been depleted. Care needs to be taken when selecting a donor to allow an engraftment of donor's immune cells and to avoid that these attack the patient's cells, leading to an acute graft-versus-host disease (aGVHD). Often, unrelated donors with HLA-matched alleles need to be considered. In most cases, a donor matching at HLA-A, -B, -C, -DRB1 and -DQB1 can be found (so called 10/10 match), but mismatch at HLA-DP is frequently encountered. Different models of permissiveness have therefore been developed to reduce the risk of aGvHD. To study the effects of HLA-DP mismatches in this context, we obtained data from an extended Swiss cohort of patients that underwent alloHSCT between 2008 and 2022. It includes 1510 patient-donor pairs that were fully HLA typed (including HLA-DPB1 and HLA-DRB3/4/5), with a mean follow-up time of 3 years. A mismatch at the HLA-I level was present for 43 patients. An HLA-II mismatch was present for 1055 patients, including 1025 with a mismatch at HLA-DPB1. Using this cohort, we compare different models of permissiveness, including the TCE groups model and the model based on differences in HLA-DPB expression between patient and donor. We also take advantage of our accurate predictor of HLA-II presentation (named MixMHC2pred) to quantify the divergence in peptides presented by the patient and donor as a surrogate of permissiveness. Preliminary analyses show that our novel model is a promising alternative to the other models, managing well to discriminate combinations patients-donors with increased risk of aGvHD. An advantage of this model is that it is directly applicable to any combination of alleles and that it could be extended to mismatch in non-HLA-DP alleles.

O30 | Cytomegalovirus antigen load as a new predictive factor after haploidentical hematopoietic stem cell transplantation

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Cytomegalovirus (CMV) is known to be a risk factor for potential life-threatening complications after allogeneic hematopoietic stem cell transplantation (HSCT). The CMV proteins IE1 and PP65 play a pivotal role in eliciting immune responses. Here we explore the Antigen Load (AL) of these two proteins as a putative new predictive variable, under the hypothesis of a better outcome associated with higher AL due to an improved CMV-derived peptide presentation by HLA molecules, thus a better infection control. Main clinical and immunogenetic data were collected from 238 adult patients who underwent HSCT from

haploidentical donors between April 2010 and June 2022 at a single center. Outcomes included overall mortality (OM), non-relapse mortality and CMV infection incidence. AL was computed on patients´ HLA typing using NetMHCpan 4.1 and NetMHCIIpan 4.0. Values were expressed as absolute numbers (strong + weak binders) for each HLA allele; class I AL for IE1 and PP65, taken together and singularly, was calculated for every patient then entered in uni- and multivariate analysis. Patients with higher class I AL (above the median) had a lower risk of OM with an OR 0.74 (95% CI: 0.56–0.96, $p = 0.028$) and OR 0.49 (95% CI: 0.28–0.85, $p = 0.011$) when AL was calculated for IE1 + PP65 or IE1 only, respectively. Multivariate analysis confirmed class I AL for IE1 + PP65 as an independent risk factor of OM (HR 0.79, 95% CI: 0.63–0.99, $p = 0.043$), together with the patient CMV serostatus (HR 1.98, 95% CI: 1.02–3.85, $p = 0.043$). The present study shows the application of CMV Antigen Load as a variable predictive of outcome after HSCT. Our exploratory results indicate that CMV class I AL for the immunodominant IE1 and PP65 peptides is an independent predictor of mortality in a setting of 238 adult haploidentical HSCT. Insights into the causality mechanisms and the impact of the recently introduced, prophylactic agent Letermovir on the predictive models need to be further investigated.

O31 | A biomarker model for non-relapse mortality (NRM) risk monitoring after hematopoietic stem cell transplantation

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Many studies have shown that some serum biomarkers could predict non-relapse mortality (NRM) in the specific days after hematopoietic stem cell transplantation (HSCT). However, concentrations of biomarkers change with time due to complications or treatment after HSCT, leading to the usage of biomarkers for monitoring of NRM risk a challenging task. In this study, we explored the monitoring of NRM risk by unifying biomarker concentrations of multiple time-points. The concentrations of ST2, REG3α, TNFR1, IL6, and IL8 were measured after HSCT in a multicenter cohort of 621 patients received HSCT between February 2019 and 2022, who were divided chronologically into training ($n = 310$), test ($n = 153$), validation ($n = 158$) cohorts. NRM risk models were developed with Fine-Gray proportional hazards regression method using the maximum concentrations of different biomarkers panels. We found that the valuable biomarkers for predicting NRM at 6-month were ST2, REG3α, and TNFR1, of which the AUCs based on the maximum concentrations reached 0.83, 0.72, 0.78 respectively. Furthermore, a model based on the maximum concentrations of sST2 and TNFR1 reached AUC of 0.86 in the test cohort, which was the highest among the models based on the maximum concentrations of other biomarker panels (sST2 + REG3a, 0.82; TNFR1 + REG3a, 0.75; sST2 + TNFR1+ REG3a, 0.83). Additionally, the model of sST2 and TNFR1 reached an AUC of 0.94 in the validation cohort. We also found that the NRM risk stratification thresholds could be directly applied on day 7, 14, 28 and aGvHD onset, with the cumulative incidence of 6-month NRM for high vs low risk being 20% versus 4% ($p < 0.01$), 15% versus 3% ($p < 0.001$), 21% versus 5% ($p < 0.001$), 26% versus 7% ($p < 0.01$) respectively. We demonstrated that the maximum concentration of biomarkers achieved higher performance of predicting NRM risk. Our algorithm of sST2 and TNFR1 could make monitoring of NRM risk operated more flexibly and precisely.

O32 | Mutations in leukemic cells may lead to difficulties in HLA typing: Report of ten challenging cases

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HLA-typing is typically conducted from DNA that has been isolated from nucleated cells. Usually, the source for these nucleated cells is EDTA blood samples. In the presence of a malignant disease with blasts in the peripheral blood, however, this procedure can be problematic. We perform high-resolution HLA typing using

fragmented long range PCR and subsequent sequencing on an Illumina MiSeq or MiniSeq sequencer. For this, an in-house kit is used to generate the long range PCR amplicons. Fragmentation as well as library preparation is performed using the commercial QIAseq FX DNA Library Kit from Qiagen GmbH, Hilden, Germany. Analysis is carried out with the software NGSengine from GenDX, Utrecht, The Netherlands. We have identified 10 samples with a challenging assignment of HLA results. Four of them showed homozygous typing results for all typed loci, one sample presented homozygous for all loci except HLA-A. Retyping from buccal swabs showed, that the homozygous HLA results from blood samples turned out to be loss of haplotype cases. Three additional blood samples were each originally typed with HLA-B null alleles, and one sample was assigned with an HLA-A null allele. These results were also not confirmed in the retyping from buccal swabs. For another sample, three alleles were found: HLA-A*02, HLA-A*02Null, and HLA-A*32. In this case, 50% of the reads were allocated to A*32 and 25% each to A*02 and A*02Null. Retyping from a swab revealed A*02, A*32 to be the correct result. HLA typing of patients with myeloproliferative diseases can contain pitfalls. If the blood sample contains a sufficient amount of blast, HLA labs are at the risk of confusing the HLA typing result of the leukemic cells somatic mutations with the actual germline HLA result of the patient. The cases described here may occur occasionally, but any incorrect HLA typing can lead to serious or even fatal complications for the patient.

IMMUNOGENETICS IN ORGAN TRANSPLANTATION

O33 | Single-cell transcriptomics reveals CD8+ T cell clonotypes with effector functions are associated with lung allograft rejection

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A major complication in lung transplantation is the development of acute cellular rejection (ACR) mediated by alloreactive T cells, which has impacted graft survival outcomes. Novel single-cell and sequencing technologies are now in play that enable simultaneous investigations of both gene and T cell receptor (TCR) transcripts. These technologies provide an opportunity to ask key questions that still remain unanswered in transplantation including (i) are there restricted T cell clonotype(s) associated with ACR episodes, (ii) what are their paired T cell receptor (TCR) signatures, (iii) do these alloreactive T cells display differential activated/cytotoxic profiles and (iv) do these T cell clonotypes persist in the first-year following transplantation? This study addresses these questions in a longitudinal (up to 14 months) cohort of lung transplant recipients that either experienced ACR or not. We undertook a combined single-cell gene and TCR transcript analysis (scRNA-Seq/scTCR-Seq) of peripheral blood mononuclear cells (PBMC) isolated from blood at different post-transplant time points using the BD Rhapsody system to identify ACR-specific T cells. Data was analyzed using a new R package 'STEGO.R'. We identified populations of expanded alloreactive T cells that were predominantly CD8+ with an effector-like phenotype (including one or more of granzymes, perforin, granulysin, Th1 cytokines [IFN gamma, TNF]) in both ACR and non-ACR patients, although they differed based on varying levels of cellular senescence. Examination of these expanded CD8+ TCR clonotypes showed a diverse polyclonal and private (not identified in other patients) repertoire, with each patient displaying dominant clonotypes that were persistent throughout their sampling time points. This approach provides key evidence that a restricted pool of alloreactive CD8+ T cells with an activated phenotype are associated with ACR episodes and remain present in the first-year post-transplantation.

O34 | PD-L1 overexpression on porcine kidneys decreases xenogeneic human T-cell immune responses

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Xenotransplantation is emerging as a promising solution to the shortage of available human organs for transplantation. Genetic modifications focusing on the expression of human protective genes or the knock-out of specific antigens prolong the survival of xenotransplants. However, newly generated modifications have been tested in

in vitro assays, humanized mouse models, or non-human primate models after xenotransplantation, which raise concerns about the reproducibility and adequacy of the data, as well as social, health, and regulatory considerations. The ex vivo kidney perfusion (EVKP) represents a promising strategy for preserving genetically modified organs while maintaining physiological parameters and evaluating the strength of human immune cell reactions. This study focused on the assessment of human T-cell responses to the kidneys of wild-type (WT) or transgenic pigs overexpressing human programmed death-1 ligand 1 (hPD-L1). During ex vivo perfusion, xenogeneic T cells specifically respond and transmigrate into the endothelium of WT and hPD-L1 tissues. Secreted transcript levels of TNF-α, IFN-γ, granzyme B, and perforin were 70%, 40%, 50%, and 65%, respectively, reduced in hPD-L1-expressing renal tissues in comparison to WT tissues. In vitro assays confirmed the weaker immune responses by co-culturing xenogeneic T cells with endothelial cells isolated from WT or hPD-L1 overexpressing transgenic pigs. Remarkably, hPD-L1 overexpression on porcine ECs shows a protective effect against xenogeneic T-cell responses through significantly reduced T-cell proliferation of CD4+ (7.42%) and CD8+ (16.98%) T cells, reduced cytokine release, and 9.2-fold decreased T-cell cytotoxicity. This strategy may allow an evaluation of the efficacy of specific genetic modifications by assessing the human response of specific immune subsets towards the whole xenograft during EVKP and thereby serve the potential to reduce and refine the number of animals used in preclinical tests.

O35 | HLA DQβ0601, a diverse HLA class II beta-chain that forms interisotypic-heterodimers with DRα and DPα

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The canonical association between the HLA class II α-chain and β-chain is DRα with DRβ, DQα with DQβ and DPα with DPβ. We have demonstrated the assembly and expression of a DQβ0601:DRα heterodimer on the cell surface of a DQβ0601:DQα0103 transfectant that expresses only DRA. Approximately 5.6% (45/797) of renal transplant candidates produced antibody specific to the purified DQβ0601:DRα protein as detected by a Luminex bead-based assay. The capability of DQβ0601 to form stable interisotopic heterodimers with DPα was further investigated. Stable transfectants expressing DQβ:DPα heterodimers were identified at varying efficiencies when DQB1*06:01 was co-transfected with DPA1*01:03, DPA1*01:04, DPA1*02:01, DPA1*03:01 and DPA1*04:01. Approximately 1% (9/797) of candidate's sera demonstrate specific reactivity to the DQβ0601:DPα heterodimer. In conclusion, we have demonstrated that DQβ0601 can assemble and express stable interisotopic heterodimers with either DRα or DPα. Furthermore, correctly folded DQβ:DRα and DQβ:DPα proteins were purified from the transfectants. Additionally, antibodies to the DQβ:DRα and DQβ:DPα proteins were detected in renal transplant candidates by a single antigen bead assay. The identification of stable interisotypic heterodimers on the cell surface suggests the potential for the presentation of additional and possibly unique peptides. The availability of such heterodimers permits novel investigation into the immunology of MHC:peptide:TCR interactions and the study of T-cell and/or B-cell mediated transplant rejection.

O36 | Preformed cytokine autoantibodies are predictive for histological features of kidney graft damage

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Preexisting neutralizing autoantibodies against type I interferons in COVID-patients are known as important immune modulators and to be associated with poor clinical outcome. Elevated cytokine levels in patient sera are a risk factor in organ transplantation. Here we asked if preformed cytokine-autoantibodies could modify these effects and serve as a predictive factor. To answer this question, we have a developed a Luminex-based multiplex assay allowing the measurement of autoantibodies against 10 different cytokines (IFN-α, IFN-γ, IFN-ω, IL-6, IL-12, IL-17A, IL-17F, IL-22, TGF-β, and GM-CSF) and the cell membrane protein Caveolin-1. Sera of 343 patients were analyzed, of whom 76 had available histological data from kidney transplant biopsies.

Antibody levels were compared to histological features of graft rejection during follow-up. The combination of different cytokine autoantibody values was able to accurately distinguish between different grades of rejection in the items used by the histological Banff classification: cellular vascular rejection, peritubular capillaritis, tubulitis and transplant glomerulopathy. Further grades of other items could be distinguished with accuracy. Notably, the serum samples were taken prior to the respective transplantation, allowing to postulate an individual inherent risk profile and potential therapeutic modifications.

O37 | Pre-transplant donor specific antibodies in ABO incompatible kidney transplantation—Data from the Swiss Transplant Cohort Study

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Living donor (LD) kidney transplantation in the setting of ABO blood group incompatibility (ABOi) has been previously reported to be associated with increased risk for antibody-mediated rejection (ABMR). It is however unclear if the presence of pre-transplant donor specific antibodies (DSA) works as an additive risk factor in the setting of ABOi and if DSA positive ABOi transplants have a significantly worse long-term outcome as compared with ABO compatible (ABOc) DSA positive transplants. We investigated the effect of pre-transplant DSA in the ABOi and ABOc setting on the risk of antibody-mediated rejection (ABMR) and graft loss in a cohort of 952 LD kidney transplants. We found a higher incidence of ABMR in ABOi transplants as compared to ABOc transplants but this did not significantly affect graft survival or overall survival which was similar in both groups. The presence of pre-transplant DSA was associated with a significantly increased risk of ABMR and graft loss both in the ABOi and ABOc setting. We could not detect an additional risk of DSA in the ABOi setting and outcomes were comparable between DSA positive ABOi and ABOc recipients. Furthermore, a combination of DSA directed at both class I and class II, as well as DSA with a high mean fluorescence intensity (MFI) showed the strongest relation to ABMR development and graft loss. The presence of pre-transplant DSA was associated with a significantly worse long-term outcome in both ABOi and ABOc LD kidney transplants and our results suggests that the risk associated with pre-transplant DSA is perhaps not augmented in the ABOi setting. Our study is the first to investigate the long-term effects of DSA in the ABOi setting and argues that pre-transplant DSA risk could potentially be evaluated similarly regardless of ABO compatibility status.

O38 | Homozygosity of any HLA is associated with dedicated HLA antibody patterns: The taboo concept 2.0

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In a collaborative study of the University Hospital Leipzig and the Charité Berlin on a large cohort of kidney transplant recipients, we analyzed the occurrence of HLA-specific antibody patterns with respect to the HLA repertoire of the patients. Patients were typed for the relevant HLA determinants using mainly next generation sequencing technology. Antibody screening was performed by the state-of-the-art multiplex-based technology using microspheres coupled with the respective HLA alleles of HLA class I and II determinants. Patients homozygous for HLA-A*02, HLA-A*03, HLA-A*24, HLA-B*07, HLA-B*18, HLA-B*35, HLA-B*44, HLA-C*03, HLA-C*04, HLA-C*07 in the class I group and for the class II group: HLA-DRB1*01, HLA-DRB1*03, HLA-DRB1*07, HLA-DRB1*15, HLA-DQA1*01, HLA-DQA1*05, HLA-DQB1*02, HLA-DQB1*03, HLA-DQB1*06, HLA-DPA1*01, and HLA-DPB1*04 were found to have a significant higher antibody production compared to the heterozygous ones. All HLA determinants are affected. Remarkably, HLA-A*24 homozygous patients can produce antibodies towards all HLA-A determinants, while HLA-B*18 homozygous make antibodies towards all HLA-B and selected HLA-A and -C antigens

and are associated with an elevation of HLA-DRB1, partly -DQB1, and -DPB1 alleles. Homozygosity for the HLA class II HLA-DRB1*01, and HLA-DRB1*15 seems to increase the risk for antibody responses against most of the HLA class I antigens (HLA-A, -B and -C) in contrast to HLA-DQB1*03 where a lower risk towards few HLA-A, and B alleles is observed. The widely observed differential antibody response is therefore to be accounted to the patient's HLA repertoire. Homozygous patients are at risk to produce HLA-specific antibodies hampering the outcome of transplantation. Including this information on the allocation procedure might reduce antibody mediated immune reactivity and prevent graft loss in the patient at risk increasing the life span of the transplanted organ.

O39 | Donor HLA class I evolutionary divergence predicts late allograft rejection after pediatric liver transplantation: A long-term retrospective study

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Allograft rejection, initiated by recognition of donor antigens by the recipient's immune system, remains a challenge in transplantation medicine. HLA evolutionary divergence (HED) between an individual's HLA alleles is a continuous metric that quantifies the differences between each amino acid of two homologous alleles and reflects the breadth of the immunopeptidome that can be presented to T lymphocytes. To assess the potential effect of donor or recipient HED on rejection after pediatric liver transplantation, we studied a retrospective cohort including 120 children who underwent liver transplantation between 1991 and 2010 with a median follow-up of 14.1 years post-transplant. Histological data from liver biopsies performed either routinely or in case of liver dysfunction were collected. HED was calculated using the physicochemical Grantham distance for donor's and recipient's class I (HLA-A, -B, -C) and class II (HLA-DRB1, -DQB1) alleles. The influence of HED on rejection was analyzed through inverse probability weighting approach and G-computation analysis. We observed that donor HED class I was positively correlated to the occurrence of late (>90 days) rejection (HR, 1.19, 95% CI: 1.01–1.40) independently of covariates related to rejection such as HLA mismatches, donor's age and initial induction. G-computation confirmed the causal effect of donor HED class I on the occurrence of rejection. These findings were not observed for donor HED class II nor for recipient HED class I or II. This study confirms in a particular population (transplanted children followed until adulthood), the previous observations showing that donor HED class I is a predictor of rejection independently of donor-recipient HLA mismatches. As donor class I HED could be considered an accurate proxy of graft immunogenicity, this novel and easily accessible prognostic marker holds potential to improve donor selection and guide immunosuppression strategies.

O40 | Confirmation of the multi-parametric cfDNA algorithm for early allograft injury events in lung transplantation

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Our team previously published results at 1 month after lung transplantation (LTx) that donor-derived cfDNA (dd-cfDNA) alone is not specific for discriminating acute rejection (AR) from other early events such as infection (INF). We demonstrated the importance of a multi-parametric cfDNA study by an algorithm that considers the dd-cfDNA % to differentiate stable from non-stable patients, and the fragmentome study of cfDNA size to discriminate the injury type (INF, AR). The aims of the study are to confirm these results and adjust the diagnosis threshold of the algorithm previously published, using a larger cohort. This was a prospective study involving

92 patients (62 first patients and 30 additional patients) from the Marseille Hospital. dd-cfDNA % was determined by NGS (AlloSeq cfDNA assay, CareDx) and size profile was assessed by BIABooster (Adelis). A biopsy at day 30 (D30) established the following groups among patients: stable and non-stable (AR, INF and AR + INF). The results of this new analysis are consistent with our previous results. The dd-cfDNA % was significantly higher in non-stable patients at D30 ($p = 0.001$). The threshold previously identified as 1.72% was adjusted to 2.19%, yielding satisfactory analytical performance (sensitivity = 72.7%, specificity = 87.2%, PPV = 76.2%), and notably, an NPV = 85.0% for discriminating stable patients. Among the "non-stable" group, the results confirmed that patients with INF had significantly higher percentages of small cfDNA fragments (80-120 bp) compared to AR patients ($p = 0.028$) and stable patients ($p = 0.044$). The initial threshold of 3.7%, adjusted to 3.4%, facilitated the identification of INF patients among non-stable patients (PPV = 88.9%). The combination of the two analyses effectively differentiates the type of allograft injury. This study, conducted on a larger cohort confirms the previous results and adjust the previously established cut-off values to increase the test reliability.

O41 | HLA-specific memory B cell detection in kidney transplantation: Results from a prospective explorative study

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Detection of presence of HLA-specific B cell memory cells in addition to serum HLA antibodies may help to refine the pre-transplant immunological risk assessment in kidney transplantation. In this single-center prospective explorative study, we included 35 kidney transplant candidates with a history of HLA-sensitizing events. Importantly, patients were only eligible for study inclusion if the immunizing HLA (previous pregnancies and/or transplantations) was known and high-resolution typing data of all loci could be obtained. Of all patients, we collected peripheral blood mononuclear cells (PBMC) in order to perform a memory B cell assay. In the latter, memory B cell-derived HLA antibodies can be detected by Luminex single antigen bead assay following in vitro polyclonal stimulation of PBMC and subsequent collection of culture supernatants. HLA antibody profiles deriving from the memory B cell compartment and serum were comparatively analyzed. By taking the mean fluorescence intensity (MFI) of the self-alleles into account, we used a biological cutoff for determination of a positive signal. In the whole cohort, there were 328 HLA mismatched alleles resulting from previous immunizing events. Of these, 59 (18%) specificities were assigned as positive in SAB assay in either serum and/or memory B cell culture supernatants. Memory B cell-derived antibodies could be detected against 26/59 (44%) specificities, either alone (10/59; 17%) or with concurrent serum antibodies (16/59; 27%), and mainly for class II. On the patient level, 14/35 (40%) had detectable HLA-specific B cell memory. Memory B cell-derived antibodies could be attributed to immunizing eplets in some cases. In conclusion, assessment of previous HLA sensitization by analyzing memory B cell-derived antibodies differs from serum antibody profiles and reveals "hidden memory" in a subset of patients. Better characterization of the clinical impact of detectable B cell memory is needed.

O42 | MICA and NKG2D gene polymorphisms influence chronic rejection, graft function, and response to therapy in kidney transplantation

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Chronic rejection is a significant cause of kidney transplant failure. Traditionally, compatibility between the HLA system of the donor and recipient has been considered the most important genetic factor in reducing the risk of rejection. Recent studies have shown that other factors, such as the major histocompatibility complex (MHC) class I MICA gene can also influence the transplantation outcome. However, the role of the primary MICA receptor, NKG2D, has yet to be explored. We aimed to investigate the correlation between recipient/donor MICA allele matching and NKG2D genotype with the risk of chronic rejection and their potential clinical effects and implications for organ maintenance therapy. From 524 patients who underwent transplantation, 148 were eligible for the study. Complete MICA allele and two key functional polymorphisms of NKG2D (rs1049174C>G and rs2255336G>A) were analyzed in transplanted patients and 168 controls. As previous demonstrated, recipient/donor MICA allele mismatches correlate with an elevated risk of chronic rejection ($X^2 = 6.95$; Log-rank = 0.031). Notably, the rs1049174 [GG] NKG2D genotype contributes to a significantly increased risk of chronic rejection ($X^2 = 13.44$; Log-rank = 0.001). Moreover, the combined effect of two MICA allele mismatches and presence of rs1049174 [GG] genotype shows the highest risk ($X^2 = 23.21$; Log-rank<0.001). Finally, our findings suggested that patients carrying rs1049179 [GG] and rs2255336 [AA] genotypes may respond less to mTOR inhibitor immunosuppressive therapy than Calcineurin inhibitors (rs1049179 [GG]; $P = 0.035$; and rs2255336[AA]; $P = 0.002$).Recipient/donor MICA allele mismatches and specific NKG2D variants, as well as their combinations, can influence kidney transplant outcomes, providing insights for personalized treatment and enhancing graft survival.

O43 | Inactivating GSK3 phosphorylation by intracellular HLA-signaling

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Donor-specific HLA-antibodies (HLA-DSA) against non-concordant antigens are known to activate several intracellular signaling pathways leading to changes of the intra-graft transcriptome. These changes mediate apoptosis resistance and enhanced proliferation of the graft endothelium and contribute to characteristic histological features of chronic graft damage such as vasculopathy and graft fibrosis. Glycogen Synthase Kinase 3 (GSK3) is a serine/threonine kinase and an important regulator of inflammation, cell mobility and apoptosis. GSK3 exist in two constitutively active isoforms, GSK3α and GSK3β, whose activity is inhibited by phosphorylation at Ser21 (GSK3α) and Ser9 (GSK3β), respectively. GSK3 participates in the PI3K/AKT/mTOR signaling pathway, known to be activated by donor specific HLA-antibodies. Moreover, GSK3 downregulates the transcription factor NFAT, the inhibition of which is an important part of established immunosuppressive protocols. However, a direct link between HLA-initiated signaling and GSK3 activity has not been elucidated so far. In an attempt to analyze a possible link between HLA-signaling and GSK3 activity, we exposed human endothelial cells (HMEC-1) to monoclonal antibodies against HLA class I and II, respectively. Our new data revealed that HLA-ligation resulted in rapid inhibitory phosphorylation of both GSK3 isoforms, whereas the amount of total proteins remained unaltered. Given the functional role of GSK3 proteins in typical complications of kidney transplantation including ischemia/reperfusion injury, our data provide novel insights into the molecular mechanisms of how donor-specific HLA-antibodies affect graft integrity and propose GSK3 as novel therapeutic target in the future.

O44 | DRB1*01:02 is associated with anti AT1R antibody production

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Kidney transplantation is a life-saving procedure for patients with end-stage renal disease. However, the

immune response to transplant can lead to complications, including the production of anti-HLA Donor Specific Antibodies (DSA) or non-HLA antibodies (such as Angiotensin II receptor type 1-AT1R-) that can compromise the outcome of the graft. Using in silico techniques, we observed that HLA-DRB1*01:02 was able to present AT1R peptides with greater binding affinity. Subsequently, a cohort of 59 kidney recipients with a common haplotype observed in the Italian population containing DRB1*01:02 (HLA-A*33:01∼B*14:02∼DRB1*01:02) was analyzed in comparison with a control group of 52 patients with other different haplotypes. Pre- and post-transplant sera of both cohorts were tested for the presence of DSA (Luminex-based bead assay) and AT1R-ab (Enzyme-linked immunosorbent assay).From the obtained results, AT1R-abs are strongly produced in patients with the haplotype containing DRB1*01:02 whereas the control cohort does not produce AT1R-abs above the defined cut-off threshold, both in pre-transplant ($p = 0.0009$) and post-transplant samples ($p < 0.0001$). No significant correlations were found in the DSA development in both cohorts. These findings shed light on a possible genetic link between HLA haplotypes and the development of AT1R-abs in kidney transplant recipients. Understanding this association may contribute to more personalized immunosuppressive therapies, also investigating clinical parameters of patients.

O45 | Molecular mismatch tools to predict events within the first year after renal transplantation

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Molecular mismatch tools using structural bioinformatics provide information beyond conventional HLA matching approaches. In this study, we aimed to define risk groups for rejection based on the combination of three different molecular mismatch scores. We calculated the eplet mismatch load, the number of high immunogenic eplets, and the PIRCHE II score based on 11 loci high-resolution HLA typing data of donors and recipient in 439 consecutive transplantations. None of these transplants had pre-transplant donor-specific HLA-antibodies, at least one conventional HLA mismatch, a standardized immunosuppression with tacrolimus/mycophenolic acid/prednisone, and a minimal follow-up of 1 year. ROC analyses were used to define cut-off for prediction of (sub)clinical rejection according to Banff 2019 classification within the first year post-transplant as a reference. Ninety-five of 439 patients (22%) experienced (sub)clinical rejection. AUC were 0.60 for eplet mismatch load (cutoff 73), 0.56 for number of high immunogenic eplets (cutoff 4), and 0.60 for PIRCHE II (cutoff 93). If all molecular mismatch scores were below the cutoff, the patient was assigned to the low-risk group (19% of all patients); if all molecular mismatch scores were ≥ the cutoff, the patient was assigned to the high-risk group (21% of all patients). The incidence of (sub)clinical rejection was 12% in the low-risk group and 33% in the high-risk group ($p = 0.003$). Internal validation of the assigned groups for prediction of clinical rejection (6% vs 24%; $p = 0.004$), ATG-treated rejection (1% vs 16%; $p < 0.001$) and development of de novo DSA at 5 years post-transplant (6% vs 25%; $p = 0.003$) revealed similar results. In conclusion, a combination of three individual molecular mismatch tools allows to distinguish low and high immunological risk groups among standard renal allograft recipients.

O46 | The Mayo clinic in Arizona virtual crossmatch algorithm: An accurate and safe alternative for assessing HLA compatibility

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The Mayo Clinic in Arizona (MCA) has established a virtual crossmatch (VXM) algorithm that allows our solid organ transplant programs to safely proceed to deceased donor transplants primarily based on the VXM. Over the past 7 years, our HLA laboratory has applied this algorithm to 9660 VXM of which 2849 VXM were performed over the past year alone. Transplant candidates are tested every 60 days for the presence of HLA antibodies using single-antigen bead (SAB) assays. We also implemented Levey-Jennings charts and Coefficient of Variation (CV %) assessments to closely monitor the accuracy and precision of the SAB assays. Serum samples are treated with EDTA (5%) and bovine calf serum (5%) to remove heterophile antibodies and other serum components that interfere with solid-phase assays. VXM assessment is primarily performed on a serum sample tested within the past 60 days; however, the patient's donor-specific antibody (DSA) history is further considered to provide a more accurate risk assessment. A VXM is considered

positive when ≥1 DSA with a cumulative MFI≥2000 is detected resulting in the recommendation for a prospective crossmatch. A prospective crossmatch is recommended for all patients with cPRA≥80% except for zero-mismatched donors at HLA-A, -B, -C, -DRB1, -DQB1 and -DPB1. Our DD kidney transplant rate is significantly higher (57.7) than the expected rate (21.4). Our median wait time for a DD kidney transplant (8.6 months) is significantly lower than the regional and national median (52.7 and 31.3 months, respectively). Also, our estimated hazard ratios (HR) for DD kidney graft failure show excellent outcomes at 1-month (HR = 1.3), 3-months (HR = 1.03), 1-year (HR = 1.02), and 3-years (HR = 1.03) post-transplant vs. expected (HR = 1.0). In conclusion, our VXM algorithm has proven to be an accurate and safe alternative to the physical crossmatch for assessing HLA compatibility before DD solid organ transplantation.

O47 | Imlifidase in highly sensitized kidney transplant recipients with a positive crossmatch against a deceased donor: Preliminary results of the French cohort

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Imlifidase is authorized for desensitization of highly sensitized adult kidney transplant candidates with a positive crossmatch (XM) against a deceased donor. French consensus guidelines have determined the following eligibility criteria: calculated panel reactive antibodies (cPRA) of 98%, and 3 years on the waiting list, immunodominant donor specific antibodies (iDSA) with mean fluorescence intensity (MFI) > 6000 (and <5000 at 1:10 dilution) and a negative post-imlifidase complement-dependent cytotoxic crossmatch (CDCXM). Here, we report on the results for the first 9 patients transplanted in this context who had at least 3 months of follow-up. All 9 patients had been on dialysis for an average of 123 months, with cPRA at 99% ($n = 2$) or 100% ($n = 7$). At transplantation, the mean number of DSA was 4.3. The median iDSA MFI was 9153 (6430-16,980). Flow cytometry and CDC XMs before imlifidase were positive in 9 and 2 patients, respectively. After one injection of imlifidase all were negative. Patients received polyclonal antibodies, intravenous immunoglobulins, rituximab, tacrolimus, and mycophenolate. Five patients had a DSA rebound within the first 14 days: 2 had concomitant clinical antibody-mediated rejection (ABMR), 2 had subclinical ABMR, and 1 had isolated positive C4d staining. No ABMR was observed in patients without rebound. CKD-EPI eGFR was 56 mL/min/1.73m^2 at the last follow-up (7 months). No graft loss or death were observed. Four patients developed at least one infection. These data demonstrate that the use of imlifidase to desensitize highly sensitized patients can have an acceptable short-term efficacy and safety profile in selected patients.

O48 | Assessing the diagnostic utility of donor-derived cell-free DNA for evaluating kidney allograft rejection: Results from the EvAcADE study

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After transplantation, the damage found in the biopsied allograft may involve different anatomopathological entities, of which immune-mediated insults (ABMR or TCMR) are the leading cause of graft loss. Non-invasive biomarkers capable of differentiating and detecting insults earlier are necessary to increase graft survival rates. We aimed to validate the performance of donor-derived cell-free DNA (dd-cfDNA) as a diagnostic tool for immuno-mediated allograft injury at the time of clinical suspicion. Fifty-three adult kidney transplant recipients (KTr) from 14 hospitals at 1 year posttransplant were enrolled at the time of an indication biopsy (T0) in this Spanish multicentric observational study. Blood was drawn at T0 for anti-HLA antibody (alloAb) and dd-cfDNA analysis. AlloAbs and laboratory and clinical data were obtained at 90 (T1) and 180 (T2) days post-biopsy. dd-cfDNA was tested by AlloSeq cfDNA (CareDx). SPSS was used for data analysis, with $P < 0.05$ indicating statistical significance. At T0, 30.9% of the indication biopsies were classified as acute rejection (AR), of which 20% ($n = 11$) were ABMR and 10.9% ($n = 6$) were TCMR. The dd-cfDNA was significantly greater in any AR (0.78% vs 0.41%, $P = 0.037$) or with active ABMR (1.23% vs 0.41%, $P = 0.017$) from non-active rejection. Importantly, dd-cfDNA performed significantly better than traditional markers (eGFR, creatinine, and proteinuria) for detecting AR (AUC: 0.694, $P = 0.037$) and ABMR (AUC: 0.769, $P = 0.017$). The optimal cut-off for this specific cohort of 0.4% dd-cfDNA showed a sensitivity of 93.75% and a specificity of 42.31% for discriminating patients with AR ($P = 0.037$) and ABMR ($P = 0.009$) from patients with no rejection. Multivariate logistic regression confirmed that dd-cfDNA 0.4% as the best biomarker for to diagnose of AR and ABMR at the time of clinical suspicion. dd-cfDNA could be used as a diagnostic biomarker for AR in KTr patients within the first year post-transplantation.

IMMUNOTHERAPY, GENE THERAPY & NK CELLS

O49 | HLA-DQ2 Chimeric HLA antibody receptor (CHAR) T cells to target HLA-DQ2-specific B cells to desensitize patients in solid organ transplantation

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Chimeric HLA antibody receptor (CHAR) T cells are a promising therapy for targeting HLA-specific B cells in sensitized patients awaiting transplantation. Previous research demonstrated that HLA class I CHAR T cells can eliminate HLA-specific B cells with exceptional specificity. Since many highly sensitized patients have antibodies directed against HLA-DQ, we aimed to develop HLA-DQ CHAR T cells to target HLA-DQ-specific B cells. Mismatched HLA-DQB1*02:01/DQA1*05:01 has previously been described as a risk allele for inducing HLA antibodies upon transplantation. Thus, we designed a viral vector encoding the CLIP peptide joined by a linker, to HLA-DQB1*02:01, CD28-CD3ζ costimulatory and signaling domains, P2A, and HLA-DQA1*05:01, and CD28-CD3ζ domains. Also, NGFR was used as a gene marker. Cells were retrovirally transduced to express CHAR molecules, which was verified by flow cytometry. Target cells were generated using the sequence of DQ2- and DQ7-specific human recombinant monoclonal antibodies (mAbs) to create B cell receptor (BCR)-expressing cells. DQ2 CHAR molecules were successfully expressed on the cell surface of both K562 cells and primary CD8+ T cells. Furthermore, we created targets by introducing two different DQ-specific BCRs into U266 and RAMOS B

cell lines. Expression was confirmed by flow cytometric analysis of the IgG, gene markers, and appropriate tetramers. Specificity was established by incubation of primary CHAR T cells and BCR-expressing cells, where HLA-DQ2 CHAR T cells specifically produced IFNγ upon incubation with DQ2-specific B cells. These tools are now further being used to investigate whether DQ2 CHAR T cells can also eliminate DQ2-specific B cells. We aim to expand the range of specificities by additional HLA class II CHAR T cells to further explore the potential of this therapy in highly sensitized patients, ultimately desensitizing sensitized renal transplant patients and treating humoral rejection after solid organ transplantation.

O50 | Identification of immune-modulating ligands and cytokine expression patterns in organoids exposed to inflammatory cytokines

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With the rise of regenerative therapies, immune diagnostics become relevant beyond conventional organ transplantation. Yet, the immunogenicity of regenerative therapies such as organoids remains poorly understood. Our multi-center study addresses this gap by evaluating the immunogenic properties of three different organoid models. We exposed the organoids to interferon gamma (IFNɣ)/interleukin-1β (IL-1β) and assessed immunogenicity through the expression of immuno-modulatory ligands and cytokine secretion. Complement mediated lysis was evaluated by cross-matching tubuloids with anti-HLA class I antibodies. Cytokines and ligands were differentially expressed in all organoid types upon stimulation. IP-10 and MCP-10, described as biomarkers of acute kidney transplant rejection, were significantly upregulated in all organoid types upon stimulation. In IFNɣ stimulated kidney organoids also IL-5 and IL-15 were significantly enhanced to concentrations, while tubuloids showed an increase of IL-6 and RANTES and a decrease of IL-8. For beta cell organoids eotaxin, GM-CSF, IL-1ra, IL-4, IL-7, IL-9, IL-10, IL-17, IP-10, MCP-1, MIP-1b, PDGF-bb and TNF-a were increased upon IFNɣ /IL-1β exposure. HLA class I, HLA-DR, co-stimulatory ligands MICB and CD54 were significantly upregulated kidney organoids. In contrast, MICA downregulation together with the elevated IL-1ra/IL-1β ratio show potential anti-inflammatory signaling. In tubuloids, CD46, CD55 and CD59 were strongly expressed, yet, non-expressing tubuloid cells were sensitive to complement mediated lysis. Furthermore, immunogenic ligand expression was passage-dependent, with significant expression of CD54 and PVR. Beta cell organoids expressed CD46 and CD58 and showed IFNɣ/Il-1β induced expression of HLA class I, HLA-E, PDL1, ICAM1 and 4-1BBL. In sum, our study delineated pro- and anti-inflammatory expression patterns, thereby enhancing the comprehension of immunomodulatory mechanisms critical for future organoid refinement.

O51 | Unprecedented survival improvement in patients with progressive multifocal leukoencephalopathy by partially HLA-matched polyomavirus-specific T-cell therapy

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Progressive multifocal leukoencephalopathy (PML) is a devastating demyelinating opportunistic viral infection of the brain caused by human polyomavirus 2 (HPyV-2) and particularly affecting patients with impaired cellular immunity, with mortality rates of up to 90% within months. There is no treatment to stop or slow down disease progression. Previous experience showed that pembrolizumab (anti-PD-1) is only effective in patients with

endogenous HPyV-2-specific T cells. For patients lacking endogenous HPyV-2-specific T cells, adoptive T-cell therapy (ACT) using allogeneic virus-specific T cells (VSTs) represents a new experimental therapy. Between 03/20 and 02/22, 28 PML patients received ACT with allogeneic HPyV-VSTs. The cause of PML were lymphoproliferative disorders ($n = 17$), systemic autoimmune diseases ($n = 5$), lymphopenia without prior immunotherapy ($n = 4$), and AIDS ($n = 2$). HPyV-VSTs were manufactured in a CliniMACS Prodigy using Cytokine Capture System IFN-gamma technology with GMP overlapping peptide pools VP1 and LT. The donors were 5–10/10 HLA-matched first-degree relatives ($n = 9$) or unrelated donors from the alloCELL registry ($n = 19$). In addition to clinical workup, T-cell immunity in the patients' blood was characterized prior and during treatment. The mean purity of HPyV-VSTs was 28.3% CD3 + IFN-γ + T cells and comparable between products from related or unrelated donors. Overall, 22 of 28 PML patients showed substantial symptom improvement or disease stabilization, along with detection of HPyV-specific T cells. Six of 28 patients did not respond to ACT. An increase in viral load in cerebrospinal fluid (CSF) and the absence of HPyV-specific T cells during follow-up correlated with a worse outcome after ACT. Personalized HPyV-VSTs from both, related and unrelated donors, were well tolerated and clinically effective. ACT with HPyV-VSTs is an unprecedented and promising therapeutic approach for PML patients for whom no treatment options have been available before.

O52 | In vitro profiling of post-transplant immunosuppressants reveals distinct impact on antiviral T-cell immunity

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Infectious complications, including widespread human cytomegalovirus (CMV) disease, frequently occur after HLA-mismatched hematopoietic stem cell and solid organ transplantation due to immunosuppressive treatment causing impairment of T-cell immunity. Therefore, in-depth analysis of the impact of immunosuppressants on anti-viral T cells is needed. We analyzed the impact of mTOR inhibitors sirolimus (SIR/S) and everolimus (EVR/E), calcineurin inhibitor tacrolimus (TAC/T), purine synthesis inhibitor mycophenolic acid (MPA/M), glucocorticoid prednisolone (PRE/P) and common double (T + S/E/M/P) and triple (T + S/E/M + P) combinations on antiviral T-cell functionality. T-cell activation and effector molecule production upon antigenic stimulation was impaired in presence of T + P and triple combinations. SIR, EVR and MPA exclusively inhibited T-cell proliferation, TAC inhibited activation and cytokine production and PRE inhibited various aspects of T-cell functionality including cytotoxicity. This was reflected in an in vitro infection model, where elimination of CMV-infected human fibroblasts by partially HLA-matched CMV-specific T cells was reduced in presence of PRE and all triple combinations. CMV-specific memory T cells were inhibited by TAC and PRE, which was also reflected with double (T + P) and triple combinations. EBV- and SARS-CoV 2-specific T cells were similarly affected. These results highlight the need to optimize immune monitoring to identify patients who may benefit from individually tailored immunosuppression to combat viral infections.

O53 | Humoral response in lung cancer patients treated with a plasmacytoid dendritic allogeneic cell line-based cancer vaccine

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We are conducting a Phase I/II clinical trial with PDC*lung01 product based on an irradiated plasmacytoid dendritic cell line loaded with 7 HLA-A*02:01-restricted tumor peptides for treatment of HLA-A*02:01+ NSCLC cancer patients in combination or not with anti-PD1 (NCT03970746). PDC*lung01 is injected intravenously

and subcutaneously 6 times at weekly interval at two dose levels, 14 million, or 140 million cells in stage II/IIIA (cohort A1 & A2, monotherapy) or stage IV (cohort B1 & B2; in combination with anti-PD1) patients. PDC*line cells express HLA-A*02:01, B*07:02, B*44:02, DRB*01:03, DRB*08:01, DPB*04:02, DPB*05:01. We describe here, both the dynamic and functionality of anti-HLA antibodies (Ab) against PDC*line that develop over time in cohort A1 ($n = 1$) and A2 ($n = 11$). One patient in cohort A1 and all patients in A2 developed anti-HLA Ab against HLA-B7 ($n = 6$), -B44 ($n = 4$), -DR103 ($n = 10$), -DR801 ($n = 12$), with MFI > 1000, starting before the last vaccine injection and reaching a peak (>20,000) 1 month after the last injection. The MFI intensity was depending on patients and HLA molecules, anti-class II molecules appearing first. Interestingly, no clinical side effect was associated to the presence of anti-HLA Ab. We demonstrated that anti-HLA Ab bind very well to PDC*line cells. Using an antibody-mediated complement-dependent cytotoxicity (CDC) assay in flow cytometry using healthy donor's or patient's sera, we have shown that PDC*line cells were resistant to the complement dependent killing mediated by patient's anti-HLA Ab despite the functionality of the patient's complement system. Interestingly, we have shown that PDC*line cells express high levels of membrane-bound complement regulatory proteins (mCRPs: CD46, CD55, and CD59) rendering them resistant to Ab-mediated CDC as shown by experiments using blocking Ab. Altogether, these results show the innocuity and the absence of deleterious effects of anti-HLA Ab on PDC*line cells used as a cancer vaccine platform.

O54 | Reducing the immunogenicity of the heart by genetic engineering during normothermic ex vivo heart perfusion

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Heart transplantation (HTx) is the only curative option to prolong the lives of patients with end-stage heart failure. However, HTx is associated with major hurdles related to the limited number of organs available for transplantation, the risk of rejection due to genetic discrepancies, and the immunosuppression (IS) burden. Previously, we showed that the silencing of swine leucocyte antigen (SLA) class I and class II expression in pulmonary grafts enabled graft survival after allogeneic lung transplantation even in the absence of IS. In this study, we aimed to evaluate the feasibility of decreasing the immunogenicity of the heart by genetic engineering towards the reduction of SLA expression. Hence, lentiviral vectors encoding for shRNAs targeting β2-microglobulin (shβ2m) and class II transactivator (shCIITA) were delivered to the heart during normothermic ex vivo heart perfusion (EVHP). Hearts perfused with lentiviral vectors encoding for non-specific shRNA (shNS) or non-transduced (non-TD) hearts served as controls. Transduction efficiency was extremely high in all types of heart tissue. Compared to shNS transduced hearts, silenced hearts showed up to 95% decreased SLA class I and up to 65% SLA class II-DQ expression, including the vascular endothelium as the major interface between donor and recipient. Histological analyses, perfusate troponin T (non-TD: 0.4 ± 0.1 vs. TD: 0.2 ± 0.1 ng/mL) and LDH levels (Abs A490-A690: non-TD: 0.961 ± 0.1 vs. TD: 0.860 ± 0.1) indicated no additional cell injury or tissue damage caused by lentiviral vector transduction in comparison to non-TD hearts after normothermic EVHP. Additionally, pro-inflammatory cytokine secretion signatures did not significantly differ between shβ2m/shCIITA and shNS transduced hearts. This study clearly shows the potential of heart genetic engineering and its use in creating immunological graft invisibility. This technology has the potential to open up a completely new direction in overcoming organ rejection.

O55 | The KIR system in a West African chimpanzee population shows limited genetic variation and skewing of the inhibitory lineage III KIR towards C2 specificity

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The human KIR family is extensively studied, displaying abundant levels of allelic polymorphism and copy number variation. Conversely, in our closest living relative, the chimpanzee, this system has been explored to a lesser extent, lacking population data and only three genomic KIR regions have been mapped. Our prior research interest focused on the genetic and functional characterization of the MHC class I repertoire in a West African chimpanzee population, revealing that this region was targeted by an ancient selective sweep, likely caused by an HIV-1/SIV-like pathogen. To comprehend the depth of the sweep, we

aimed to identify the full-length KIR repertoire in the same chimpanzee cohort using SMRT sequencing. Additionally, we resolved the genomic organization of KIR haplotypes using Cas-9-mediated enrichment combined with long-read ONT sequencing. This effort led to the identification of 35 unreported alleles and expanded the number of fully characterized genomic KIR haplotypes from 3 to 12. We show that most West African chimpanzee KIR genes experience purifying selection (dN/dS <1). Detailed comparative analysis of the various KIR regions demonstrated diversification by recombination events that introduced or deleted genes, affecting copy number variation and promoting the formation of fusion genes. Overall, the limited variation observed in the chimpanzee KIR system aligns with the MHC class I repertoire reduction. The population data we gathered allowed to examine the frequency distribution of inhibitory lineage III KIRs with either C1 or C2 specificity, revealing a predominant C2 specificity in West African chimpanzees. This matches with the higher frequencies of C2-positive MHC-C across African human and great ape species, suggesting that similar selective forces are at play, most likely driven by endemic pathogens such as HIV-1/SIV and the malaria parasite *Plasmodium falciparum*.

O56 | Disparities between inhibitory KIR receptors and their HLA ligands influence the clinical outcome of haploidentical hematopoietic stem-cell transplantation with post-transplant cyclophosphamide

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Natural-Killer cell (NK) alloreactivity impacts clinical outcomes after hematopoietic stem-cell transplantation (HSCT). However, in the context of haploidentical HSCT (haplo-HSCT) with post-transplant Cyclophosphamide (PTCy) this influence is still under debate. Our aim was to examine NK-alloreactivity after haplo-HSCT with PTCy accordingly to graft-versus-host disease and relapse-free survival (GRFS).A cohort of 145 haplo-HSCT with PTCy were included. Patients were HLA-typed by NGS (NGSgo® MX-11-3, GenDx). Donors were KIR genotyped by SSO (Lifecodes® KIR-SSO kit, Immucor). NK-alloreactivity was evaluated accordingly to missing-ligand model, through the absence of recipients' HLA ligand for a particular inhibitory KIR (iKIR) expressed by the donor. The median age of patients was 55 years, with 55% males. Acute Myeloid Leukemia and Myelodysplastic syndromes were the main diagnosis (52%). Peripheral blood was the main source of cells (98%), and comorbidity index (HCT-CI) was ≥3 in 30%. The followed up was 36 months. Our findings revealed that HLA ligand absence in the recipient for a particular iKIR expressed by the donor (iKIR-mm) associated with adverse events included in GRFS (HR = 2.46, 95% CI: 1.40–4.31, $p = 0.002$), and this could be predominantly due to the absence of C2 when the donor exhibited KIR2DL1 (HR = 1.79, 95% CI: 1.09–2.91, $p = 0.020$). HCT-CI (HR = 1.19, 95% CI: 1.04–1.35, $p = 0.009$) was also associated with worse GRFS, while complete-remission status showed lower risk of developing GRFS events (HR = 0.61, 95% CI: 0.37–0.98, $p = 0.043$). The multivariate analysis demonstrated that iKIR-mm and HCT-CI index were independent risk factors for the occurrence of GRFS events. Disparities between donor iKIR and their recipients' HLA ligands associate with clinically significant post-transplant events. Therefore, incorporating information of NK-alloreactivity into the selection criteria for haploidentical donors could improve the outcome of haplo-HSCT with PTCy.

MHC EVOLUTION, POPULATION GENETICS

O57 | Characterizing functional complementarity and the impact of natural selection on HLA class I haplotypes

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The main divergence in HLA class I (HLA-I) peptide binding preference is maintained by HLA-A and -B molecules, between which a complementary relationship at both functional and structural levels has been revealed by our previous findings. This has raised intriguing questions about the extent to which the component alleles of HLA-I haplotypes, some of which known to result from strong linkage disequilibrium (LD) and widely present in

various populations, demonstrate such a pattern. Conflicting views have arisen on this subject: while some suggested no more important functional differences between high-frequency HLA-A∼B haplotypes compared to random ones, others argued for more distinct functional attributes in high-frequency A∼B and A∼C haplotypes compared to random ones. In this context, we explored worldwide HLA-A∼B, A∼C and B∼C haplotype distribution in relation to the peptide binding functions of the proteins they encode, using available HLA-I genotypic data from 115 populations, as reported to the 13-18th IHIWs. Advanced in silico prediction and statistical methods were applied to estimate the functional relationship between molecules corresponding to each HLA-I haplotype. Interestingly, while populations typically exhibit significant positive LD among functionally divergent allele pairs, our analysis of high-frequency A∼B haplotypes indicates a negative effect on both the most and least functionally divergent allele pairs, which is compatible with a strong purifying selection acting at the haplotype level. In contrast, a similar phenomenon was not observed for A∼C and B∼C haplotypes. These results provide a new understanding of the evolutionary dynamics of HLA-I haplotypes. Despite expectations of positive selection for haplotypes with highly diverse alleles, our findings suggest that evolutionary pressures favor a more balanced complementarity of A∼B haplotypes, indicating the influence of additional evolutionary factors.

O58 | Unraveling the architecture of MHC class II haplotypes in rhesus macaques: Towards understanding the biological relevance of a pseudogene

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The regions in the genome that encode components of the immune system are often featured by polymorphism, copy number variation and segmental duplications. There is a need to thoroughly characterize these complex regions to gain insight into the impact of genomic diversity on health and disease. Here we resolve the organization of complete major histocompatibility complex (MHC) class II regions in rhesus macaques by using a long-read sequencing strategy (Oxford Nanopore Technologies) in concert with adaptive sampling. This examination successfully assembled and annotated a total of 24 unique Mamu-class II haplotypes, which comprise 17 distinct DR region configurations. In comparison, only five HLA-DR region configurations have been identified. Humans and rhesus macaques do not share a single region configuration, although some of the DRB genes and their lineages predate their speciation. The reason why pseudogenes, such as DRB6, are also conserved over long evolutionary time spans remained a puzzle. In particular, the expansion and contraction of the macaque DRB-region appears to be a dynamic process that involves the rearrangement of different cassettes of paralogous genes. These chromosomal recombination events are associated with a conserved pseudogene, Mamu-DRB6, which features the integration of two retroviral elements that may drive rearrangements. The human equivalent of this pseudogene, however, possesses only one retroviral element, which may be a more stable entity, aligning with the limited diversity recorded in the HLA-DRB region. In contrast, the DRA locus appears to be protected from rearrangements, which may be due to the presence of an adjacently located truncated gene segment, DRB9. Due to our sequencing strategy, the annotation, evolutionary conservation, and potential biological relevance of pseudogenes can be reassessed, an aspect that was neglected by most genome studies in primates.

O59 | Unraveling the evolutionary history of human and great ape Mhc-DQA1 lineages through full-length third-generation sequencing

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In humans, the MHC class II DQA1 gene exhibits abundant levels of polymorphism, with over 700 documented allelic variants in the IPD-IMGT/HLA Database. Based on their similarity, these alleles are grouped into six different lineages, namely DQA1*01 to *06. Some of these lineages are shared with great apes, designated as trans-species lineages. In addition, species-specific DQA1 lineages are documented. Currently, information regarding the DQA1 gene in great apes is limited and primarily derived from cDNA analysis. In our aim to gain a better

understanding of how evolution has been shaping immune genes and their diverse lineages, we conducted a comprehensive characterization of DQA1 gene variation at the genomic DNA level in a panel of chimpanzees ($n = 40$), bonobos ($n = 8$) and orangutans ($n = 14$) using a long-read Pacific Biosciences sequencing protocol, and compared this data to the available full-length HLA-DQA1 alleles. To get further insights into deeper evolutionary relationships, we paid particular attention to comparing intron sequences. Our analysis uncovered genetic patterns in introns 1 and 3, suggesting that the transspecies Mhc-DQA1*01 and species-specific DQA1*02, *03, *20 and *21 lineages originated from a shared common ancestral lineage. Similarly, the data highlights that the transspecies Mhc-DQA1*05 and HLA-DQA1*04 and *06 originated from a shared common ancestral lineage. Furthermore, the findings align with the previously documented strong linkage found between the chimpanzee Patr-DQA1*20 and Patr-DQB1*03, which mirrors that found between HLA-DQA1*02/*03 and HLA-DQB1*03.

O60 | Evolution trajectories of the major histocompatibility complex (MHC) class I genes in humans, chimpanzees and bonobos

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Research on the Major Histocompatibility Complex (MHC) in non-human primates (NHP) is carried out because of conservation biology challenges, but also because of the important role that some NHP species may serve as preclinical models for human diseases, including AIDS, malaria and tuberculosis. True orthologues of HLA (the human MHC) class I genes (MHC-A, -B and -C) are present in chimpanzees and bonobos. However, in comparison to HLA, the genomic data available for chimpanzees and bonobos is scarce, and often only includes exon 2 and 3 of MHC class I genes, where MHC-A, -B and -C have 8, 7 and 8 exons, respectively. In order to provide further insights into the processes that have shaped the evolution of MHC class I genes of different primate species and populations, we compare patterns of diversity of aligned sequences of 8129 MHC-A, 9588 MHC-B and 8699 MHC-C sequences, representing 25 human population samples, 3–9 bonobo population samples, and 4–7 chimpanzee population samples (including three subspecies of chimpanzees). MHC-B is the gene that shows the highest values of nucleotide diversity. Exons tend to show a higher nucleotide diversity than introns in humans. This is however not the case for MHC- C in bonobos and chimpanzees. While it has been hypothesized that bonobos underwent a species-specific selection pressure to explain a lower nucleotide diversity at their MHC class I genes in comparison to chimpanzees and humans, this trend is not observed across all bonobo samples. We observe that levels of nucleotide diversity do not differ strongly between species, but more so between MHC class I genes, where MHC-B shows the highest values of nucleotide diversity, followed by MHC-A and MHC-C. Observed similarities between MHC diversity patterns indicate that some evolutionary mechanisms are conserved across these species, while the evolution of MHC-C is peculiar in humans in comparison to bonobos and chimpanzees.

O61 | Maternal-fetal HLA compatibility and pregnancy outcome in a genetically isolated population

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In pregnancy, the semi-allogenic fetal trophoblast expresses a specific HLA profile (HLA-C, -E, -F, -G), crucial for placentation and outcome, as it mediates contact with maternal leukocytes. Paradoxically, maternal immunomodulation also requires paternal antigen recognition and thus histo-incompatibility. Indeed, pre-eclampsia, a severe pregnancy complication, is suggested to correlate with antigenic similarity. Also, combinations of fetal HLA-C and its KIR receptor on uterine NK cells correlate with poor placentation, characteristic for pre-eclampsia. We recently found increased total HLA, HLA class I and HLA-C matching was associated with pre-eclampsia, suggesting a role of HLA mismatches in immune regulation leading to uncomplicated pregnancy. We aim to determine if there is a preferential selection for HLA compatibility and specific KIR/HLA-C combinations in a genetically-isolated population and its relation to the development of hypertensive complications, in particular pre-eclampsia. This case–control study was performed in a genetically-isolated Dutch population with a high inbreeding coefficient (FROH 1.3 (recent) & 3.1 (ancient), 6–14 times the overall Dutch population). Maternal and fetal HLA-A, -B, -C, -DR, and -DQ, and maternal KIR genotyping was done for 261 pregnancies, including 41 complicated by pregnancy-induced hypertension (PIH) and 23 by pre-eclampsia. The number of maternal-fetal HLA antigen (mis)matches was compared to expected values obtained by randomization of paternal HLA haplotypes over maternal haplotypes of the fetuses. Similar methodology was executed for KIR/HLA-C data analysis. Due to the expected high degree of parental HLA matching, we hypothesize that uncomplicated pregnancies show less HLA compatibility compared to expected values. For the pre-eclamptic cases, we expect high HLA-C matching, specifically the combination of activating KIR genes with fetal HLA-C2 genotype.

O62 | Prognostic role of HLA alleles and cytokine single-nucleotide polymorphisms in chronic myeloid leukemia patients treated with tyrosine kinase inhibitor drugs

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Tyrosine kinase inhibitor drugs (TKIs) has significantly improved chronic myeloid leukemia (CML) outcomes. Peptides from CML leukemic cells may induce specific immune responses, crucial for deep molecular remission (DMR) and treatment-free remission (TFR). In this study, Ethiopian CML patients' ($N = 151$), HLA alleles and single-nucleotide polymorphism of five cytokines revealed significant associations with clinical outcome. Clinically unfavorable outcomes correlated with HLA alleles A*03:01/02, A*23:17:01, B*57:01/02/03, and HLA-DRB4*01:01 ($p = 0.0347$, $p = 0.0285$, $p = 0.037$, $p = 0.0127$). HLA-DRB4*01:03:01 was associated with favorable outcomes ($p = 0.0058$). After assigning values for 'low,' 'intermediate,' and 'high' gene expression of SNPs' respective cytokine genes, Kaplan–Meier estimates for relapse-free survival, adjusted for age, treatment duration, and relapse risk among post-TKI patients, indicated that a gene expression ratio above the overall median of {TNF-α+, IL-6+, TGF-β1}/{IL-10+, IFNγ} and {IL-6/IL-10 + TGF- β1} correlated with a higher likelihood of treatment failure (RR: 3.01; 95% CI: 1.1–8.3; *p*-value: 0.0261) and (RR: 2.4; 95% CI: 1.1–5.2; *p*-value: 0.022), respectively. Multi-SNP, surpassing single-SNP, and HLA allele polymorphism showed promise in predicting CML patient outcomes during TKI treatment, prompting further exploration for their potential utility in TFR trials.

O63 | Major histocompatibility complex (MHC)-I molecules and intron 2 of wild songbirds around world show striking differences with other vertebrates

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MHC molecules in vertebrates are responsible for presenting peptides in order to start an immune response. The MHC of *Passeriformes* or Songbirds is more complex than that of chicken and it has longer introns. Introns are known to be involved in different kind of processes during DNA replication and transcription, and in some further mRNA processing steps. We have found that

these birds present conserved nucleotide positions, which have remained invariant throughout the evolution of Songbirds and do not appear in any other studied vertebrates (including birds of *Galliformes* order). MHC positions 10 and 96 of class I MHC molecule show Val and Leu instead of Thr and Gln respectively. In the present work, we have studied MHC class I (MHC-I) introns in different species of *Carduelis* and *Serinus*, and we have compared them with those of chicken (MHC-B) and human MHC (HLA). DNA samples studied in present work were extracted directly from blood of wild birds captured in their natural habitats around world. MHC-I intron 2 characterization was done by analyzing nucleotide frequencies, genetic distances between species and frequencies similarity percentage by using MEGA 7 and BioEdit software. MHC-I intron 2 sequences from *Carduelis* and *Serinus* species covering most of the world species habitats range were analyzed to ascertain conserved positions; 267 positions out of 312 were conserved. The closest genetically related birds showed the smallest genetic distance between them, indicating a more similar MHC-I intron 2, which was consistent with the similarity percentage data. In conclusion, the hypothesis of the "minimum essential MHC" of chicken (*Galliformes* order) cannot be applied to other birds such as *Passeriformes*, whose introns are much longer. Furthermore, MHC-I intron 2 has remained stable during millions of years of evolution, which indicates that it plays a possible fundamental role in transcriptional regulation or other processes.

O64 | High-resolution HLA haplotype frequencies of four population groups in South Africa

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In the context of hematopoietic stem cell transplantation, HLA haplotype frequencies are of crucial importance for search algorithms designed to find matching unrelated donors in a large donor registry. Haplotype frequencies are also utilized to estimate the overall probability for finding a matching donor in a registry of a given size in order to assess donor recruitment strategies. Here, we present high-resolution five-locus (HLA-A, -B, -C, -DRB1, -DQB1) haplotypes for four population groups based on $n = 56{,}710$ individuals registered with the DKMS Africa donor center located in Cape Town, South Africa. Upon recruitment, donors self-assign their and their parents' population group as either "Black," "Colored," "Indian/Asian," or "White". Here, we included donors with both parents originating from South Africa and donor and parent population groups being identical, yielding sample sizes of $n = 19{,}999$ for Blacks, $n = 4982$ for Colored, $n = 4699$ for Indians/Asians, and $n = 27{,}030$ for Whites. Haplotype frequencies were computed using Hapl-o-Mat, our open-source implementation of the expectation–maximization algorithm. The most common haplotypes within the Black, Colored, Indian/Asian, and White populations were A*30:01g∼B*42:01g∼C*17:01g∼DRB1*03:02g∼DQB1*04:02g ($f = 3.5\%$), A*01:01g∼B*08:01g∼C*07:01g∼DRB1*03:01g ∼DQB1*02:01g ($f = 1.9\%$), A*01:01g∼B*57:01g∼C*06:02g ∼DRB1*07:01g∼DQB1*03:03g ($f = 3.4\%$), and A*01:01g∼ B*08:01g∼C*07:01g∼DRB1*03:01g∼DQB1*02:01g ($f = 6.4\%$), respectively. The cumulated frequency of the 50 (100) most common haplotypes per population was $f = 38.4\%$ (50.4%) for Blacks, $f = 20.7\%$ (28.7%) for Colored, $f = 27.4\%$ (35.7%) for Indians/Asians, and $f = 38.2\%$ (49.8%) for Whites. The cumulated frequencies for Blacks and Whites were almost identical and the highest, indicating a relatively low intra-population diversity. On the other hand, the low cumulated frequency for the Colored population hints at its heterogeneous origins, including contributions of the other populations.

NEW TECHNOLOGIES IN IMMUNOGENETICS

O65 | Differential expression of HLA-DM and HLA-DO genes in normal cells, tissues and leukemia: A review of public data

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HLA class II (HLA-II) antigen presentation is modulated by the peptide editor HLA-DM and its negative regulator HLA-DO, determining the peptide repertoire presented by normal and malignant cells. Lack of DM function was suggested as escape mechanism of leukemia from autologous T-cell immunity, while the same was shown to increase in vitro T-cell alloreactivity. Both phenomena have potential implications for the outcome of allogeneic hematopoietic cell transplantation (alloHCT) as leukemia treatment, but a comprehensive analysis of HLA-DM and HLA-DO mutual expression in different cell types and tissues is still lacking. Here we collected bulk and single cell RNA sequencing data from public databases and published studies to explore the expression of HLA-DM (DMA and DMB) and HLA-DO (DOA and DOB) coding genes along with classical HLA-II, CIITA and CD74 in 204 different cell types (including subsets of dendritic cells and B cells), 50 normal tissues (including gut, liver and skin as common targets of T-cell alloreactivity), and leukemia/lymphoma either primary ($N = 16$ AML and $N = 7$ ALL) or cell lines ($N = 169$). As previously reported DMA, DMB and DOA were coregulated with HLA-II, CIITA and CD74 in all HLA-II expressing immune cells, while DOB was found only in B cells and certain subsets of DC in peripheral blood. Interestingly, DOB was also expressed by CD34+ hematopoietic stem cells from bone marrow, subsets of medullary thymic epithelial cells of thymus, Schwann cells in adipose tissue, and Langerhans cells in skin. In non-hematopoietic tissues, DOB expression was found in association with presence of B cells, including gut and liver. In leukemia/lymphoma, DOB expression was mainly restricted to B-cell derived malignancies, with very limited expression in primary AML. In conclusion, these data provide a comprehensive atlas of DM and DO expression which might help future investigation on the role of these molecules in different clinical context and in particular in alloHCT.

O66 | Comparative performance assessment and validation of a decentralized donor-derived cell-free DNA assay for kidney allograft rejection monitoring

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A potential breakthrough in the diagnostic of post-transplantation allograft injuries is the emergence of donor-derived cell-free DNA (dd-cfDNA) as a promising, non-invasive biomarker with clinical relevance in solid organ transplantation. This study seeks to assess the effectiveness of a novel decentralized, NGS-based, dd-cfDNA testing kit, AlloSeq cfDNA, in comparison to the established standard, AlloSure Kidney, aiming to provide valuable insights into their diagnostic capabilities and potential implications for improving the current diagnostic landscape in allograft rejection monitoring. Kidney transplant recipients ($n = 580$) from 3 referral centers underwent measurements (603 total evaluations) with AlloSeq cfDNA and AlloSure Kidney dd-cfDNA measurements alongside allograft biopsies. Correlation between assays was evaluated using r-squared (r^2) and Spearman's rank correlation test, and associations with rejection using logistic regression analyses. Mean dd-cfDNA levels from AlloSeq cfDNA and AlloSure Kidney were 0.51 $\pm$ 0.81% and 0.43 $\pm$ 0.78%, respectively. The assays were highly correlated, with $r^2 = 0.95$ and Spearman's rank correlation 0.88 (p-value <0.0001). Mean dd-cfDNA levels were 1.15 $\pm$ 1.60% with and 0.39 $\pm$ 0.48% without rejection ($p < 0.0001$) for AlloSeq cfDNA, and 1.06 $\pm$ 1.47% with and 0.31 $\pm$ 0.49% without rejection ($p < 0.0001$) for AlloSure Kidney. Both tests showed significant association with allograft rejection ($p < 0.0001$). Consistency between the assays was also confirmed across clinical scenarios including post-transplant timepoint, allograft stability, and allograft rejection subcategories (antibody-mediated and T-cell mediated or mixed rejection). The AlloSeq cfDNA assay delivers precise results within 24 hours, with low input requirements and with a flexibility that accommodates centers of all sizes, ensuring convenience in clinical practice and enhancing patient care.

O67 | Comparing HLA loss detection by NGS versus qPCR

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Genomic loss of mismatched HLA alleles (HLA loss) is a possible driver of relapses after hematopoietic stem cell

transplantation, especially in a haploidentical transplant. Early detection of HLA loss impacts clinical decision making. In case of non-HLA loss relapse, donor lymphocyte infusion or modulating immunosuppression is a treatment option. In the case of HLA loss, a second allotransplant from a different donor may be considered. HLA loss can be measured by quantifying the levels of markers within the region of HLA gene loci. This can be measured by qPCR, which is highly accurate, but the number of markers currently available in commercial kits are insufficient to adequately discriminate between individuals. Here, we demonstrate a method using Next-Generation Sequencing (NGS) for increased informativity in determining HLA loss. PCR assays were developed for 15 STRs interspersed between HLA-A (5′ region of the MHC cluster) and HLA-DPB1 (3′ region). The amplicons are sequenced on an Illumina MiSeq and data is analyzed in a new TRKengine software (GenDx) using a new feature for analysis of HLA loss. The software reads the FastQ files, extracts the information on STR counts, and visualizes the STR lengths in graphs. Artificial chimeric samples were created and analyzed using a qPCR method and the new NGS method. Here we show that STR markers within the MHC region can be applied to quantitatively monitor loss of specific HLA alleles within chimeric samples and can be an accurate indication of loss of a specific haplotype. In summary, STR markers within the MHC region can be used as highly informative, sensitive and specific markers to detect HLA loss by NGS.

O68 | Identification and characterization of the novel fusion gene LILRB3-LILRB5 by long-read sequencing

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The leukocyte immunoglobulin (Ig)-like receptors (LILRs) are a multigene family present in primates but absent in rodents. Human LILRs encode 11 immunoglobulin superfamily receptors, showing genetic diversity within and between human populations, and are composed of five activating receptors (LILRA1, LILRA2, LILRA4, LILRA5, and LILRA6), five inhibitory receptors (LILRB1, LILRB2, LILRB3, LILRB4, and LILRB5), and one soluble form (LILRA3). Among the LILR genes, LILRA6 displays a broad range of copy number variations, ranging from 0 to 6 copies per individual. However, the complete characterization of the genomic region surrounding the LILRA6 gene remains elusive owing to the considerable sequence homology between LILRA6 and its adjacent gene LILRB3. Here we discovered a novel large deletion (33,692 bp) in the LILRA6 region by using long-read sequencing technology. This deletion encompassed the LILRB3, LILRA6, and LILRB5 genes, causing the loss of LILRA6 and positioning LILRB3 exons 12–13 immediately downstream of LILRB5 exons 1–12. This result suggests that the genomic structure could produce a fusion gene comprising LILRB3 and LILRB5. Based on this finding, we developed an algorithm for copy number determination from short-read sequencing data and found that the fusion gene LILRB3-LILRB5 is more frequent in the Japanese population than in other populations. Our findings suggest that LILRB3-LILRA6-LILRB5 genomic region is a hotspot for non-allelic homologous recombination, creating human genome diversity.

O69 | Different strategies to resolve complete KIR haplotypes using long-read ONT sequencing

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Killer cell immunoglobulin-like receptors (KIR) modulate the activity of NK cells by engaging with MHC class I molecules. The genetic region encoding KIR receptors displays extensive variation, reflected by copy number variation, allelic polymorphism, and haplotype rearrangements. The characterization of KIR genes are of importance, as the interplay of KIR and their ligands may have clinical relevance. Current methods for the identification of human KIR genes have primarily focused on discerning their presence or absence. However, information on complete KIR haplotypes at an allele level resolution may further improve the understanding of the functional properties of these receptors. We explored different strategies using long-read sequencing on an Oxford Nanopore Technologies (ONT) platform to unravel KIR haplotypes in rhesus macaques. This species displays more diverse KIR haplotypes than humans, representing a valuable model to validate characterization strategies. Two long-

read approaches employ different types of target enrichment, with the first relying on directed Cas9 nuclease activity whereas the second utilizes adaptive sampling, a computational tool for reference-based enrichment. Both strategies yield relatively low coverage at present but are in most cases sufficient to phase haplotypes. We also successfully applied the guided Cas9 strategy to resolve human KIR haplotypes. In a third strategy, the complete rhesus macaque genome is sequenced and assembled, enabling the annotation of phased KIR haplotypes through an automated pipeline. This approach not only constructs detailed KIR haplotypes but also provides insights into the entire genome, including the MHC genes. Our different strategies highlight the value of long-read ONT sequencing, offering a comprehensive understanding of KIR genetics. These approaches also demonstrate the potential for implementation into clinical settings and the potential to finetune association studies.

O70 | Genotyping of MICA and MICB using a full-length gene sequencing strategy for a large panel of International Histocompatibility Workshop cell lines

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MICA and MICB are class I chain-related genes found in the beta-block of the major histocompatibility complex. They are highly polymorphic genes with 531 and 246 alleles documented so far (IPD-IMGT/HLA Database v3.55.0). We developed full-gene typing strategies for MICA and MICB using PacBio's Single Molecule Real-Time DNA sequencing technology, using them to type a panel of 178 International Histocompatibility Workshop (IHW) B-lymphoblastoid cell lines to validate this methodology and improve the characterization of IHW cell lines. Only 47.2% (MICA) and 50.0% (MICB) had previously reported DNA typing (two field resolution or higher) in the IPD-IMGT/HLA Database. Typing concordance was 96% for MICA and 93% for MICB, once cell lines with incomplete gene sequences were considered. For MICA most of these were due to polymorphisms in exon 1, 6 or introns, whilst for MICB most arose from intronic regions, all of these being outside the regions commonly sequenced historically. Any discrepant alleles were within those permitted by known linkage disequilibrium with HLA-B. Forty-two novel MICA and 34 novel MICB alleles were observed, which were predominantly intronic variants. A non-synonymous MICA variant was identified in exon 2 (Glycine to Arginine at residue 70). Of all novel alleles, 44 differed from an existing allele by one single nucleotide polymorphism (SNP), 11 differed by two SNPs and the remaining 21 differed by ≥3 SNPs or a combination of SNPs and indels. We extended or confirmed the sequences of 13 MICA and 32 MICB alleles in the IPD-IMGT/HLA Database. Twenty-one unique MICA and 9 unique MICB proteins were observed, the most common being MICA*008 (27%) and MICB*005 (52%). In total 94 unique MICA alleles and 72 unique MICB alleles were observed, with the most common being MICA*008:04:01 (8%) and MICB*004:01:01 (10%). This work further characterizes this well-established panel of cell lines for the MICA and MICB genes, improving their usefulness to the immunogenetic community.

O71 | Sensitive NGS-based chimerism monitoring using ONT sequencing

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With improved sensitivity of chimerism monitoring, a relapse after hematopoietic stem cell transplantation can be detected earlier. The NGS-based chimerism monitoring assay NGStrack® (GenDx) uses 34 bi-allelic indel markers, employing Illumina sequencing. Illumina sequencing is robust, but relatively slow and requires a large investment. Oxford Nanopore Technologies (ONT) sequencing combines rapid analysis and scalability with a small footprint and low investment requirements. Here, we complement the design of NGStrack to be compatible with ONT sequencing. Artificial chimerism samples were created by diluting (cell line) DNA in DNA, resulting in 9 series, each consisting of 16 chimeric samples. NGStrack primers were used for amplification of the 34 markers, followed by indexing, sample pooling and custom ONT library preparation. Libraries were sequenced on a MinION flow cell (R10.4.1) on a MinION Mk1B or GridION (400 bps, 5 kHz). Basecalling was performed real-time with MinKNOW Super-accurate (SUP)

basecalling algorithm. Data was analyzed in TRKengine 1.4 software (GenDx) using default settings. Generating ~300.000 reads, chimerism levels as low as 1% could be accurately measured, approaching the sensitivity of NGStrack using Illumina (0.1%–0.5%). Compared to Illumina, ONT sequencing results in relatively high sequencing noise, which may be a limiting factor for sensitivity that could be further improved with changes in sequencing and basecalling, and/or analysis. In 2.5 h, enough reads were generated to genotype 96 samples. When sequencing for 24 h up to 50 samples can be monitored. For a single monitoring sample, enough reads can be obtained within 1 h of sequencing, resulting in a single sample turnaround time of just over 4 h from DNA to results. In conclusion, ONT sequencing with NGStrack is a suitable approach for both high-throughput and samples requiring fast turnaround times, while obtaining a sensitivity of at least 1%.

O72 | Enhancing PCR stability for HLA genotyping from buccal swab samples through the incorporation of bovine serum albumin

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In our custom next-generation sequencing (NGS) workflow for HLA genotyping, we process over 50,000 samples monthly. This substantial sample volume provides a unique opportunity to meticulously analyze factors influencing PCR success and evaluate strategies for enhancement. Upon DNA extraction from buccal swabs and quantification, samples with a concentration below 2 ng/μL are excluded from further processing. Previously we observed variations among countries in terms of samples with insufficient DNA concentration (<2 ng/μL), likely due to varying compliance rates with collection instructions. However, for samples with sufficient DNA, we maintained a relatively stable PCR sample failure rate (defined as failure of amplification in several targets) of approximately 0.3%. Anomalies emerged in October 2022: PCR sample failure rates suddenly spiked, primarily driven by samples from Germany, where failure rates exceeded 2% during the first 5 months of 2023. Notably, this increase was not linked to reduced DNA concentrations. Instead, we observed elevated failure rates across the entire spectrum of DNA concentrations. Dilution experiments indicated the presence of inhibitory substances persisting after DNA purification, significantly compromising PCR success. Our solution involved the addition of Bovine Serum Albumin (BSA) to the PCR reaction. BSA effectively counteracted the inhibition observed in affected samples. Following extensive testing and validation, we implemented a protocol that incorporates BSA in the PCR. As a result, the PCR sample failure rate for samples from Germany decreased from 2.4% (Jan–May 2023) to 0.1% (Jul–Dec 2023). Notably, also samples from unaffected countries benefitted considerably, reducing failure rates to 0.1% overall. In summary, the incorporation of BSA emerged as a highly effective strategy in countering inhibitory substances, resulting in markedly enhanced PCR stability for HLA genotyping from buccal swab samples.

POSTER PRESENTATIONS

AUTOIMMUNITY, INFECTION, REPRODUCTION & CANCER

P1 | Exploring the germline diversity of various macaque B-cell receptor regions

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Macaque species are widely applied as a model for the study of infectious diseases and vaccine efficacy. Despite their importance, genomic characterization of some macaque immune regions remains limited, such as regions encoding the highly variable B-cell receptor (BCR). Specificity of each BCR is dictated by the rearrangement of variable (V), diversity (D), and joining (J) gene segments, the pairing of heavy and light chains, combinatorial and junctional diversity, and somatic hypermutations. The well-characterized human V(D)J segments are located on three chromosomes, forming distinct regions encoding the heavy chain (IGH) and two light chains (IGK, IGL). At an individual level, these BCR regions are highly diverse, featuring insertions, deletions, and allelic variations. The macaque BCR regions, also distributed across three chromosomes, seem to display more diversity, especially in IGK and IGL V segments. To refine

translatability of immunological research from macaques to humans, a deeper understanding of the macaque BCR layout is essential. In this study, long-read sequencing was performed using ONT and PacBio platforms to characterize complete BCR regions. Using a custom bioinformatic pipeline, macaque BCR clusters were assembled and annotated. Despite abundant repetitive sequences and highly similar segments, phased haplotypes were resolved for IGH, IGK and IGL regions. This allowed identification and mapping of previously reported and novel gene segments, including potentially functional and pseudo entities. Our sequencing approach enabled a comprehensive characterization of the heavy and light chain BCR regions in macaques, revealing extensive allelic and structural variations. Further implementation of this strategy would generate accurate germline references of V, D, and J segments, which allows assessment of mutational rates and BCR affinity maturation in response to infection or vaccination, thereby refining the macaque model in biomedical research.

P2 | HLA allele frequencies and susceptibility to Bullous Pemphigoid in a group of 116 Italian patients

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With the aim to identify alleles that may reflect a higher susceptibility to the disease, in the present study we analyzed the HLA allele and phenotype frequencies distribution in 116 Italian patients affected by Bullous Pemphigoid (BP): 86 receiving Gliptin for their concomitant Type 2 diabetic (T2D) pathology, 30 with no Gliptin administration. A strong significant association was found between both allele and phenotype frequencies for HLA-DQB1*03:01 (69.19% and 95.35%; respectively) in the 86 patients affected by BP and treated with Gliptin, comparing the results with a reference group of 1017 Italian healthy individuals (32.01%, pc = 0.00012; 54.47%, pc = 0.00012) and with a group of patients affected by T2D, but not suffering for BP (28.33%, pc = 0.00012; 46.76, pc = 0.00012, respectively). In the same group of patients, we also observed higher allele and phenotype frequencies for the HLA class II DQA1*05:01P (70.93% and 95.19%, respectively) and for both DRB1*11:01:01 (24.42% and 40.79%, respectively) and DRB1*11:04:01 (29.07% and 45.35%; respectively), showing statistically significant differences compared to healthy controls or to patients affected by T2D but not by BP. Moreover, we also observed a statistically significant difference in the allele and phenotype frequency of the B*18:01:01 (21.51% and 36.05%, respectively) towards the healthy individuals (14.06%, $p \leq 0.00076$ and 25.76%, pc = 0.035188, respectively). These data suggest that the increased specific allele and phenotype frequencies observed identify potential markers of susceptibility to BP in particular when associated to a Gliptin treatment.

P3 | Genetic variants associated with narcolepsy

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HLA-DQB1*06:02 remains the main genetic risk factor for narcolepsy. However, single nucleotide polymorphisms (SNPs) in genes encoding proteins interacting with HLA or other elements of the immune system are also implicated as a genetic risk factors. We investigated previous GWAS defined SNPs to determine association with disease onset and symptoms experienced in local cohorts. Initially, we investigated three SNPs (rs1154155, rs12587781, rs1263646) encoded within the genes for the TCRα chain with diseased onset, of which, rs1154155G was significantly associated ($P = 0.017$). Further investigation of rs1154155 in a familial setting emphasized the contribution of HLA-DQB1*06:02 homozygosity combined with rs1154155G resulting in an increased risk of narcolepsy. In addition, we observed a significant association between rs306336 ($P = 0.018$), rs4290147 ($P = 0.023$) and rs2834168 ($P = 0.036$) with an increased rate of long-term memory decline implying that cognitive issues experienced are due to genetic predispositions in addition to neurodegenerative loss. In addition, rs4290173, located upstream of the APOBEC1 complement factor (A1CF) genes was also nominally associated with a decline in sleep efficiency ($P = 0.021$). We performed additional

GWAS testing which resulted in no significant SNP candidates following statistical correction. However, 32/100 of SNPs with the lowest *P*-values were located on chromosome 6 outside the MHC, suggesting a rational for further investigation. Our research highlights the impact of the genetic risk factors in narcolepsy onset and the symptoms experienced. Inclusion of additional SNPs alongside conventional genetic screening to support a diagnosis would allow the clinician to tailor treatment and combat both the traditional and non-traditional symptoms.

P4 | NKG2D receptor and MICA and MICB ligand polymorphism in CALR mutation—Driven myeloproliferative neoplasms

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The relevance of activatory NKG2D receptor and its ligands—MICA and MICB in MPN remains unexplored. The aim of our study was to investigate the role of these polymorphic genes in CALRmut driven MPN. MICA and MICB genotyping was performed by NGS in 42 CALRmut+ and 139 JAK2 V617F+ MPN patients and 150 healthy controls from the Bulgarian population. Five SNPs in NKG2D, previously shown to be associated with the level of natural lymphocytotoxicity were genotyped by TaqMan Assay. By fitting additive generalized linear models, we observed statistically significant increased frequency of 3 alleles: MICA*004:01, *009:01, MICB*008:01 and 3 haplotypes: MICA*009:01∼MICB*004:01, MICA*009:01∼MICB*005:02, and MICA*008:01∼MICB*008:01 in CALR mut.+ MPN patients compared to healthy controls. On the other hand, haplotypes MICA*016∼MICB*005:02 and MICA*008:01∼MICB*005:08 showed possible protective association. Additionally, we identified 2 new MICB alleles in CALRmut+ MPN patients. When alleles were grouped according to functionally relevant polymorphisms, MICA A6 exon 5 variant was significantly more frequent in CALR+ patients. Comparison between 2 MPN cohorts did not show any statistically significant differences, except for the borderline increased frequency of MICB*004:01 allele in CALR mut + patients. Despite of the prevalence of NKG2D haplotypes, associated with low level of cytotoxicity, no statistically significant differences were observed between patients and controls. Analysis of NKG2D and MICA/B ligand combination showed statistically significant correlation between NKG2D rs1049174 C/G and rs2617160 A/T heterozygotes and MICA 129Val weak binders in CALRmut+ patients. Taken together our data suggest significant role of MICA/B polymorphism, and NKG2D receptor-ligand combinations in CALR mut + MPNs. It is possible that MICA/B targeting approaches could be of clinical benefit for some of MPN patients. Supported partly by grant KP-06-H41/2.

P5 | A non-invasive method for fetal trophoblast HLA typing in early pregnancy

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Maternal-fetal HLA compatibility influences pregnancy outcome as it is associated with severe complications such as pre-eclampsia. Currently, no non-invasive technique for fetal HLA typing exists, while it may facilitate obstetric risk assessment. Trophoblast Retrieval and Isolation from the Cervix (TRIC) is a promising non-invasive method for fetal genome analysis based on retrieval of extravillous trophoblasts (EVT) from the maternal cervix. The present study aims to test the feasibility of applying TRIC to determine fetal HLA typing at early gestation. Cervical samples of 36 pregnant women were collected using a cytobrush. Saliva and umbilical cord blood, from 13 pregnancies, served as maternal and fetal HLA genotype controls, respectively. Cervical samples from non-pregnant women, primary cultured EVTs and cryo-sectioned term placenta served as controls for cell phenotype, protein expression and effect of fixation. TRIC was applied by HLA-G-coupled nanoparticle immunomagnetic separation of EVT from maternal cells. EVT presence before and after isolation was determined by HLA-G, β-hCG and Cytokeratin-7 (Cyt-7) immunofluorescence. The method was optimized by comparison between 3 types of (nano)beads and 2 magnets and was further compared to Flow Cytometry Cell Sorting. HLA-

G+ EVTs (0.01%–0.04% among maternal cells) were detected in cervical samples of every trimester of gestation, with the earliest detection at 6 weeks. Maternal cells showed Cyt-7 expression, indicating its unsuitability as a specific trophoblast marker. Although β-hCG+ Cyt-7+ EVTs were detected after TRIC in nine samples, none resulted in successful fetal HLA typing, while there was maternal HLA genotype confirmation. Thus, EVT yield and purity must still be increased in order to successfully use TRIC as a non-invasive technique for fetal HLA typing. Optimization of the isolation technique is performed to assess its usability for fetal genomic analysis and the feasibility for clinical application.

P6 | Differential modulation of mutant CALR and JAK2 V617F-driven oncogenesis by HLA genotype in myeloproliferative neoplasms

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We aimed to explore further the role of HLA in CALRmut + MPNs using association, gene expression analyses, and neoepitope predictions. NGS HLA genotyping was conducted in 42 CALRmut+, 158 JAK2 V617F+ MPN patients and 1083 healthy controls. HLA-I genotype diversity revealed no significant differences between CALRmut+ patients and controls. However, HLA-C*06:02 showed an inverse correlation with CALRmut. This was supported by the identification of protective haplotypes HLA-A*01:01∼C*06:02 and HLA-B*13:02∼C*06:02. A meta-analysis, including 138 CALRmut+, 479 JAK2V617F+ patients from 4 independent cohorts and 2693 healthy individuals from the 1000 Genomes project identified alleles which were significantly more frequent in CALRmut+ versus JAK2 V617F+ patients. Additionally, an inverse correlation between presentation of JAK2 V617F and CALRmut-derived peptides was observed in both patients and healthy individuals. Molecular dynamics simulations suggested that some CALRmut peptides may bind stably specific HLA-I alleles. Analysis of HLA-II diversity showed that CALRmut + MPNs had higher DQB1 evolutionary divergence compared to both JAK2 V617F+ patients and healthy controls. The association analysis showed that HLA-DQA1*01:02; DQB1*05:02; DQA1*04:01; DRB1*16:01; DRB1*08:01 alleles were significantly enriched in CALRmut+ patients and none of these were predicted to bind CALRmut derived 15-mer peptides. Further we identified predisposing and protective for CALRmut+ MPNs HLA-II haplotypes. Finally, scRNA-Seq analysis revealed low expression of TAP1 and CIITA genes in CALRmut+ hematopoietic stem and progenitor cells. In conclusion, our study indicates that HLA-I genotype differentially restricts JAK2 V617F and CALRmut-driven oncogenesis, potentially explaining the mutual exclusivity of the two mutations and differences in their presentation latency. These findings have practical implications for the development of neoantigen-based vaccines in MPNs. Supported by grant KP-06-H41/2.

P7 | Effects of HLA-G molecules in primary biliary cholangitis: The Sardinian experience

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Primary biliary cholangitis (PBC) is a rare autoimmune cholestatic liver disease. To date PBC causes are still unclear. However, both genetic factors and environmental triggers could contribute to the development of the disease. The Sardinian population due to their homogeneous traits, is particularly suitable for genetic studies, especially for rare diseases such as PBC. In this study, we

investigated the role of soluble HLA-G (sHLA-G), HLA-G alleles, and 3′ UTR haplotypes, and their potential impact on the onset and therapy response in PBC patients. A cohort of 166 Sardinian PBC patients was compared to 180 healthy individuals and 205 autoimmune hepatitis-1 (AIH-1) patients. The HLA-G alleles and 3′ UTR haplotypes of the patients and the healthy controls were analyzed. Plasma sHLA-G levels were measured, and the results were categorized based on HLA-G 3′ UTR haplotypes known to affect HLA-G expression. Our data showed that the HLA-G UTR-1 haplotype was significantly more frequent in PBC patients compared to the control population [160/332 (48.2%) versus 123/360 (34.3%), respectively; OR = 1.79 (95% CI 1.32–2.44); $p < 0.0001$; pc = 0.0008]. Moreover, we observed a strong correlation between sHLA-G levels and both therapy response and disease severity. Notably, patients with an inadequate therapy response exhibited significantly lower sHLA-G levels compared to those with a favorable response [25.58 (0.0–60.9) U/mL versus 15.53 (6.29–24.77), respectively; $p = 0.010$]. Our study suggests that in PBC patients, HLA-G UTR-1 extended haplotypes may contribute to disease onset, therapy response, and disease severity. Reduced levels of sHLA-G in PBC patients could be implicated in these processes.

P8 | Role of HLA-G in Sardinian idiopatic pulmonary fibrosis patients

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Idiopathic pulmonary fibrosis (IPF) is a rare disease of unknown origin that results in lung scarring (fibrosis). IPF typically worsens over time, with varying rates of progression. Both rare and common genetic factors, including those within the HLA genes, are known to influence susceptibility to the disease. However, the role non-classical HLA genes have not been fully elucidated. In this study, we explored the impact of HLA-G alleles and 3′ UTR haplotypes, as well as plasma soluble HLA-G (sHLA-G), on disease progression in Sardinian IPF patients. The genetic isolation and homogeneity of this population offer an exceptional research setting. With few confounding variables, even a small sample size of patients can generate significant findings, particularly important for rare diseases like IPF. We compared a cohort of 180 healthy individuals and 106 Sardinian IPF patients, categorized based on disease severity, from stable (SP) to rapid disease progression (RP). Analysis of HLA-G alleles and 3′ UTR haplotypes was conducted for both patients and controls. Additionally, plasma sHLA-G levels were measured. Our findings revealed an overlap of the HLA-G allele frequency between patients and controls. Interesting, the HLA-G*01:01:01:08 allele was significantly more frequent in group SP compared to group RP [23/84 (27.38%) versus 14/128 (10.77%), respectively; OR = 3.124 (95% CI 1.501–6.503); $p = 0.003$].Our study suggests that the genetic variability of HLA-G may influence disease progression. In particular, the presence of HLA-G*01:01:01:08 allele is associated to slower evolution of the disease. Further investigations are necessary to elucidate the complex mechanisms underlying the role of HLA-G in IPF pathogenesis and progression.

P9 | Islet antibodies among North Indian Type 1 diabetes patients: Prevalence and persistence pattern

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The evaluation of the autoimmune response among youth onset/type 1 diabetics (T1D) includes assessment of autoantibodies to glutamate acid decarboxylase (GAD65), insulinoma-associated antigen 2 (IA-2) & Zinc transporter 8 (ZnT8). Temporal patterns of antibody levels may vary according to the type of antibody with inter-individual differences. HLA predisposition for T1D is well established. Residual C-peptide "micro-secretion" among T1D subjects suggests that beta cells have either escaped immune attack or have regenerated. The present

study aimed to investigate i) the prevalence of islet autoimmunity at varying time points since diagnosis, ii) persistence of individual autoantibodies and Fasting C-peptide (FCP) levels & iii) HLA association with islet antibody positivity & survival. A total of 366 participants with clinical T1D and a median diabetes duration of 6 years were evaluated for baseline status. The natural course of islet cell antibodies and C-peptide levels was assessed at intervals of 2–5 years, 5–10 years and >10 years from the baseline. Persistence of islet antibody and C-peptide secretion was tested in participants who had antibody positivity or detectable C-peptide levels in their initial sample. Overall positivity of 67% was observed for any of three antibodies tested, which increased to 76% in subset of patients ($n = 125$) with diabetes duration of <2 yrs. The persistence analysis revealed that autoreactivity to ZnT8 and IA2 declined earlier compared to GAD65Ab (median survival of 31 years). Fasting C-peptide had a significantly shorter survival in islet antibody positive versus negative group (4 vs 7 years, $p < 0.01$). Among this T1D cohort, 74.9% were HLA-DRB1*03 positive while only 30.6% were DRB1*04 positive. Significant associations of DRB1*03 alleles with presence of GAD antibodies and DRB1*04 alleles with ZnT8 antibody positivity were observed. Antibody persistence did not reflect any HLA association. A unique antibody profile was observed in this T1D cohort.

P10 | Nanopore sequencing reveals high resolution HLA alleles associated to autoimmune neutropenia in early childhood

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Autoimmune neutropenia (AIN) in early childhood is caused by antibodies against epitopes on the Fc receptor type 3b (FcγIIIb). Previous studies have shown very consistent results for an association between AIN and HLA class II alleles found with low resolution. This study aimed to examine the association between AIN and HLA class II alleles with higher resolution, and to contribute with novel investigation of HLA class I alleles. High-resolution 3rd-field level HLA genotyping of six loci of HLA class I (HLA-A, -B and -C) and class II (HLA-DRB1, -DQB1 and -DPB1), was performed with nanopore sequencing on 107 AIN patients with NanoTYPE™ 24/11 kit v2 RUO HLA typing (Omixon Biocomputing Ltd.) on a MinION (Oxford Nanopore Technologies) and compared at G-resolution level with 1000 healthy controls genotyped with high-resolution by next-generation sequencing at Histogenetics. HLA genotyping with resolution of 1st-field level of class II had previously been performed on the patients with the HLA-FluoGene DRDQ Kit on a real-time platform (inno-train Diagnostik GmbH). The investigation of class I alleles revealed a higher risk of disease associated with A*02:17:01G and C*01:02:01G, while a protective effect was observed for C*03:04:01G. Regarding class II, a higher risk was observed for DRB1*10:01:01G, DRB1*14:01:01G, DRB1*16:01:01G, DQB1*05:02:01G and DQB1*05:03:01G, and a protective effect for DRB1*04:01:01G, DRB1*13:02:01G, DQB1*03:02:01G and DQB1*06:04:01G. The most disease associated two-locus haplotype was DRB1*10:01:01G-DQB1*05:01:01G and DRB1*16:01:01G-DQB1*05:02:01G, while the DRB1 shared epitope S2 was associated with protection. The high-resolution HLA genotyping performed with nanopore sequencing was in concordance with the previous performed low resolution genotyping results for HLA class II. Our results suggest that specific HLA alleles and haplotypes might play a role in susceptibility to and protection against AIN.

P11 | Detection and identification of platelet antibodies using a Luminex bead assay

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Accurate detection of human platelet antibodies (HPA) is critical in the diagnosis and treatment of several

immune-mediated platelet disorders in which HLA class I antibodies may also play a role. The Luminex bead-based assay enables the detection and identification of HPA antibodies against antigens within the HPA-1, -3 and -4 (GPIIb/IIIa), HPA-2 (GPIb/IX) and HPA-5 (GPIa/IIa) systems as well as against GPIV and HLA class I antigens. We performed a retrospective analysis of HPA/HLA antibody testing results performed with Luminex, LIFECODES® Pak Lx™ Assay (Immucor GTI Diagnostics, Inc., USA) at the Tissue Typing Laboratory in Rijeka, Croatia, from 2019 to 2023. Among 566 patients with thrombocytopenia, excluding neonatal alloimmune thrombocytopenia, who were screened for antibodies, HPA and/or HLA class I antibodies were detected in 78 (13.78%) samples, of which 57 (73.08%) were from female and 21 (26.92%) from male patients. HPA antibodies only were detected in 45 (57.69%) samples, HLA class I antibodies only in 17 (21.79%) sera and both, HLA plus HPA antibodies in 16 (20.51%) samples. The most common specificity of HPA antibodies was anti-GPIIb/IIIa (33; 50%), anti-HPA-5b (12; 18.18%), anti-GPIa/IIa (9; 13.64%), followed by anti-GPIb/IX (5; 7.57%), anti-HPA-1a (2; 3.03%), anti-HPA-1b (2; 3.03%), anti-HPA-5a (2; 3.03%) and anti-GPIV (1; 1.52%). In conclusion, HPA and/or HLA antibodies were detected in a small proportion of patients with thrombocytopenia, mostly in women. HPA antibodies were mainly directed against GPIIb/IIIa antigens. Almost half of the patients were positive for HLA class I antibodies. Considering their role in the immune response, it is necessary to further investigate the association with immune-mediated thrombocytopenia. Luminex is a sensitive technique that enables the simultaneous detection of HPA and HLA class I antibodies, which makes it an important method in the diagnosis and treatment of platelet disorders.

P12 | Distinguishing anti-3-hydroxy-3-methylglutaryl-CoA reductase immune-mediated necrotizing myopathy from non-immune-mediated statin myotoxicity: The role of HLA-DRB1*11:01 association

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A subset of patients with statin therapy may develop severe anti-3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) immune-mediated necrotizing myopathy (IMNM), suggesting genetic involvement. Previous studies noted a high prevalence of HLA-DRB1*11:01 carriers among anti-HMGCR IMNM individuals. It remains unclear if this allele specifically associates with anti-HMGCR IMNM or extends to non-immune-mediated statin myotoxicity. Our study aimed to investigate HLA-DRB1*11 association with anti-HMGCR IMNM vs non-immune-mediated statin myotoxicity. HLA-DRB1 typing was performed using SSO and NGS in 11 anti-HMGCR IMNM patients, 20 non-immune-mediated statin myotoxicity patients, and 29 matched statin controls. Comparative analyses were conducted across the three groups. Differences in demographic characteristics, clinical features, and laboratory abnormalities were noted between patients with anti-HMGCR IMNM and those with non-immune-mediated statin myotoxicity. HLA-DRB1*11 frequency was significantly higher in anti-HMGCR IMNM patients compared to controls (81.8% vs. 17.2%; $p < 0.001$; odds ratio [95% CI] = 21.6 [2.87–237.4]). Particularly, more HLA-DRB1*11 carriers were identified in anti-HMGCR IMNM patients compared to non-immune-mediated statin myotoxicity (81.8% vs. 25%; $p < 0.001$; odds ratio [95% CI] = 13.5 [1.73–153.21]). This association primarily came from HLA-DRB1*11:01. Patients with non-immune-mediated statin myotoxicity and controls showed no significant differences in HLA-DRB1*11 allele distribution. Our findings suggest a strong association between HLA-DRB1*11, particularly HLA-DRB1*11:01, and anti-HMGCR IMNM, not observed in non-immune-mediated statin myotoxicity. Identifying HLA-DRB1*11:01 may help identify those at high risk of anti-HMGCR IMNM.

P13 | The impact of HLA-DRB1 alleles in a Hellenic Pediatric Onset multiple sclerosis cohort: Implications on clinical and neuroimaging profile

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Pediatric-Onset Multiple Sclerosis (POMS) is considered a rare, demyelinating, complex disease, in association with genetic, epigenetic and environmental aetiopathogenetic factors. Linkage studies in Caucasian both adult and pediatric cohorts have consistently suggested that HLA-DRB1*15:01 represents the allele most strongly linked to MS development and clinical phenotypic characteristics. The present study aims to investigate the prevalence of HLA-DRB1 alleles among a cohort of Hellenic patients with POMS and any possible association with clinical and imaging disease features. 100 POMS patients fulfilling the IPMSSG (International Pediatric Multiple Sclerosis Study Group) criteria, 144 Adult-Onset MS (AOMS) patients, and 246 Healthy Controls (HCs) have been enrolled. HLA genotyping was performed with a standard low-resolution sequence-specific oligonucleotide (SSO) technique. POMS patients display a significantly increased HLA-DRB1*03 frequency compared to both HCs [24% vs. 12.6%, OR [95%CI]: 2.19 (1.21–3.97), $p = 0.016$) and AOMS (24% vs. 13.1%, OR [95%CI]:2.1 (1.1–3.98), $p = 0.034$] respectively. HLA-DRB1*03-carriers display reduced risk for brainstem lesion development (OR [CI 95%]:0.19 (0.06–0.65), $p = 0.011$). A significantly lower frequency of HLA-DRB1*07 (4% vs 13.4%, OR (95% CI): 0.27 (0.09–0.78), $p = 0.017$) and HLA-DRB1*11 (37% vs 52%, OR [95% CI]: 0.54 (0.34–0.87), $p = 0.016$) was observed in POMS patients compared to HCs. The HLA-DRB1*03 allele was associated with an increased risk for POMS, replicating our previous results, and with a lower risk for brainstem lesion development, a common clinical and neuroimaging feature in POMS, while HLA-DRB1*07 and HLA-DRB1*11 display a protective role. These findings expand the existing knowledge of HLA associations in POMS, aiming at an ongoing better understanding of the underlying immunopathogenic process and therapeutic options.

P14 | Availability of CD36 negative platelets and the importance of CD36 donor screening

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A CD36 deficiency is virtually unknown in Caucasians while comparatively frequent in African, Arabian and Eastern Asian populations. CD36 type 1 deficiency enables the formation of CD36 isoantibodies due to pregnancy or platelet transfusions. However, transfusion of compatible platelets is challenging when CD36 negative donors can rarely be found within the local donor population in central Europe. Here we describe our strategy to provide CD36 negative platelet concentrates for immunized patients. Anti-CD36 was found in two patients by a commercial Luminex assay (Immucor, Dreieich, Germany). One patient suffers from AML, the other patient from glioma. Both show refractoriness to platelet transfusions. Flow cytometry with anti-CD36 FITC (clone FA6-152, Stem Cell Technologies, Vancouver, CAN), anti-CD42b PE (clone HIP1, Biolegend, San Diego, USA), and anti-CD14 PE (clone M5E2, Biolegend) was used to test the CD36 expression on the patients' platelets and monocytes. For the second patient a platelet crossmatch was performed by MASPAT assay (Sanquin, Amsterdam, NL). Both patients showed a CD36 type 1 deficiency. Therefore, no compatible donors were available within our regular apheresis donor cohorts. For patient 1 we could refer one donor with a marginal CD36 expression who agreed to give platelets by apheresis. For patient 2 we identified one potential unrelated donor and luckily, some family members were identified as CD36 negative, too. Supply of CD36 negative platelets to immunized patients is a challenge for middle European blood services. Fortunately, as a result of an earlier research project, we were able to supply both patients with compatible CD36 negative platelets. We

will continue our efforts to identify and recruit active CD36 negative platelet donors and to pursue methodological approaches to detect CD36 deficiency.

P15 | Investigation of the interaction of HLA-G with natural killer inhibitory receptors in colorectal cancers

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The immune system is an organized system that defends the host against external and internal threats. Tumor cells can carry some surface antigens different to the normal cells and they can be perceived as 'unfamiliar' cells, therefore these antigens might cause an immune response. Most cancer cells do not express surface antigens, or reduce their expression to escape immunity, therefore they are not eliminated by the host. An efficient immune surveillance requires surface antigens along with the expression of HLA. The differences of HLA antigens in tumor cells have been suggested as a mechanism that result with the escape of tumor cells from natural killer (NK) cells. Colorectal cancer (CRC) can cause poor prognosis and numerous changes in individual's immune system like the increase of HLA antigens in cell surface, the deterioration of NK cell function and escape from the immunity. In our study, we aimed to determine the soluble HLA-G (sHLA-G) antigens using the ELISA method from peripheral blood serum samples, to investigate the loss or absence of HLA-G expression in tumor tissue through immunohistochemistry (IHC) and the infiltration of KIR (killer-cell immunoglobulin-like receptor) indicators on NK cell surface to the tumor tissue. The result of this study showed that the expression of HLA-G and KIR markers in patients with CRC increased and it might be a strong, independent and useful prognostic marker in colorectal cancer. We found no difference between the sHLA-G levels. Our results suggest that HLA-G and KIR markers may be a promising target for future immunotherapeutic approaches.

P16 | Potential actionable somatic variants in chronic lymphocytic leukemia

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Chronic Lymphocytic Leukemia (CLL) is a mature B cell malignancy with clinical & genomic heterogeneity. Many variations occur in CLL but have not been explored fully for their actionability / immunotherapy. Tumor associated antigens (TAAs) & Neoantigens (Neoags) arise through somatic variations & could induce potential antitumor T cell responses. There are few reports on TAAs/Neoags in CLL &, to the best of our knowledge, none have been reported from India so far. To identify TAAs & Neoags in CLL patients using next generation sequencing (NGS). This study was approval by institute's ethics committee. Paired malignant B cells & Neutrophils were sorted from 42 CLL patients. These were processed for Whole Exome (Twist Whole Exome) & Whole Transcriptome (NEB Ultra II directional RNA-Seq) NGS on NovaSeq6000. In-house NGS discovery pipeline was used to analyze coding somatic mutations, predict HLA–epitope-TCR binding & immunogenicity. Median of 26 coding somatic mutations were observed/CLL patient. Median nonsynonymous TMB was 0.7. More than 2200 coding variants were identified in driver genes SF3B1 (19%), NOTCH1 (15%), TP53 (8%), CHD2 (6%) & others. Putative Neoags restricted by HLA-A2 & CDR3 loops of TCRαβ were mapped in FNDC3B, C16ORF57, SF3B1. Genes, for example, LAG3, MAPK4, TIMD4 involved in cellular pathways such as signaling, cell communication, adaptive immune responses & RNA processing were differentially expressed in CLL. This study has identified putative actionable TAA & Neoag specific peptide sequences in CLL for the first time in the Asian Indian population. Future studies like dendritic cell mediated stimulation of CD8 + T cells will be able to establish immunogenicity of these epitopes in CLL. Targeting TAA/Neoags along with checkpoint blocking antibodies

may aid augment anti-tumor immune responses. Acknowledgements ICMR grant 2020-0012, 5/13/4/2020/NCD-III.

P17 | Two-locus haplotypes HLA-DRB1∼IL-17A and HLA-DRB1∼IL-17F—Protectors of rheumatoid arthritis in the Russian population

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Rheumatoid arthritis (RA) is a systemic autoimmune disease with a genetic predisposition. The alleles HLA-DRB1*01 and HLA-DRB1*04 (encoding shared epitopes) are main risk factors for RA. But there are HLA-DRB1*11 and HLA-DRB1*13, encoding the DERAA sequence, and could be protective for the RA development. The main cytokine genes IL-17A and IL-17F play an important role in the RA immunopathogenesis and are located on chromosome 6. Previously, we established linkage disequilibrium and the formation of haplotypes HLA-DRB1∼IL-17A and HLA-DRB1∼IL-17F in the Russian population of the Chelyabinsk region. The aim is to investigate linkage disequilibrium and HLA-DRB1∼IL-17A, HLA-DRB1∼IL-17F haplotypes frequencies in RA patients and healthy control Russian population. DNA typing of HLA-DRB1* genes was performed in 88 RA patients and 115 healthy control (HC) by PCR SSP using sets of Protrans. SNPs -197*G/A IL17A, 7488T/C IL-17F were detected by allele-specific PCR (Litekh LLC, Russia). The haplotypes frequencies (HF) and linkage disequilibrium (D′) were analyzed using Arlequin 3.5. For association study p values were calculated by the exact two-tailed Fisher's criterion, the criterion RR with 95% CI was established. The 7 two-locus haplotypes HLA-DRB1∼IL-17A, 2 HLA-DRB1∼IL-17F were found in RA group. In the control group there were 6 haplotypes HLA-DRB1∼IL-17A and 4 HLA-DRB1∼IL-17F. The protective specificities HLA-DRB1*13 and HLA-DRB1*11 formed the following haplotypes: HLA-DRB1*13∼IL17A -197*A (5% versus 6% $p = 0.7$); HLA-DRB1*11∼IL17A -197*G (3% vs 8% $p = 0.02$ OR = 0.3 CI 0.1–0.9); HLA-DRB1*11∼IL17F 7488*C (0% vs. 0.7% $\chi^2 = 12.75$ $p < <0.001$); HLA-DRB1*13∼IL17F 7488*T (0.7% vs. 1% $p = 0.3$). According to our data, it is possible to form haplotypes between IL-17 and HLA-DRB1 alleles with the protective sequence DERAA. HLA-DRB1*11∼IL17A -197*G, HLA-DRB1*11∼IL17F 7488*C could be protective factors against rheumatoid arthritis.

P18 | Genetic insights into COVID-19 severity: HLA-E and HLA-G alleles as potential determinants

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Coronaviruses (CoVs) are a group of infectious agents that periodically emerge, posing significant obstacles for the well-being of both humans and animals. The expression of HLA-I molecules, essential for the control of both natural and acquired immune system components, is thought to potentially influence susceptibility or resistance to COVID-19. Our study focuses on genotyping HLA-E and HLA-G in SARS-CoV-2-positive individuals, comparing data between those with mild and moderate/severe symptoms. A total of 97 COVID-19 patients and 100 healthy controls were included in this study. We used peripheral whole blood samples obtained from both patients and control subjects. Genomic DNA extraction was performed using the QIAamp DNA Blood Mini Kit, followed by polymerase chain reaction (PCR) amplification and DNA sequencing. The M/F ratio of the patients was 52/45 and the mean age was 52.78 ± 14.08, while healthy individuals M/F: 42/58 and mean age was 54.2 ± 6.82 years. Patients were classified as mild (*n*: 52), moderate (*n*: 24) and severe (*n*: 21) according to clinical findings. There was no statistically significant difference between gender and disease progression. No statistical significance was observed between the patient and control groups in terms of HLA-E alleles. E*01:01 allele was increased in mild cases and E*01:03 allele was increased in severe cases. The G*01:04 allele was statistically lower in all patients and in those with mild severity. In conclusion, our analysis of HLA-E and HLA-G genotypes in SARS-CoV-2-positive individuals, we identified distinct patterns, especially concerning disease severity. These findings contribute to our understanding of host

genetic factors that may influence susceptibility to severe outcomes in COVID-19 patients, which could inform future research and therapeutic strategies aimed at mitigating the impact of coronaviruses on human health.

P19 | Understanding the role of HLA-G polymorphism in causing susceptibility to cervical cancer

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HLA-G is a non-classical HLA class I antigen exhibiting inhibitory actions against natural killer (NK) cells and T-lymphocytes. Aberrant expression of HLA-G is associated with several cancers. The production of HLA-G is controlled by several polymorphisms and this could plausibly affect the expression of HLA-G. The study aimed to analyze HLA-G allele polymorphism in women with cervical squamous cell carcinoma (SCC). Blood and tissue samples were available from 38 women with cervical carcinoma and 28 with healthy cervix. DNA extraction from tissue samples was followed by Human Papillomavirus (HPV) genotyping. The DNA extracted from blood was subjected to sequencing for HLA-G locus using commercially available kits for Next generation sequencing (GenDx). The library preparation was performed as per manufacturer's instructions and run on Nova Seq platform. The output data was analyzed using Fisher exact test. All cases of carcinoma cervix were positive for high-risk HPV subtype 16. The frequency of the HLA-G allelic groups in controls were HLA-G*01:01 29 (51%), HLA-G*01:03 5 (8.9%), HLA-G *01:04 14 (25%) and HLA-G*01:06 8 (14.2%). In cervical carcinoma frequency was HLA-G*01:01 56 (73.6%), HLA-G*01:03 4 (5.2%), HLA-G*01:04 12 (15.7%), HLA-G*01:05 1 (1.7%) and HLA-G*01:06 2 (3.5%). HLA-G *01:01 was associated with susceptibility ($P = 0.001$) and HLA-G*01:06 was protective ($p = 0.016$). Genotype distribution of the HLA-G 14-bp polymorphism (+3142 position): controls had frequency of 14-bp del/del 8 (28.5%), ins/del 10 (35.7%), ins/ins 10 (35.7%). In cases del/del 7 (18.4%), ins/del 10 (60.5%), ins/ins 10 (21%). On comparison no statistical significance was observed. The study demonstrates HLA-G*01:01 to be associated with susceptibility ($P = 0.001$) and HLA-G*01:06 to be protective allele ($p = 0.016$) in our cohort. Further investigation in a larger cohort could explore their use in prognostication.

P20 | Sudden cardiac death and HLA variation—Report from the initial phase of investigations

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Sudden cardiac death (SCD), natural unexpected death of unknown or cardiac case, has increasing burden and could account for up to 20% of overall mortality. In addition to traditional factors contributing to risk of SCD such as inherited heart muscle diseases and/or arrhythmic syndromes, inflammatory response has also been discussed as an independent risk factor. An important role in inflammatory reaction has been attributed to the immune system genes, the most polymorphic of which are HLA genes. In a pilot group of 10 patients who died of sudden cardiac death, six HLA loci (HLA-A, -B, -C, -DRB1, -DQA1, -DQB1 and -DPB1) were genotyped using NGS. Observed allele frequencies were then compared with those determined in a control group of 141 healthy individuals. All investigated subjects were unrelated and of the same (Czech) ethnicity. The HLA-DQB1*06:09 allele was more frequent in the patients than in the healthy population with an odds ratio 18.5 ($p = 0.02$). To exclude the possibility that this observation was due to chance, we have been expanding the patient group to comprise at least 60 individuals for the initial phase of the study, the obtained frequency comparisons for the extended patient group will be presented at the meeting. We will report pilot data from the initial phase of our study of a possible association between HLA and sudden cardiac death. Being aware of their limited validity, by their presentation we wish to disseminate the idea of investigating a plausible role of HLA in this complex medical condition, and thus also search for eventual collaborators in a multicenter project which would be most relevant for addressing properly the research question whether HLA variation could be involved in SCD pathobiology. Supported by the Min. Health Czech Rep.—RVO (FNOL, 00098892), Palacky University IGA PU_LF_2024_005.

P21 | Association between HLA-markers and the COVID-19 severity in residents of Saint-Petersburg, Russia

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The aim of the study was to investigate the association between HLA-markers of St. Petersburg residents and the COVID-19 severity. Seventy-eight residents of St. Petersburg (median age 55; range 20–84) with COVID-19 were included in the study. The control group included 1563 HSC donors living in St. Petersburg (median age 32; range 18–60). Low resolution HLA-typing of HLA-A, -B, -C, -DRB1, -DQB1 genes was performed by PCR-SSP and PCR-SSOP. HLA typing of the control group was carried out before the SARS-CoV-2 pandemic. Statistical analysis was performed using two-sided Fisher's exact test, logistic regression, Arlequin ver. 3.5 and Statistica ver. 10. The distribution of the examined persons according to the disease severity was the following: mild ($n = 41$), moderate ($n = 32$), severe (5). Individuals with moderate and severe course of COVID-19 were combined into one group ($n = 37$) for further analysis. A lower frequency of HLA-A*01 was found in St. Petersburg residents with mild COVID-19 compared to the group with moderate/severe course (0.0366 vs. 0.1351; $p = 0.04$) and the control group (0.0366 vs. 0.1193; $p = 0.02$). A higher frequency of HLA-A*11 was found in individuals with moderate/severe disease course compared to those with mild course (0.1081 vs. 0.0244; $p = 0.048$). There was also a trend towards an increased frequency of HLA-A*11 in individuals with moderate/severe COVID-19 course compared to controls (0.1081 vs. 0.0582; $p = 0.08$). Multivariate analysis included age, gender, SARS-CoV-2 strain and immunogenetic parameters. The severity of COVID-19 depended on the presence of HLA-A*11 (OR 7.38, CI 1.15–47.3, $p = 0.032$) and patient age (OR 1.05, CI 1.01–1.09, $p = 0.008$). According to multivariate analysis, there was a trend of HLA-A*01 influence on the COVID-19 severity (OR 3.88, CI 0.88–17.09, $p = 0.068$). Thus, HLA-A*11, HLA-A*01 may be HLA-markers associated with severe COVID-19 in St. Petersburg residents. A larger cohort needs to be examined to confirm the conclusion.

P22 | Haplotype frequencies and linkage disequilibrium of HLA-DRB1 and TNFα SNPs in Russian patients with irritable bowel syndrome living in the Chelyabinsk region of the Russian South Urals

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Irritable bowel syndrome (IBS) is a complex functional disorder that involves multiple interactions of genetic inheritance, environmental and psychosocial factors. Previously, we established the presence of linkage disequilibrium and formation of HLA-DRB1- SNPs TNFA haplotypes among Russians of the Chelyabinsk region. The aim is to investigate linkage disequilibrium between HLA-DRB1 and SNPs in TNFα haplotypes in patients with IBS and a healthy control Russian population. DNA typing of HLA-DRB1 alleles genes and SNP-238G/A, -308G/A, -863 C/A, -1031 T/C TNFA was performed in 75 IBS patients and 132 healthy control (HC) Russian population by PCR SSP using sets of Protrans and the RFLP-method. The haplotypes frequencies (HF) and linkage disequilibrium (D′) were analyzed using Arlequin 3.5. For association study p values were calculated by the exact two-tailed Fisher's criterion, the criterion RR with 95% CI was established. In the IBS group we found out 9 haplotypes: HLA-DRB1*14–TNFA-238*A; HLA-DRB1*01 – TNFA- 308*G; HLA-DRB1*03 – TNFA- 308*A; HLA-DRB1*04 – TNFA- 308*G; HLA-DRB1*16 – TNFA-308*A; HLA-DRB1*10 – TNFA-863*A; HLA-DRB1*14 – TNFA-863*A; HLA-DRB1*01 – TNFA-1031*T; HLA-DRB1*10 – TNFA-1031*C. Of these 9 haplotypes, 5 were rare and found only in the IBS group: HLA-DRB1*14-TNFA-238*A; HLA-DRB1*16 – TNFA- 308*A; HLA-DRB1*10 – TNFA-863*A; HLA-DRB1*14- TNFA-863*A; HLA-DRB1*10 – TNFA-1031*C, but not in control group. The HLA-DRB1*16 – TNFA- 308*A (RR = 2.9 95% CI 2.4–3.5) contain mutant allele -308*A and HLA-DRB1*16, previously was shown as risk factor for IBS predisposition.

P23 | HLA characterization of 115 type 1 diabetes children by next generation sequencing

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Type 1 diabetes (T1D) is an autoimmune disease characterized by pancreatic beta cell destruction triggered by environmental factors in genetically predisposed individuals. DR3-DQ2 and DR4-DQ8 haplotypes are strongly associated with T1D, but also HLA class I alleles and other genes resulted associated to the disease. In this study, 115 European T1D children were typed by next generation sequencing (NGS) for HLA-A, -B, -C, -DPA1, -DPB1, -DQA1, -DQB1, -DRB1/3/4/5 genes, using Immucor MIA FORA NGS MFlex HLA typing kits and MIA FORA NGS EXPRESS software for data analysis. Whereas 100 subjects (87%) were carriers of DQ2 and/or DQ8 haplotypes, the remaining 15 presented other haplotypes, of which the most frequent were: DRB1*07:01:01∼DQA1*02:01:01∼DQB1*02:02:01 (7/30), DRB1*16:01:01∼DQA1*01:02:02∼DQB1*05:02:01 (6/30), DRB1*13:02:01∼DQA1*01:02:02∼DQB1*06:04:01 (5/30) and DRB1*08:01:01∼DQA1*04:01:01∼DQB1*04:02:01 (3/30). These haplotypes are known to play a neutral or protective role in the development of T1D, suggesting a possible role of other genetic risk factors, inside and outside the Major Histocompatibility Complex (MHC). Regarding HLA class I genes, the most frequent allele in non-DQ2/DQ8 with respect to DQ2/DQ8 T1D subjects was HLA-B*39:06:02, already described in literature as the HLA class I allele most predisposing to T1D, especially when associated with DR1, DR8 and DR16 haplotypes. Moreover, also HLA-C*07:02:01 allele resulted more frequent in T1D subjects without high-risk haplotypes. This allele has been associated with increased expression of the T cell receptor complex variable region gene and autoimmune diseases including T1D and psoriasis. Our findings confirmed, albeit in a limited case study, that HLA genes are the major susceptibility genetic factors to T1D, with DR3-DQ2 and DR4-DQ8 haplotypes conferring the higher risk, and that also HLA class I alleles are involved in susceptibility to disease.

P24 | Evaluation of clinical-laboratory parameters in patients with autoimmune renal disease after transplantation

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Rare autoimmune disorders, such as lupus nephritis (LN), granulomatosis with polyangiitis (GPA), and anti-glomerular basement membrane (anti-GBM) disease, can progress to end-stage renal disease (ESRD). Renal transplantation (RT) is the most effective replacement therapy, improving survival and quality of life. Despite the low prevalence of recurring nephritis in allografts, clinical and laboratory surveillance is necessary for successful management of both grafts and patients. The purpose of this study was to record and present the data from clinical and laboratory monitoring of patients with autoimmune-mediated kidney disease after RT. Five patients who had undergone RT due to autoimmune mediated ESRD were included in the present study. Specifically, we enrolled 2 females (aged 50 and 37) with SLE and elevated levels of anti-dsDNA antibodies (Abs), 2 males (aged 40 and 53) with GPA and positive anti-C-ANCA Abs, and 1 male (aged 43) with positive anti-GBM Abs. Immunosuppressive agents, such as tacrolimus, mycophenolic acid, and corticosteroids, were administered to all patients. In the 5-year follow-up period after RT, we recorded good rates of graft survival and no case of antibody or T-cell mediated rejection. Importantly, no recipient has developed donor-specific Abs. There was no documented case of disease recurrence, and Abs followed a downward trend over time until normalization. A single patient passed away 5 years after transplantation from necrotizing CMV colitis, maintaining normal graft function until the time of death. Given our small sample size, we acknowledge the limitations of our study. However, it appears that RT is the definitive treatment option for ESRD of autoimmune origin. Despite the low prevalence of post-RT disease recurrence, recipients should be monitored closely, enabling timely diagnosis and early management to prevent transplant rejection.

P25 | TNFA G308A polymorphism in COVID-19 Russian patients of the Chelyabinsk region

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TNFα is the main pro-inflammatory cytokine involved in the immunopathogenesis of COVID-19. There is evidence of an association of elevated TNFα levels with an increase in the number of deaths from COVID-19. The TNFA gene encoding TNFα is located on the short arm of chromosome 6 inside a cluster of MHC class III genes. One of the most studied polymorphic variants in this gene is TNFA G308A, the functional significance of which is to change the transcriptional activity of the TNFA gene. The purpose of the work was to evaluate the alleles and genotype frequencies of the G308A in TNFA in COVID-19 patients in comparison with healthy Russians of the Chelyabinsk region. The study group consisted of 100 COVID-19 patients without concomitant severe diseases of Russian nationality. The control group consisted of 238 healthy donors of the Chelyabinsk Regional Blood Transfusion Station of the Russian population. Allele-specific PCR was performed to detect G308A TNFA. To calculate the reliability of differences in the allelic variants and genotypes frequencies between groups, the PAST program was used (v3.16), in which the criterion χ^2 was evaluated. The reliability of the obtained values was considered statistically significant at $p < 0.05$.The TNFA −308*G allele is found in the groups of COVID-19 patients and the control group with a higher frequency. As a result of the analysis of the distribution of TNFA -308G/A genotypes, it was found that TNFA -308G/G prevailed in patients compared with the control group (88.0% and 75.2%, respectively, $\chi^2 = 6.942$, $p = 0.009$, OR = 2.417, CI 1.236–4.729). At the same time, the TNFA -308G/A genotype is more common in the control group compared with patients (23.5% and 11.0%, respectively, $\chi^2 = 6.955$, $p = 0.009$, OR = 0.402, CI 0.201–0.804). The results obtained require further work and consideration.

BIOINFORMATICS, DATA ANALYSIS IN IMMUNOGENETICS

P26 | Uncovering the immunogenetic landscape of 2609 multiple sclerosis patients of the OFSEP-HD cohort

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The French Observatory for Multiple Sclerosis—High-Definition (OFSEP-HD) cohort, is a multi-centric French cohort including 2609 patients with multiple sclerosis (MS). Immunogenetics has a well-known influence on the susceptibility of an individual to develop MS, and more recently genetic influence on severity was reported. Yet the immunogenetic component of this cohort has not been described. Thus, here we describe the genetics of the OFSEP-HD cohort. This includes ancestry analysis for identifying superpopulation clusters as well as admixed ancestry analysis to quantify the genetic admixture proportions within individuals. Genetic data from 2609 MS patients were collected using the Affymetrix genotyping platform and preprocessing steps were performed using PLINK and Python. We performed ancestry analyses using principal component analysis (PCA) and admixture software. We identified 4 major clusters, reflecting the genetic heterogeneity within the OFSEP-HD cohort, and thereby in the general French MS population. We performed HLA imputation from Single Nucleotide Polymorphisms (SNPs) using the HIBAG software. We highlighted a variety of observed HLA alleles and suggested potential trends between specific alleles and MS subpopulations. In addition, we pinpointed discordances between self-reported geographical origins and genetic ancestries. Indeed, self-reported North-African ancestries are underestimated by half compared to inferred North-African ancestries ($p < 10–3$). Overall, our study elucidates the genetic diversity within the OFSEP-HD cohort shedding light on its potential implications in MS susceptibility and severity. It paves the path for using genetic data to empower personalized medicine applications and lead to a better understanding of MS with respect to inherited factors.

P27 | HLA-DRB3, DRB4, DRB5: Three loci or a single locus?

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In order to understand the diversity of the region spanning the HLA-DRB genes and pseudogenes in the delta block of the MHC, we have performed a multi sequence alignment of 94 haplotypes from 47 samples from the human pangenome sequence assemblies. Alignment of ~120 kb region from HLA-DRB9 to HLA-DRB1, containing the expressed HLA-DRB1, HLA-DRB3, HLA-DRB4, and HLA-DRB5 genes and the HLA-DRB2, HLA-DRB6, HLA-DRB7, HLA-DRB8 and HLA-DRB9 pseudogenes revealed a region of high structural complexity interspersed by regions of high conservation. The alignment placed HLA-DRB3, HLA-DRB4 and HLA-DRB5 (DRB345) loci in the same location, perfectly aligning their coding regions. The DRB345 region of ~15 kb was absent in DRB1*01, 10 haplotypes and a deletion of ~50 kb was observed in the DRB1*08 alleles consistent with previous studies. Similarly, HLA-DRB2, HLA-DRB6 and HLA-DRB7 (DRB267) were aligned to the same region. Phylogenetic analysis of the region spanning DRB345, and the HLA-DRB1 loci, including 5 kb upstream and downstream sequence showed that the DRB345 sequences clustered together as distinct lineages separately to the HLA-DRB1 sequences. This data, as well as countless HLA typing results from clinical and research laboratories that show that DRB3, DRB4, DRB5 are never seen on the same haplotype (except for rare examples) also supports DRB345 as being a single locus. A hypothesis originally suggested by Andersson et al. DRB267 are rarely typed in HLA laboratories. We analyzed 3200 samples from the 1000 genomes project and did not identify examples where these genes were present on the same haplotype (with a few exceptions, likely to be the typing mistakes), also suggesting that DRB267 are lineages at the same locus rather than unique loci.

P28 | Pan-major histocompatibility complex and pan-leukocyte receptor complex reference graphs containing 246 fully contiguous phased sequences enable precision immunology

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The major histocompatibility complex (MHC) and the leukocyte receptor complex (LRC) are gene-dense regions on chromosome 6 and 19, respectively, of the human genome. Both regions are key to immune system function, highly variable among individuals, and frequently refractory to genomic analyses due to their extreme polymorphism and structural variation. We have developed novel methods for targeted capture and de novo assembly of the MHC and LRC regions, and we have applied these methods to create 246 highly accurate, fully contiguous, and haplotype-phased sequences of each region. We obtain PacBio HiFi sequencing reads either via a custom targeted PacBio library preparation method, or from datasets provided by the Human Pangenome Reference Consortium (HPRC). In our MHC regional assemblies, we call alleles across 39 loci including the class I and II HLA genes, discovering 1246 putative novel allele sequences. We identify copy number variation in the C4A and C4B genes and find significant linkage disequilibrium between C4A~C4B haplotypes and 14 MHC loci. We build our sequences into a novel "pan-MHC" reference graph, and we demonstrate that this reference improves the accuracy of short-read variant calling. In the LRC region, which includes the killer-cell immunoglobulin-like receptor (KIR) gene complex, we create fully contiguous and phased 1.6-Mb sequences and identify KIR phased alleles and haplotypes. We find 18 unique KIR haplotypes and call 2346 alleles of KIR genes, of which 61% (1422) are absent in the IPD-KIR Database, including 1011 unique putative novel KIR allele sequences. Our haplotypes and graphs contain significantly more population diversity than preexisting MHC and LRC sequences, thus improving the prospects for global health equity in these clinically important genomic regions.

P29 | A bioinformatic pipeline to assemble complete genomes and to unravel phased immune regions in rhesus macaques using long reads

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Rhesus macaques are applied as model in biomedical research, offering valuable insights into evolutionary biology and mechanisms underlying various human diseases. A comprehensive understanding of their genome is crucial for the advancement and refinement of these disease models. The current rhesus macaque reference

genome, released in 2019, is of high quality, but represents sequences of collapsed chromosomes. Consequently, it features mis-assemblies, especially in the complex immune regions. We applied an affordable hybrid sequencing approach, utilizing the strengths of both ONT and PacBio long-read sequencing, to successfully assemble a high-quality rhesus macaque genome. Overall, the new genome assembly displays a contiguity and completeness that is largely consistent with that of the current reference genome. To streamline the process, we have developed a pipeline that outputs not only a complete collapsed genome, but also phased sequences of regions of interest, including the MHC and KIR regions of maternal or paternal descend. Also, the automatically phased MHC and KIR haplotypes have been validated by targeted characterization and segregation analysis. Our pipeline is build using Snakemake, designed to be user-friendly with customizable options for adaptability. Steps for quality control, read filtering, assembly, polishing, validation and annotation are included. We utilize a combination of established bioinformatic tools with settings proven to enhance assembly outcomes. Users have the flexibility to choose to assemble the genome based on an existing reference genome or with a de novo approach. In addition, users can define their own regions of interest, which will be extracted and assembled as phased sequences. In conclusion, the hybrid long-read sequencing approach in combination with our custom assembly and annotation pipeline allows a user-friendly construction of reference-quality macaque genomes in addition to phased immune regions.

P30 | HLAtools: An R package for HLA region informatics

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The IPD-IMGT/HLA Database curates ~40,000 allelic variants for 46 genes in the HLA region, and makes protein and DNA alignments for these genes available for public use, along with accessory data about allele names and their changes over time, on the ANHIG/IMGTHLA GitHub repository. The static text files in this repository are updated every 3 months. These resources are central to the application of HLA genotype data for stem-cell and organ transplantation therapies, as well as disease association, evolutionary biology, and population genetics research. To foster the standardized use of these key resources, we have developed HLAtools, an R package that automates the consumption of IPD-IMGT/HLA Database resources, making them available for computation. The HLAtools package includes computable versions of IPD-IMGT/HLA reference data files, alongside a suite of reference resources and functions that foster computation on these data-objects. The package identifies IPD-IMGT/HLA loci with peptide, nucleotide or genomic sequence alignments, the names of all HLA alleles for all IPD-IMGT/HLA Database release versions, and includes a guide to the non-standard features found in some gene fragments. Package functions validate, trim and convert allele names across IPD-IMGT/HLA Database releases, build customized peptide, codon, nucleotide and genomic alignments for specific alleles at user-specified positions, including alleles at different loci, and convert between GL String/GL String Code and UNIFORMAT data formats. Extensions for other data-analysis packages are also included. The companion HLAtools data package includes computable versions of the peptide, codon, nucleotide and genomic alignments for all polymorphic loci, and an "atlas" that identifies the boundaries between gene features (exons, introns and untranslated regions) at each gene, pseudogene and gene fragment. All packaged data objects can be updated with each IPD-IMGT/HLA Database release.

P31 | epiArt: A graphical epitope amino acid repertoire translation of HLA allele disparities

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The immune response towards allo-HLA-antigens depends, as already demonstrated, on the HLA repertoire of the recipient and the incompatible epitopes on the donor. However, transplant allocation still focusses on mismatches on the antigen level instead of epitopes. To enhance our understanding of the similarity relations of HLA alleles in terms of amino acid polymorphism and shared epitopes, we mapped epitope amino acid sequences and calculated the pairwise sequence

difference between HLA alleles. In particular, we translated amino acid sequences of all currently proven epitopes into a sequence atlas comprising all classical class I and II antigens. These sequences were then transferred into a differential amino acid space, followed by visualization of the pairwise allele distances by means of antigen-specific graphs. They give an overview of relationships of all alleles of the antigen, corresponding similarity/dissimilarity structures, outlier alleles, and alleles with similarity to different antigen groups. Additionally, we calculated prevalence of the different amino acids for each polymorphic sequence position and visualized them in term of stacked bar plots, representing amino acid motif plots of all alleles belonging to an antigen. Through our visualizations, the complexity of the different allele groups and their cross reactivity becomes evident. We see strongly varying intra-group heterogeneity of the HLA class I and II alleles, as well as joint intergroup and even inter-locus epitopes. These findings indicate a benefit of epitope-based transplant matching: Single allele mismatches between donor and recipient potentially refer to identical epitopes, or to a set of multiple mismatched epitopes. This data adds a new level of quality to allocation, allowing for the definition of potentially tolerable and of taboo allele mismatches, respectively.

P32 | epiTOol: A browser-based HLA epitope (mis)matching application for small to large cohorts

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Handling large numbers of transplant donors and recipients in routine diagnostics is often still a manual transfer process and a source for potentially fatal mishap. Moreover, allele matching, processing of antibody data, and epitope assignment requires separate tools with partially missing direct interfaces. Here we present 'epiTOol', an interactive analytics tool for all-in-one processing of HLA typing and antibody data. At first, data import functionalities create a database of individuals collecting HLA typing and antibody data. Large numbers of several thousand or tens of thousands of measurements can be loaded simultaneously. For the individually present alleles and antibodies, epitopes are then automatically assigned using incorporated data previously extracted from 'epregistry'. Based on these epitopes, matches and mismatches between the individuals can be assessed. Thereby, two modes are available: (i) Single individual mode: comparison of a single donor to a list of potential recipients (i.e., one available organ to a waiting list of patients), or, vice versa, a single patient to a list of potential donors (usually close relatives). (ii) Pair mode: pairwise comparison of donors and assigned recipients. Both modes can be applied in routine diagnostic tasks in immunology laboratories as well as for scientific retrospective evaluation. Matching and mismatching epitopes are displayed for each comparison by means of absolute numbers and of present allele, antibody and epitope information, respectively. Finally, export functionalities for spreadsheet and pdf files allow for easy and comfortable utilization of the evaluation results in routine processes such as issuing medical reports. Screening of a test cohort of 220 persons led to first interesting results regarding epitope match and mismatch constellation, prevalence and antibody production. Details will be presented separately.

P33 | Structural and dynamic analyses of the differential binding of the hypoxia-inducible factor-1 (HIF1) into the HF1 regulatory elements at coding region of the HLA-G gene

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The immune checkpoint HLA-G molecule, primarily expressed in tumors and during pregnancy, may be expressed under hypoxic conditions, particularly by the hypoxia-inducible factor-1 (HIF1). The HLA-G gene presents hypoxia regulatory elements (HREs) at the promoter (-966/-962 bp) and at exon 2 (+281/+285 and +291/+295 bp) regions, where HIF1 synergistically promotes gene transcription. A variation site at the -966 HRE (-966A allele) influences HLA-G expression in tumor cell lineages, during hypoxia-mimicking treatment and reduce HIF1-HRE complex binding and stability. The +291 HRE at the HLA-G exon 2 presents two mutations (+292A > T and +293C > T) that may contribute to HIF1-HRE binding. We analyzed the HIF1-HRE complex stability at the atomic level, using structural

bioinformatics tools, taking into account the +291 HRE polymorphism, and taking advantage of the previous 3D constructions of three distinct HIF1-HRE structures, using molecular docking methods. These protein-DNA complexes varied based on the +291 HRE sequence, determined by the +292A > T and +293C > T allele combinations. Then, the complexes were submitted to molecular dynamic simulations to evaluate stability and dynamics over 200 ns (duplicate run) in a physiological environment. Conformational parameters of the DNA were calculated using the Curves+ webserver. No differences were detected in the fluctuation and flexibility of HIF1-HRE conformational changes among molecular complexes concerning the +292A > T and +293C > T polymorphisms, as they exhibited conformational variances ranging between 2 and 3 Å over time. Both polymorphic sites induced a less energetically favorable binding of the DNA double helix to HIF1; however, this effect appears to be more evident for the +292 T and +293C allele combination, potentially influencing HRE recognition and HLA-G gene regulation by HIF1 under hypoxic conditions.

P34 | Using biobank data to identify full-length haplotypes of 15 MHC genes in the Finnish population

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Large and high-quality biobank collections of up to hundreds of thousands of samples can provide a vast amount of valuable data. Advanced molecular biology techniques and improved computational power allow cost-effective and rapid processing of large datasets. Together, these create an excellent resource for databased research. In this study, we used genomic data from the Blood Service Biobank to determine HLA type of blood donors registered in the Biobank. We used the HiBAG algorithm on a Finnish reference dataset to impute alleles of 15 MHC genes with two-field accuracy for 35,400 donors. HLA-F, -G, -A, -E, -C, -B, MICA and MICB genes of MHC class I and HLA-DRB5, -DRB3, -DRB4, -DRB1, -DQA1, -DQB1 and -DPB1 genes of MHC class II were included in the study. Based on the imputed HLA and MIC allele data, we determined the most common long HLA-MIC haplotypes in the Finnish population using Genetic Analysis Package version 1.2.1 in R. A total of 10,570 putative haplotypes were generated, of which 139 had a frequency ≥0.001. A closer look at reported ancestral haplotypes (AH7.1, AH8.1, AH18.2, AH35.2, AH35.3, AH44.1, AH44.2, AH47.1, AH57.1, AH60.2, AH60.3 and AH61.1) confirmed the strong frozen block structure of the MHC; long haplotypes were built from known allele combinations in the beta and delta blocks. The haplotype structure was usually broken between blocks at the known recombination sites in the MHC region, mostly before the epsilon block. Within the blocks, there was remarkably little allelic variation, although some variation was observed within the beta block between the MICA and MICB genes and within the alpha block between all three HLA genes (HLA-F, -G and -A). In this study, by typing of 13 HLA and 2 MIC genes of the 35,400 biobank donors in silico, we not only determined the frequencies of the most common full-length haplotypes in the Finnish population, but also determined the allele combinations of alpha and beta blocks in the 12 ancestral haplotypes.

P35 | A new bioinformatics tool providing robust methods for HLA case–control studies

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Case–control studies are a type of observational study commonly used to look at factors associated with diseases or outcomes. In the context of the human MHC (HLA), these studies aim to investigate the role of specific HLA alleles or haplotypes in disease susceptibility or resistance by comparing frequencies between individuals with the disease (cases) and individuals without the disease (controls). However, the high level of polymorphism that characterizes HLA genes and the lack of Hardy–Weinberg equilibrium arising frequently in the cases often lead to the violation of the basic assumptions of simple methods such a chi-square tests. To address this, we present a new user-friendly bioinformatics tool powered by R and shiny to assess HLA and disease associations based on case–control samples and show how users can easily upload data, analyze it using robust methods and easily visualize and download results. The originality of our approach is two-fold: first, it considers three

complementary representations of the data, i.e. alleles (or haplotypes), genotypes and phenotypes, to address associations; second, it uses a resampling scheme based on permutations of the aggregated data to obtain empirical distribution estimates from which p-values are directly obtained, thus allowing to stay away from issues with voided asymptotic distributional properties. The tool also offers several options for multiple testing adjustment (with the well-known Holm-Bonferroni as a default), an essential step for sound statistical conclusions. All the features of this new tool are illustrated with an example case–control study on an undisclosed tropical disease containing 479 individuals typed at high resolution for DQA1, DQB1, DPB1 and DRB1. Here, we see how in some cases it is possible to find associations directly at the allele level while other times it is necessary to use genotypic or phenotypic levels.

P36 | Modeling an international donor recruitment strategy in a collaborative pilot study between a UK and Indian donor registry

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The UK registries for stem cell donors are increasingly recruiting more people from minority ethnic backgrounds, diversifying the HLA types on these registers. However, previous work indicates that the ethnic minority populations in the UK cannot deliver the diversity of HLA types needed for all UK ethnic minority patients. As the British Asian population is the largest ethnic minority in the UK (∼7%), we are collaborating with DATRI, an established registry based in Chennai, India, in a three-year pilot study to investigate whether the directed recruitment of 10,000 Indian donors can benefit patients in India, UK Asian patients, and beyond. DATRI's register has nearly 550,000 donors located throughout India, with the majority of donors residing in the southernmost states. We estimated the HLA haplotype frequencies of 18 geographical regions, based on the 36 states and union territories of India. We compared four UK South Asian populations to these regions; one was most similar to the North East region (where most donors reside in West Bengal), the other three were most similar to Delhi and Punjab, in northwest India. Using the regional haplotype frequencies, we ran simulations of the recruitment of 10,000 donors and matched them to simulated UK patient models. We created an impact score for each match based on how many donors the patient already had on the DATRI register and a UK register. The highest scoring regions were Haryana & Chandigarh, Rajasthan, Madhya Pradesh, Punjab, and Delhi. For the first year's recruitment, DATRI rapidly recruited and typed over 3000 people in 2023 based on our recommendations. An initial impact assessment shows a newly recruited donor from Rajasthan has donated domestically, several further donors are 10/10 matches to international patients, and 67% of the donors have HLA types that are new to DATRI. These are promising early data that international collaborations can result in effective donor recruitment strategies.

P37 | HLA-DRB3, -DRB4, -DRB5 allele frequencies of 12 populations from Germany, United Kingdom, Poland, Chile, United States, India, and South Africa

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The class II HLA genes DRB3/4/5 potentially play an important role in hematopoietic stem cell transplantation and were therefore added to the DKMS standard typing profile in 2019. Since then, more than 2.9 million newly registered donors have been typed for HLA-DRB3/4/5, around 1.7 million in Germany, 400,000 in the UK, 400,000 in Poland, 180,000 in Chile, 170,000 in the USA, 70,000 in India, and 70,000 in South Africa. DRB3/4/5 allele frequencies were analyzed for different ethnic groups in the DKMS donor centers in Germany (German, Turkish), UK (British, South Asian), Poland (not differentiated), Chile (Non-Indigenous, Mapuche), USA (African American, European), India (not differentiated), and South Africa (Black, White). DRB3 is most abundant in haplotypes in most ethnic groups (exceptions: UK/British and Chile/Mapuche) with frequencies up to 62.4% (South Africa/Black), followed by DRB4 with up to 35.1% (UK/British) and DRB5 that shows the lowest abundance

in haplotypes in all populations with frequencies up to 26.2% (India). The frequency of DRB3/4/5 absence in haplotypes ranged from 10.3% (Germany/Turkish) to 18.1% (Chile/Mapuche). DRB3*02:02 g was the most frequent DRB3 allele in most populations (except UK/British and Chile/Mapuche) with frequencies between 40.1% (UK/British) and 71.7% (UK/South Asian). In DRB4, we observed very little variability with DRB4*01:01g being the most frequent allele with frequencies between 97.7% (Germany/Turkish) and 99.96% (South Africa/Black). In DRB5, DRB5*01:01g was the most frequent allele in all populations except Chile/Mapuche with frequencies from 27.6% (Chile/Mapuche) to 97.8% (South Africa/Black). Even though DRB3 and DRB5 showed more variability than DRB4, this was not too pronounced, as shown by the cumulative frequencies of the three most frequent alleles: These ranged from 95.8% (Germany/Turkish) to 99.8% (USA/African American) in DRB3 and from 94.6% (Chile/Non-Indigenous) to 100% (South Africa/Black) in DRB5.

P38 | IDS: A clinical decision support system for kidney transplantation with precision medicine tools to enhance bedside manners

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In the field of kidney transplantation, a great deal of data has been collected over the past few decades. However, data accessibility remains complicated for clinicians. There are no tools to provide the right information at the right time, or to explain his/her condition to a patient. As part of the KTD-innov project, we have created a clinical decision support system (CDSS) for kidney transplantation that combines data analysis and biomarker approaches, and provides visualizations of specific data. The idea is to have on a single tool, access to important patient information and calculate predictive scores on demand to help clinicians make the right decision for their patient. In addition, we propose a comparison with the historical French kidney transplant database, using precision medicine algorithms to tailor a population subselection to patient characteristics. All these data are secured by a specific three-layer infrastructure with distributed access to the databases of several centers. Based on 596 follow-up visits, the pipeline for each visit has been studied to determine whether data can reach the CDSS in a maximum of 20 days, including the return of biological analyses results for samples collected and sent to the various platforms. The entire pipeline was completed on time for only 31.6% of visits. However, if we look at platform analyses individually, 4 out of 5 platforms have an average of less than 20 days (One-tailed Student's *t*-tests: $p < 0.001$).We built a CDSS to track kidney transplant patients and present empirical results of its use in hospital context. The specific architecture enables data and algorithms to be updated including with synthetic reference data, and new algorithms or tools to be added. This concept may extend other fields.

P39 | Evaluating HLA imputation clinical utility compared to classical genotyping

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HLA compatibility between donor and recipient is crucial to long-term graft survival and an essential criterion in organ allocation. While HLA genotyping remains the gold standard for accurately identifying individuals HLA alleles, exploring HLA imputation could bring additional genetic information about donor and recipient compatibility. We can distinguish 2 different starting points in HLA imputation: HLA haplotypes-based imputation which generates high-resolution HLA alleles from low-resolution, and SNP-based imputation. We aimed at assessing the most effective method, and evaluate its potential clinical utility compared to classical methods. We compared 4 HLA imputation tools consisting of 2 haplotype-based: HLA-Upgrade (easyhla.univ-nantes.fr), HaploSFHI (sfhitools.fr/HaploSFHI); and 2 SNP-based: HIBAG (zhengxwen.github.io/HIBAG), DEEP*HLA (github.com/tatsuhikonaito/DEEP-HLA); using data

from 5 different populations within the 1000 Genome project ($N = 2504$). Then, to explore the feasibility of using HLA imputation in a real-life kidney transplantation clinical setting, we used the best method to impute the KiT-GENIE dataset ($N = 1986$ donor/recipient pairs). Our results favored the HIBAG approach for its predictive accuracy in all populations gaining between 5% and 50% of accuracy. Using this method, we calculated and compared the number of mismatches between donor and recipient from KiT-GENIE between regular clinical HLA typing and HLA imputed typing. First, we analyzed HLA-A, HLA-B and HLA-DRB1 data and found no differences between the 2 methods (mean of 3.8 (typed) versus 3.9 (imputed) mismatches). Moreover, among a subset of individuals ($N = 70$) with high-resolution HLA typing (HLA-A, HLA-B, HLA-C, HLA-DQB1, HLA-DRB1), we obtained similar results (mean of 5.8 (typed) versus 5.9 (imputed) mismatches). This study suggests SNP-based imputation as the preferrable approach, offering promising implications for a clinical transplantation setting, notably for graft allocation.

P40 | PRIMUS: A distributed web application architecture to benchmark sets of analytics based on reuse of multi-source clinical research data in multiple sclerosis under the umbrella of precision medicine

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The treatment response of patients with multiple sclerosis (MS) is highly heterogeneous. The PRojection In MUltiple Sclerosis (PRIMUS) project aims to create a clinical decision support system (CDSS) for neurologists to inform treatment selection for MS patients. Thus, we constructed an elaborated distributed infrastructure as a robust pillar of the CDSS. As its user, the neurologist can enter the patient's characteristics and history, leading to a visualization for the next 2 years of the evolution of MS using routine clinical markers (incidence of relapses, MRI T2 lesions, disability progression) subject to a given therapeutic scenario based on a database of 5061 patients. Three major building blocks characterize the fully functional CDSS architecture (online on https://primusruotest.chu-nantes.fr/). (1) Decentralized algorithms pre-process the miscellaneous, origin-dependent data into a harmonized multi-source database. This includes the data format, that is, use the unperturbed data or anonymize it with the avatarization method under the Octopize license or augment its quantity via synthetic data generation. (2) Centralized algorithms report the data according to different analytics, incorporating herein an expert-defined filter approach (11, each with 2–13 options), a reduced version thereof (8, each with 2–3 options) to increase the number of queried patients and a distance-based approach consisting of a factor analysis of mixed data yielding the first K nearest neighbors derived by the shortest Euclidean distance to overcome filter limitations. (3) A hub communicating with the different algorithms and relaying the query and data formats to a dedicated frontend. The total infrastructure encompasses various data pipelines, alternative analytics and intelligible interfaces to be benchmarked to converge towards a flexible CDSS prototype tailored for a future clinical investigation, well beyond the use case of MS and located at the heart of personalized medicine.

P41 | Neoantigen recognition by HLA class I and II: Relationship between hematological malignancies and specific HLA distribution in Greek population

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Neoantigens comprise the result of non-synonymous somatic mutations accumulated in malignant cells. Successful recognition of tumor-associated antigens from the HLA class I and class II are considered as a pivotal step for the induction of anti-tumor responses. In addition, the highly immunogenic neoantigens are described as targets of immune checkpoint inhibitors anti-tumor mediated T cell response. Therefore, neoantigens are representing promising new targets for personalized immunotherapy. The introduction of NGS has assisted in better understanding the HLA biology and their association with the neoantigens. This study aimed to investigate the potential association between the HLA class I / II with the neoantigens found in several hematological malignancies. For this purpose, patients suffering from

hematological malignancies, including acute/chronic myeloid leukemia, myelodysplastic syndromes, multiple myeloma and Hodgkin lymphoma, were typed for the HLA class I and class II, using an NGS method (AlloSeq Tx17, CareDx). HLA allele frequencies were obtained, by analyzing the NGS data. The most frequent HLA alleles were submitted to Cancer Antigenic Peptide Database and caAtlas database, to find potential associations with the neoantigens. The results of this study showed more than 100 mutant proteins that are associated with the most frequent HLA class I and class II alleles of hematological patients. Among them, PAX3, RET, GAS1, CYP26A1, SLC16A14, SPATA4, WNT10B, NKD2, TIMM22, TAZ and TEAD2 are responsible for key functions of the malignant cells and can be recognized by the HLA class I and II. Moreover, these proteins are orchestrated in a specific network, where the recognition by the HLA is of paramount importance for the T cell mediated immune responses. In conclusion, the above results indicated that this set of neoantigens targeted by the HLA, may result in finding precisely aimed novel therapies, which will contribute to better disease administration.

P42 | Limited incremental value of routine flow crossmatch prior to kidney transplantation

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The Genome Canada Transplant Consortium is evaluating the use of routine pre-transplant cytometric flow crossmatch (FCXM) to determine the incremental value of this assay for: (a) detecting donor-specific antibodies (DSA), (b) providing actionable information to guide clinical practice, and (c) optimizing health outcomes and economics. We here present the first phase of this program. A total of 10,437 FCXM tests performed pre-transplant during 2016–2021 were compared with the corresponding prior single antibody bead (SAB) test. One-Lambda SAB results were reported using a threshold of 1000 MFI, confirmed by Immucor as required, and informed by epitope reactivity, hot-bead patterns, prior sensitization and other confounders. T-cell FCXM agreed with the class I SAB for 10,192 samples (97.6% overall, 97.4% FCXM−/SAB− and 0.2% FCXM+/SAB+) and 10,106 samples for class II DSA (96.8% overall, 96.6% FCXM−/SAB− and 0.2% FCXM+/SAB+). B-cell FCXM agreed with the class I SAB for 10,055 samples (96.3% overall, 96.2% FCXM−/SAB− and 0.1% FCXM+/SAB+) and 10,001 samples for class II DSA (95.8% overall, 95.6% FCXM−/SAB- and 0.2% FCXM+/SAB+). Less than 4.2% of recipient/donor pairs showed disagreement (either FCXM+/SAB− or FCXM−/SAB+) between the assays. For class I SAB tests 191 (1.8%) were FCXM+/SAB- for T-cell FCXM, and 318 (3.1%) for B-cell FCXM. Figures were comparable for class II SAB tests (Table 1). Sensitivity analysis performed using thresholds of 1000–5000 MFI to inform SAB interpretation marginally increased the agreement of FCXM for HLA class I DSAs from approximately ∼20% to ∼40% for T and B FCXM but did not alter concordance for class II DSA. Routine pre-Tx FCXM shows >95% agreement with prior SAB DSA but only contributes potential incremental actionable information in ≤3% of cases. Selective FCXM testing for patients with questionable SAB results thus may be more viable to guide transplant decisions.

P43 | New measure of interallelic linkage disequilibrium (LD) for highly polymorphic pairs of loci

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Linkage disequilibrium (LD) is the non-random association of alleles at different loci. In genetic systems with only biallelic loci, unique measures of LD can describe association of both the loci as a whole (global LD) and their alleles (haplotype LD). However, in systems with higher genetic diversities where there are multiallelic loci, like in the human MHC (HLA), and current LD measures can only capture either the overall (global) associations or the interallelic (haplotypic) associations. Despite the importance of both approximations, we aimed at improving LD measures for studying subtle associations between specific alleles into particular haplotypes. Traditional measures rely on the difference between observed and expected haplotype frequencies, but these are known to have flaws due to this simplistic approach. Here we provide a more realistic perspective where multiple alternative valid solutions are shown including the previous simple as a particular case. The idea behind our new method involves considering the difference between observed and expected haplotype

frequencies as an observable pattern stemming from a hidden perturbation, termed 'seed LD' (sLD). For instance, introducing disequilibrium in a specific haplotype, say A1∼B1, into a population in equilibrium for A1, A2, A3, B1, B2, B3, would alter all haplotype frequencies finally leading to lower frequencies for A1∼B2, A1∼B3, A2∼B1, A3∼B1, and higher frequencies for the rest. These alterations lead to a system-wide perturbation, even with the introduction of just one haplotype. Measures derived solely from the difference between observed and expected haplotype frequencies may be misleading. By carefully considering the effects of changes produced by each haplotype throughout the system, we can frame this problem under the mathematical concept of a "linear system" and find optimal solutions using well-known linear solvers and base decisions on criteria such as parsimony or external information.

P44 | Structural insights into the CTLA-4 T17A signal peptide mutation

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Mutations in the immune-checkpoint CTLA-4 protein have been associated with susceptibility to autoimmune diseases and cancer. Notably, the presence of an Alanine instead of a Threonine at position 17, a missense signal peptide (SP) polymorphism at exon 1 (T17A), induces protein structural changes, leading to the elimination of a vital glycosylation site, crucial for maintaining the CTLA-4 tertiary structure. We investigated the physicochemical and dynamic characteristics of the CTLA-4 T17A mutation, using computational approaches. We used the 3D structure model of the CTLA-4 molecule (Protein Data Bank ID: AF-P16410-F1) as a template for homology modeling of the native human CTLA-4 SP 3D structure (T17), encompassing residues 1–35 (UniProt ID: P16410), employing MODELLERv9.14. The most structurally similar template model was employed to generate the mutated SP 3D structure (A17), also using MODELLER. Evaluation of both native and mutated 3D structures was based on protein stereochemical properties using PROCHECK, while differences in hydrophobicity and electrostatic potential surface were analyzed using PyMol. Structure stability was assessed by comparing entropy vibration (ΔSvib) differences, utilizing the DynaMut server. Native and mutated CTLA-4 3D structure showed similar conformation to the template after template alignment, and presented more than 90% of the residues in the core region of phi-psi torsion angles, indicating they have good stereochemical quality. The CTLA-4 T17A: i) altered the surface electrostatic profile, reducing positive charge at the N-terminus, ii) increased surface hydrophobicity that potentially affects protein folding, and iii) slightly increased vibrational entropy, reducing the overall protein stability due to increased flexibility. In conclusion, T17A mutation is likely to disrupt CTLA-4 function by altering its physicochemical properties and stability.

P45 | PIRCHE application versions 3 and 4 lead to equivalent T cell epitope mismatch scores in solid organ and stem cell transplantation modules

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The PIRCHE algorithm and web service predict the number of potential allogeneic HLA-derived T-cell epitopes in the context of transplantation. Elevated PIRCHE scores between recipient and donor in organ and stem cell transplantation have been shown to correlate with increased risk of donor-specific HLA antibodies and graft-versus-host disease, respectively. To optimize these predictions, the PIRCHE algorithms and resulting predictions are occasionally improved by (1) refining the specific definition of how T-cell epitopes are defined compared to self-epitopes, and by (2) improving the web service infrastructure. With each revision of the PIRCHE application server, it is critical to completely evaluate the predicted scores, and compare with previous revisions to ensure continuity for researchers and clinicians. This study presents the methodology and results of a comparison between the newly introduced PIRCHE version 4.2 with its predecessor version 3.3, which is already in use in retrospective studies. To perform this validation, we simulated a virtual cohort of 10,000 transplant pairs from major ethnicity groups based on NMDP haplotype frequencies. These transplant pairs were analyzed using both the solid organ and stem cell transplantation modules within the PIRCHE webservice, and results were compared between the versions 3.3 and 4.2. In the stem cell transplantation module, both versions yield identical results for 100% of the test population. In the solid organ transplantation module, 97% of the test population has identical PIRCHE scores in both versions. The deviating cases could be attributed to a refinement in the PIRCHE algorithm's specification. For the 3% of cases with

deviations, the determined magnitude of the difference is likely to be below the detection limit for clinical effects. We therefore confirm the equivalence in PIRCHE scores generated by the application server versions 3.3 and 4.2.

P46 | Decoding the influence of donor age, HLA matching, and relatedness on hematopoietic stem cell transplantation success for leukemia patients

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In this research, using retrospective data from the National Marrow Donor Program (NMDP) database, we sought to ascertain the influence of donor age, relatedness, and HLA matching status on the overall survival (OS) and event-free survival (EFS) probabilities of patients diagnosed with acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) undergoing hematopoietic stem cell transplantation (HCT). We employed a dual-methodology approach, harnessing both classical statistical inference and machine learning techniques, complemented by explainable AI, to derive actionable insights about these critical variables, including advanced statistical inference based on the Cox proportional hazards models and Shapley analysis based on the machine learning models. The results of this study offer practical implications for HCT decision-making, providing valuable perspectives on selecting the most suitable donors to optimize patient outcomes.

P47 | The role of HLA class I alleles in predicting hepatocellular carcinoma risk and patient outcomes: An in-depth TCGA-based study

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Leveraging a rigorous comparative genomic approach, this study illuminates the variance in HLA class I allele frequencies between individuals diagnosed with hepatocellular carcinoma (HCC) and a pan-cancer cohort, excluding HCC, from a dataset of 8998 patients within The Cancer Genome Atlas (TCGA). Our investigation, inspired by the foundational work of Filip Orenbuch et al., delves into the genetic distinctions that separate HCC from a wider array of cancers, pinpointing specific alleles with significant prognostic implications. Employing Fisher's exact test, we determined the statistical significance of the disparities in allele frequencies, uncovering that alleles HLA-A*11:01, A*33:03, A*24:02, B*46:01 and C*01:02, C*07:06, C*08:01 are markedly linked to an elevated risk of HCC. This analysis further explored the prevalence of these alleles within diverse ethnic groups registered with the National Marrow Donor Program (NMDP), revealing a notable dominance among individuals of Chinese and Japanese descent, who are disproportionately affected by liver cancer. Additionally, our findings emphasize the role of alleles such as A*11:01, A*33:01, A*02:06, A*02:07, B*15:25, B*46:01, B*55:02, C*01:02, C*04:03, C*07:06, and C*08:01 in increasing the risk of hepatitis B virus infection among HCC patients. A particular focus on the allele A*01:01's higher frequency in women suggests an increased disease risk for female carriers. Notably, the allele HLA-B*51:01 demonstrated a significant correlation with improved survival outcomes (Log HR −0.769, *p*-value 0.035), further highlighting the crucial role of HLA class I alleles in the clinical prognosis of HCC and suggesting pathways for developing tailored therapeutic strategies.

P48 | Revival of the HLA-Cw11 story despite modern typing techniques

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HLA-Cw11 was defined in the 1990s using serological reagents as a new HLA-C antigen with epitopes of both Cw1 and Cw3. Cloning of the involved C and B locus genes revealed that the Cw3 serological reactions could be attributed to Cw3 sequences in the B*46 allele. The associated HLA-C antigen appeared to be a normal Cw1, explaining the combined serological reactions. The Cw11 antigen and Cw*11:01 allele were subsequently removed by the WHO HLA Nomenclature Committee. Modern typing techniques, like NGS, might be expected to prevent this nowadays. However, we recently failed to

correctly type a regional organ. The results of a RT-SSP HLA typing, nor a SSOP performed the next day provided a clear typing result for the HLA-B allele of this regional organ donor. Subsequent NGS analysis, using MX11-3 (GenDx) also failed to clarify the typing result. Hemizygous sequencing of the specific allele revealed an HLA-B*51 allele until the end of exon 3, followed by an apparent C*01:02:01 sequence with an additional mutation in exon 4 position 1788 resulting in a silent T to G substitution in codon 251. All our standard typing techniques failed to give a reliable result: Firstly, RT-SSP resulted in a rare B*51 allele and an additional Cw*01 reaction did not fit to the other results. Secondly, the PCR used for HLA-B in the SSOP failed to amplify exon 3 resulting in an aberrant result. Thirdly, there were only a few B51 specific sera reacting weakly. Finally, the software of NGS failed to properly align the generated sequences because part of the exon 3 sequences was assigned to HLA-C, and HLA-B sequences were missing. Single strand direct sequencing might have resulted here in a correct (new) typing result, although no defined allele name was yet available for this specific allele. The sequence has been submitted to the WHO HLA nomenclature committee and awaits decision.

P49 | cPRA.cz—Web based algorithm for automated Luminex analysis

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Kidney allocation in the Czech Republic, until the end of the year 2023, depended on cell-based PRA testing for defining hypersensitized recipients. From the beginning of the year 2024 the transplantation program started using calculated PRA as evaluated by the Luminex single antigen bead assay. Our goal was to ensure consistency in the calculation method across the seven transplant centers in the country and to avert discrepancies in results among individual centers. To achieve this, we developed a publicly available website, cpra.cz, which hosts an algorithm for the automatic analysis of raw data exported from the analysis software Fusion and MatchIT. Due to interlaboratory variation, every center determines and validates its own cut-off. Our parsing algorithm allows users to choose the threshold level and also the type of cut-off when entering raw data. In addition, the website facilitates the uploading of results for multiple patients simultaneously, either in a single XML file or across several files. Our algorithm then organizes the data from these uploads into individual patients and further processes it is using the TXMatching algorithm, which we developed in 2021 for the international kidney paired exchange program. Subsequently, our system sends the positive alleles via API to the vPRA calculator, established by Eurotransplant. This calculator maintains a database of 10,000 donors typed at high resolution. Following the receipt of results from vPRA, cPRA.cz prepares processed results for real-time download in PDF, CSV, and Excel formats. These results can then be uploaded to the Czech Donor and Transplants Information System responsible for organ allocation. The entire process is completely anonymous and no source data are saved during the calculation. AS was supported by the Ministry of Health, Czech Republic—conceptual development of research organization (IKEM, IN 00023001).

P50 | SNV in MHC class III genes. Detection by exome sequencing in samples with homozygosity in HLA-A∼C∼B∼DRB1∼DQB1 loci

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Single Nucleotide Variants (SNVs) within class III genes in the MHC region can impact transplantation outcomes serving as markers for various diseases. Exome sequencing of samples exhibiting homozygosity (Hz) for class I and II alleles enables the characterization of extended haplotypes within class III. A total of 89 samples obtained from participants in the Induced Pluripotency Stem Cell Biobank at UERJ, underwent exome sequencing. NGS (Omixon HLA Twin) confirmed HLA-A, -C, -B, -DRB1, -DQB1 Hz in 74 samples. SNVs were identified using the Emedgene Illumina Nextera. Among a total of 1187 genes (class I: 11 genes, $n = 467$; class II: 20 genes, $n = 699$), 21 SNVs were identified in class III genes, including 4 in LTA (rs2229094; rs3093542; rs2229092; rs909253), 6 in C2 (rs7746553; rs367996721; rs9332739; rs1042663; rs9332730; rs36221133), and 11 in CFB (rs41516673; rs12614; rs641153; rs1048709; 31947367; rs113197809; rs144812066; rs151651; rs150920440; rs4151659;

rs199518433). Thirteen were missense variants (CFB, $n = 7$; C2, $n = 3$; LTA, $n = 3$), 5 were synonymous variants (CFB, $n = 4$; LTA, $n = 1$), and 2 were splice donor region variants & intron variants in C2, with 1 splice polypyrimidine tract variant & intron variant in the LTA gene. Eighty extended A∼C∼B∼rs2229094∼rs2229092∼rs909253∼rs7746553∼rs1042663∼rs12614∼rs641153∼rs1048709∼DRB1∼DQB1 haplotypes were determined using Arlequin®. Four Hz samples exhibited different haplotypes within class III. The T∼A∼C∼C∼G∼C∼G∼A class III haplotype was identified in 88 out of 178 genotypes, with 6 samples sharing this haplotype exhibiting heterozygosity in HLA-C or -DQB1 loci. Thirty different haplotypes were observed. The haplotype A*01:01:01∼C*07:01:01∼B*08:01:01∼DRB1*03:01g∼DQB1*02:01:01 was present in 12 Hz samples, with 9 having T∼A∼C∼C∼G∼C∼G∼A. The number of class III SNVs detected by exome sequencing represented less than 1.8% in a sample of A-C-B-DRB1-DQB1 Hz individuals.

P51 | Argentina's leap into the cPRA Era: Embracing transplantation advancements

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In pursuit of enhancing transplantation opportunities for sensitized patients in Argentina, we developed the Argentinean Calculated Panel Reactive Antibody (cPRA) calculator, leveraging a prototype database with HLA antigen data from deceased donor donations in 2022. Our methodology, designed to capture the specific HLA antigens and frequencies unique to Argentina, reflects the nation's diverse genetic landscape. Distinguishing between HLA class I and II, as mandated by the National Procurement and Transplantation Information System of the Argentine Republic (SINTRA) for the upload of differential values, our cPRA calculator exhibited robust agreement with OPTN cPRA (rc = 0.949 for PRA I, 0.960 for PRA II) and Eurotransplant vPRA (rc = 0.919 for PRA I, 0.985 for PRA II). The high concordance coefficients signify a robust alignment between the Argentinean cPRA calculator and its international counterparts, affirming its precision and reliability in assessing sensitization phases. This study serves as a pioneering effort, introducing a population-specific cPRA algorithm grounded in Argentina's unique HLA frequencies. Future refinements, including the unification of PRA I and PRA II into a single value, are planned to align Argentina's leap into the cPRA era with calculators worldwide. The Argentinean calculator offers valuable insights for organ allocation systems and ensures accurate assessments in the diverse landscape of sensitized patients in Argentina, ultimately optimizing transplantation access and outcomes.

P52 | Comparison of two semi-quantitative single antigen bead methods for the detection of anti-HLA antibodies with a machine learning algorithm

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The detection of anti-HLA antibodies and their reactivity based on binding of complement fractions is of great importance for the successful outcome of kidney transplantation. However, the results of these analyses are often complicated and multidimensional and therefore difficult to be interpreted. The purpose of this study was to compare the two commercially available single-antigen bead (SAB) methods in terms of detecting anti-HLA antibodies and complement binding specificities using machine learning methods. Sera from 97 patients with a positive Panel Reactive Antibody Test (>5%) was tested by two SAB methods, Immucor (IC) and One-lambda (OL), for class I and II anti-HLA antibody specificities and their ability to bind complement. Inferential statistics and Principal Component Analysis of the results followed. In analysis of specificities that were positive by both methods, a strong positive linear correlation was shown between the mean fluorescence intensity (MFI) of anti-HLA antibodies as detected the two methods ($r = 0.61$, $p < 0.001$ and $r = 0.54$, $p = 0.001$ for class I

and II, respectively). In Principal Component Analysis, negative results were also included. In the projection of the plane defined by the first two principal components, the loading corresponding to the IC method for class I antibodies was congruent with the loadings of the complement binding detection methods, C3d and C1q. In contrast, the OL loading was nearly perpendicular to the rest loadings, indicating the existence of non-complement-binding specificities that are not detected by IC. A similar result was shown for class II anti-HLA antibodies. OL detected a number of anti-HLA class I and II antibody specificities not detected with IC. These specificities potentially do not bind complement. The two complement-based methods were equivalent in detecting complement binding specificities, both class I and II.

P53 | Advancing HLA typing on nanopore sequencing platforms: A novel phasing-based bioinformatics software for high resolution and accuracy

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Nanopore sequencing platforms offer rapid, accurate, and cost-effective solutions for characterizing HLA genomic sequences, making them invaluable for local laboratory testing and expedited HLA typing of deceased donors. However, challenges persist due to the relatively poor signal-to-noise ratio of nanopore data, which can impact the accuracy of HLA typing results. Here, we introduce HA-HLA, a novel phasing-based bioinformatics software that leverages long-read sequencing to pre-phase reads and construct haplotype consensus sequence for HLA gene typing. Our pilot study involved 11 HLA genes in 8 samples using the AllType NGS 11 loci sample prep flex kit (One Lambda) on QitanTech nanopore platform QNome-3841. Remarkably, compared to our conventional NGS-based HLA typing results, HA-HLA achieved precision rates of 100% at the 2-field resolution and 97.9% and 98.8% at the 3-field resolution for class I and class II genes, respectively. Furthermore, HA-HLA demonstrates adaptability to other long-read platforms, suggesting its potential for widespread adoption in HLA typing. This advancement holds promise for enhancing precision and efficiency in various clinical and research applications, although further validation and optimization may be necessary for broader implementation.

P54 | Laboratory reporting of DQB1*03:05 (DQ8) and DQB1*03:19 (DQ7) in UK NEQAS for H&I's external proficiency testing scheme for Coeliac disease

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UK NEQAS for H&I's scheme 8 HLA Genotyping for Coeliac and Other HLA Associated Diseases primarily assesses participants' ability to HLA genotype to aid in the diagnosis of HLA associated diseases. Participants that register for Coeliac Disease (CD) are required to report their HLA genotype findings for 10 samples a year, which are assessed against a reference type allowing labs to report at a resolution that is applicable to the needs of their clinical users. Laboratories can also report interpretative comments although these are not currently assessed. There are a high number of errors for CD genotyping with >20% of laboratories making at least one error each year 2018–2023. Two samples in this timeframe, which had lower frequency DQ7 and DQ8 alleles (i.e., not DQB1*03:01 or *03:02) had particularly high number of errors in reporting. In 2022 15/54 labs made an error for a sample with reference type DRB1*04:03, 11:01; DQB1*03:01 (DQ7), 03:05 (DQ8); DQA1*03:01, 05:05. In 2023, 8/54 labs made an error for a sample with reference type DRB1*07:01, 11:02; DQA1*02:01, 05:05; DQB1*02:02, 03:19 (DQ7). Labs with errors are asked to submit a root cause investigation. Review of the responses found the errors in these two samples were caused by a number of issues: 11% due to transcription errors, 25% due to interpretation of results, 32% were caused by problems with commercial kits such as insufficient resolution of HLA type and ambiguous results, 4% due to a technical failure, 29% gave no response. It is important that labs can perform accurate HLA typing to support the diagnosis of CD. UK NEQAS for H&I in collaboration with the British Society for Histocompatibility and Immunogenetics have produced guidelines on reporting HLA results for CD and stratifying risk to aid participants and standardize reporting.

P55 | Performance of participants of UK NEQAS for H&I pharmacogenetics schemes over the last 5 years

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The UK National External Quality Assessment Service for Histocompatibility and Immunogenetics (UK NEQAS for H&I) run a number of Schemes to assess participants' ability to correctly determine HLA types in relation to drug hypersensitivities. These include Scheme 7 – B*57:01 Typing, to assess participants' ability to correctly determine HLA-B*57:01 status for abacavir hypersensitivity and Scheme 8—HLA Genotyping for Coeliac and Other HLA Associated Diseases, to determine participants' ability to correctly determine HLA status for allopurinol, carbamazepine and phenytoin hypersensitivity (B*58:01, A*31:01/B*15:02 and B*15:02/B*57:01/B*58:01 respectively). This combination of schemes offers laboratories the option to select relevant external proficiency testing (EPT) to their laboratory repertoire. An analysis of performance by participating laboratories from 2019 to 2023 was performed in Scheme 7 and from 2021 to 2023 in Scheme 8 (when drug hypersensitivity options were included in the Scheme). There were between 50 and 67 participant labs in Scheme 7 and 1–6 in Scheme 8 for pharmacogenetic testing. In total, 10 errors were made (error rate 0.3%); 7 errors for abacavir (4 false negative, 3 false positive) involving 5 laboratories, and 3 errors for carbamazepine (2 false negative, 1 false positive), involving 1 laboratory. There were no errors in results for allopurinol or phenytonin testing. The highest number of errors was made in 2023 ($n = 4$). Significantly, 6/10 errors were reported as due to sample mix up, highlighting the importance of robust laboratory procedures and participation in EPT schemes to help ensure laboratory quality during all stages of testing. Performance in HLA pharmacogenetic schemes over the last 5 years is encouraging, with 99.7% results reported correctly. In the future a single combined scheme for HLA typing and pharmacogenetics is planned, including further options as the number of known HLA drug-interactions expands.

P56 | Enhancing organ transplantation outcomes through standards-compliant software solutions

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Accurate monitoring of organ transplantation requires clinical expertise and technological support in the form of reliable software. Software safety and usability standards are crucial to obtain efficient medical procedures and to improve patient care. Advyser Solid organs, a software for monitoring donor-derived cell-free DNA (dd-cfDNA), has been developed through rigorous adherence to international standards for software as a medical device. Our procedure for post-transplantation surveillance utilizes sequencing technologies for accurate monitoring of dd-cfDNA, a biomarker of tissue damage with probable connection to graft rejection. Analysis is facilitated by the Advyser software and its easy-to-use workflow, resulting in a process that is efficient and robust. We implemented a holistic approach that integrates development, planning, and testing processes to comply with IEC 62304: medical device software lifecycle processes, IEC 62366: usability engineering for medical devices, IEC 82304: health software product lifecycle, ISO 13485: quality management systems (QMS) for medical devices. Implementing and following international standards has resulted in enhanced patient safety. Adherence to ISO 13485 minimizes risks associated with software malfunctions and improved software quality. Following IEC 62304 and IEC 62366 ensures meticulous development, rigorous testing, and user-centered design, resulting in a more reliable product, faster and more accurate analysis. Efficient development processes enable quicker analysis and more timely decision-making. The adoption of a compliant QMS and the implementation of state-of-the-art standards makes Advyser Solid organs a safe and robust software, a best-in-class solution when it comes to patient safety. Advyser empowers healthcare professionals with reliable results for improved transplant outcomes.

P57 | Avocado as a supplement in Afro-Colombian patients with obesity, diabetes and who express the HLA-DQB1*02:01

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In Colombia, a high prevalence of the HLA-DQB1*02:01 allele has been observed in the Afro-Colombian

population, especially in regions such as Chocó, Cauca, Antioquia, San Basilio de Palenque and San Andrés Islas. This allele is associated with specific metabolic characteristics in African Americans, as discussed in a study that classifies diabetes in this population based on insulin secretion, immunological and genetic markers. The results suggest that the majority of obese African Americans with diabetic ketoacidosis have type 2 diabetes, characterized by increased insulin secretion, absence of autoimmune markers, and lack of HLA genetic association, while lean African Americans with diabetic ketoacidosis tend to have characteristics of diabetes type 1. On the other hand, the production and variety of avocado in Colombia has been investigated, highlighting its use as fresh food and its different varieties. This study reveals that avocado not only has valuable nutritional properties, such as monounsaturated fatty acids, phytosterols and antioxidants, but also shows morphological and metabolic variations between different cultivars. Additionally, the effect of daily consumption of avocado, a source of monounsaturated fatty acids, has been studied in people with dyslipidemia. The results indicate significant benefits, such as a decrease in total and LDL cholesterol, an increase in HDL, and a reduction in the risk of cardiovascular disease. Together, these findings suggest that avocado, with its healthy properties, could be considered as part of the diet for African-American people with genetic predisposition to conditions such as diabetes and dyslipidemia, providing metabolic and cardiovascular benefits.

HEMATOPOIETIC STEM-CELL TRANSPLANTATION (HSCT)

P58 | Effect of Killer-cell Immunoglobulin-like receptors and their cognate HLA ligands on tumor disease-free survival after allogeneic hematopoietic stem cell transplantation

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Different HLA and KIR combinations may influence NK cell activation and education states and alter antitumor immune surveillance, including relapse and survival in patients undergoing unrelated or haploidentical hematopoietic stem cell transplantation (HSCT). In a group of 481 patients, we assessed which donor KIR receptors, recipient cognate HLA ligands (C1, C2, and Bw4), inhibitory (i)KIR-HLA pairs or post-transplant iKIR-HLA pair number variation have the greatest impact on disease-free survival (DFS). Among KIR donors, the association with DFS was found for the KIR2DL2 molecule (134 [53%] vs. 92 [40.5%]; $p = 0.0066$; OR = 1.65; 95% CI 1.15–2.37), and among KIR receptors for which the ligands are unknown, a statistically significant adverse effect on DFS was demonstrated for KIR2DS2 (134 [52.1%] vs. 92 [41.6%]; $p = 0.022$; OR = 1.53) and KIR2DL5 group 2 (54 [56.8%] vs. 78 [44.1%]; $p = 0.045$; OR = 1.67; 95% CI 1.01–2.76). A protective effect on DFS was demonstrated for KIR2DL4 del (97 [44.5%] vs. 34 [64.2%], $p = 0.011$, OR = 0.45, 95% CI 0.24–0.83). There was no association between patient HLA ligand groups and DFS, just as no association was detected between pairs of donor inhibitory KIR with the cognate recipient HLA ligand and DFS in post-transplant constellations. Of importance, a decrease in the number of iKIR-HLA pairs post-transplant had a significant adverse effect on DFS compared to an increased number (18/20 [90.0%] vs. 6/17 [35.3%]; $p = 0.0010$; OR = 13.09; 95% CI 2.82–60.67). Maintenance of tumor immune surveillance after HSCT is mediated by NK cells through receptor-ligand interactions rather than the presence of the receptor or ligand itself. NK cell function depends on a constellation of a series of iKIR-HLA pairs rather than on any particular pair. Modulation of the effectiveness of tumor immune surveillance by iKIR-HLA pair number variation after HSCT may indicate the existence of a mechanism for setting and resetting NK cell education.

P59 | HLA-loss relapse detection using NGS data from standard HLA genotyping in haploidentical stem cell transplantation

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HLA-loss relapse is a specific type of relapse after stem cell transplantation from haploidentical family donors (haplo-SCT). This type of relapse is characterized by deletion of the patient-specific non-inherited HLA haplotype in leukemic cells at the time of relapse. We developed a software algorithm to detect patient-specific HLA haplotype loss in post-transplant chimeric samples using NGS

data (AlloSeq Tx kit designed for 17 loci, CareDx). The group tested by this algorithm included 34 patients (a total of 87 samples) in whom relapse was detected after haplo-SCT. Using pre-transplant HLA typing data, unique markers were identified for all haplotype combinations in this group. In 26 out of 34 patients only classic relapse occurred, the patient-specific HLA read counts were in accordance with non-HLA chimeric markers. In 8 out of 34 patients (23.5%), HLA-loss relapse (with or without previous classic relapse) was detected. In samples from these patients, patient-specific HLA reads were undetectable or below the noise level. The limit for detection of HLA loss was set to 5% of the patient's genotype in non-HLA markers. HLA-loss relapse was unequivocally diagnosed if patient-specific HLA values were below the noise level (set to 1%). This criterion was met for all tested samples. Based on these data, the procedure was included in the standard post-transplant monitoring after haplo-SCT. The 23.5% of HLA-loss relapses after haplo-SCT detected in our cohort corelates with published data. However, the detection limit is not completely satisfactory for clinical practice. Infusion of donor-specific lymphocytes is the standard treatment in the early stages of relapse. This treatment is not recommended for HLA-loss relapse. Therefore, further improvement of the detection limit of our procedure is necessary to be able to detect this type of relapse at earlier stages. Supported by MH CZ–DRO (00023736, UHKT).

P60 | HLA Loss detection after haplo-identical hematopoietic stem cell transplantation using a next generation sequencing based specific approach

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HLA loss is found in approximately 30% of relapse cases after haplo-identical stem cell transplantation. The detection of cause of relapse is of interest due to the therapeutic impact of these relapses, as donor lymphocyte infusions don't have any impact on it. Nevertheless, it may be difficult for HLA laboratories as the number of cases is quite low in a lot of centers and specific chimerism based techniques may have several blind alleles. We developed a new NGS based method specific for HLA loss detection. Briefly, the polymorphic markers at telomeric and centromeric region of each HLA classical locus are selected ($n = 22$). All markers are amplified in one multiplex PCR (47 min). The amplicons for each sample are ligated to an index (35 min) before purification with magnet bids (20 min). Library is then quantified and sequenced in a MiSeq (Illumina) and can be mixed with classical HLA Typing libraries. Analysis is performed on a software developed by EFS (Loss_Profiler). Results obtained on 5 first samples with the new method were compared to a classical HLA typing approach using NGMIX HLA typing reagents and TypeStream Visual (One Lambda) software. The new reagents gave fully concordant results confirmed HLA loss detection for patients with HLA loss and those with classical relapse. Also, sensitivity may be higher than those derived from the HLA typing method to reach 1.25% if there is a sufficient number of informative markers while HLA loss detection is difficult with less of 10% blasts with HLA typing derived method. Especially, the interpretation is quite easier with the specific approach. In conclusion, HLA loss detection is feasible using specific NGS based approach and may be pooled with HLA typing. It may help laboratories to manage specific cases of relapses that occur after a long delay post haplo-HSCT.

P61 | DSA determination and eplet mismatches analysis in pediatric patients affected by hemoglobinopathies treated with haploidentical hematopoietic stem cell transplantation

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A limitation to the success of allogeneic hematopoietic stem cell transplantation (HSCT) for the cure of β-thalassemia major is represented by graft rejection. Clinical studies indicated that the presence of Donor Specific Antigens (DSAs) is the most important risk factor for rejection, in particular in haploidentical transplantation

(h-HSCT) settings. In the present study we determined the presence of DSAs in the sera of 19 pediatric patients affected by Hemoglobinopathies treated with h-HSCT, on a Luminex platform (One Lambda, CA, USA). Graft rejection was observed in 6 out of 19 patients (31.5%) after h-HSCT. Among the 6 patients that lost the graft we determined the presence of DSAs with MFI > 1000 in 4 patients (66.6%): 2 with MFI between 1000 and 5000, 2 with MFI higher than 5000, against both HLA class I and II. Although the difference was not statistically significant, it was interesting to observe that among the patients with a successful outcome, the presence of DSA values between 1000 and 5000 MFI was observed only in 3 out of 13 patients (23.07%). We further investigated the potential role in this group of transplanted patients of the eplets mismatches, that are known to represent the functional component of HLA epitopes recognized by antibodies. We used the MatchMaker software, in which we loaded the data of the HLA high resolution typing for 11 loci of both patients and donors, determined in NGS (One Lambda, CA, USA) establishing a media value of 10.6 for HLA class I and 7.3 for HLA class II verified epitope mismatches. No statistically significant difference was observed comparing the outcome of the patients based on the numbers of verified eplets mismatches considered higher than the media. However, we found that 4 of the 6 patients (66.6%) that rejected the transplant had a number of verified eplets mismatches for class II above the median, while only 4 out of 13 (30.7%) with a successful outcome had the same characteristics.

P62 | Eplets mismatched analysis in a group of pediatric patients affected by hematological malignant diseases treated with haploidentical HSCT

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The level of the HLA polymorphism is an obstacle to the search of compatible donors for patients affected by hematological diseases proposed for a hematopoietic stem cell transplant (HSCT). Nevertheless, recent development of new manipulation strategies for HSCT from haploidentical donors (haplo-HSCT) has shown that despite the large HLA antigenic disparities, impressive clinical results have been obtained in pediatric and adult patients. However, few studies investigated the potential role of eplet mismatches between in haplo-HSCT. Very briefly, eplets represent the "functional" component of HLA epitopes recognized by antibodies. Results relative to kidney transplantation showed that high numbers of eplet mismatches were associated with the development of donor-specific antibodies and shortened graft survival. The aim of this study was to investigate a potential association between the clinical outcome in 213 pediatric patients affected by malignant hematological diseases treated with a haplo-HSCT in a single Centre with the amount of verified eplets mismatches present in the patient/donor pairs. We determined a median of 10 (range 0–27) verified different eplets mismatches for class I and 7 (range 0–19) for class II, using Epitope Matching (Thermo Fisher, Canoga, CA, USA). Although little advantages observed for non-related mortality and survival in patients with lower HLA class I and II eplets mismatches, no statistically significant differences were observed in the clinical outcome of the patients. A potential explanation for the lack of clinical improvements in this haplo-HSCT setting could be found in the adoption of the ex vivo TCRαβ/CD19 depletion that supposedly minimizes the potential advantage of lower eplet mismatches in patient/donor pairs. It will however be interesting in the future to observe the potential impact of the eplet mismatches load in a larger court of patients treated with haplo-HSCT or with different conditioning regimens.

P63 | HLA-DQ heterodimers and graft failure after haploidentical stem cell transplantation in patients with acute leukemia

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Both chains (α and β) of HLA-DQ molecules (heterodimers) show extensive polymorphism. Additional HLA-DQ diversity is afforded by the trans-dimerization of the α chain from one parent with the β chain from the other parent. The α chains from DQA1*02, 03, 04, 05, 06 alleles form stable heterodimers with the β chains from DQB1*02, 03, 04 alleles (group 1—G1) as do the α chains from DQA1*01 with the β chains of DQB1*05 and 06 (group 2—G2). Multivariable models identified significantly higher relapse risk in G1G2 and G2G2 patients compared with G1G1 patients after HLA-A, -B, -C, -DRB1 matched unrelated hematopoietic stem cell

transplantation. The aim of our study was to investigate the association of HLA-DQ heterodimers with graft failure (GF) after related haploidentical stem cell transplantation in patients with acute leukemia. One hundred and thirty-four patients (84 with acute myeloid leukemia and 50 with acute lymphoblastic leukemia) in first or second complete remission were included in our study. All patients and donors were HLA-A, -B, -C, -DRB1,-DRB3/4/5, -DQA1, -DQB1, -DPA1, and -DPB1 typed at high resolution. In each patient and donor cis-encoded genotype (HLA-DQ G1G1, G1G2, and G2G2), trans-dimers and number of unique molecules were determined. The end point of the analysis was the assessment of the development of GF depending on the number of unique HLA-DQ G1 and G2 molecules in the recipient and on donor/recipient HLA-DQ G group matching (G1G1, G1G2, G2G2). We found a trend ($p = 0.06$) to less frequent development of GF (primary or secondary) in patients with 4 HLA-DQ G2 molecule: number of G2 = 0, hazard ratio (HR)—1; number of G2 = 1, HR—0.49, 95% CI—0.17–1.41; number of G2 = 2, HR—1.95, 95% CI—0.22–17.4; number of G2 = 4, HR—0.03, 95% CI—0.00–0.74. So, the patients with 4 HLA-DQ G2 had the lowest risk of developing GF. Further study is needed to understand the role of HLA-DQ heterodimers in GF after allogeneic hematopoietic stem cell transplantation.

P64 | Validation and implementation of HLA loss relapse detection by NGS-based HLA typing

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HLA loss relapse (HLA-LR) consists of genomic loss of mismatched HLA molecules in re-emerging leukemic cells after hematopoietic stem cell transplantation (HSCT). The implementation of HLA-LR detection in clinical laboratories is crucial for guiding therapeutic decisions since donor lymphocyte infusion is not effective as a rescue therapy for HLA-LR. In this sense, the applications of next-generation sequencing (NGS) HLA typing could be expanded in this field providing complete HLA gene and allele coverage and the possibility to combine chimerism study in the same NGS run. In this study, we validate and implement HLA loss detection by NGS-based HLA typing in the routine of our laboratory. Previously, we had assessed the behavior and sensitivity of NGS-based HLA typing in chimeric samples by analyzing the signal noise ratio in seven artificial chimeric curves. Optimization consisting in deep coverage increase was required to improve the sensitivity of samples with more extreme chimerism. For the validation phase, 41 post-HSCT patient samples with known chimerism were analyzed according to the optimized procedure. The low-represented HLA allele was successfully detected until 5% of chimerism level, so we established that HLA-LR can be assessed in a chimerism range from 0% Donor—100% Patient to 95% Donor—5% Patient. Furthermore, correlation between signal-noise ratio and chimerism level was determined for each HLA gene (R^2 range: 0.34–0.77). By linear regression, the expected signal-noise ratio value can be calculated according to the level of chimerism of relapsed sample and the concordance of observed and expected value determines relapse type. Since the implementation as a new test in our lab, we have analyzed 4 cases of post-HSCT relapses 2 classified as HLA loss and 2 as classical. In conclusion, NGS-based HLA typing applications can be expanded in the context of post-HSCT relapse classification providing complete HLA gene and allele coverage.

P65 | Impact of KIR genotype on clinical outcome of hematopoietic stem cell transplants: A single center experience

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Allogeneic stem cell transplantation (allo-SCT) is a life-saving therapy for patients with hematological malignancies. A fully HLA matched donor is the best option, but

the probability of finding an identical donor is around 30% and matched unrelated donors (MUD) are available on average within 2–3 months of starting the search of the donor. For this reason, the use of alternative transplants from haploidentical donors has increased significantly over the last decade and is still increasing. Several studies have demonstrated that the presence of particular KIR genotypes (B/x), some KIR activating genes (Cen B) and an alloreactive NK population in the donor are associated with a better clinical outcome of transplants. Patients receiving allo-SCT from haploidentical ($n = 35$) and MUD ($n = 53$) since 2019 to 2023 were included. We typed KIR genes of the selected donors by PCR-SSO Luminex method using the KIR SSO genotyping test (One Lambda Inc., Canoga Park, CA). The aim of this study is to evaluate the impact of donor KIR B status on the clinical outcome of allo-HSCT. Twenty haploidentical donors (57%) were Best/Better and 15 Neutral (43%); 23 MUD (43%) were Best/Better and 30 Neutral (57%). We found that acute myeloid leukemia (AML) patients transplanted from donors with donor KIR B status Best/Better have a better overall survival at 3 years for both haploidentical and MUD transplants. We found no correlation between KIR ligand mismatch in GvH direction and the clinical outcome of haploidentical transplantation. There was no difference in the incidence of acute GvHD in recipients of Best/Better or Neutral donors. Even if in a small cohort, our data suggest that the presence of some KIR activating genes (Cen B) in donors can improve the outcome of transplants: this further confirms that the typing of KIR genes is an important tool that should be considered to select the best donor when more than one is available.

P66 | The impact of donor-specific antibodies presence on the outcome post-allogeneic hematopoietic stem cell transplantation: A survey from a single center

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Donor-specific antibodies refer to anti-HLA antibodies that specifically correspond to a mismatched antigen of the donor. DSA's role in alloSCT is controversial: several studies reported their association with primary graft failure in the setting of mismatch unrelated or haploidentical donor SCT. International guidelines recommend to test patients for DSA before transplant and if possible, to choose donor with negative screening. Our study aims to evaluate the impact of DSA presence on the outcome post-transplant. We collected clinical data of 236 recipients of alloSCT, performed at our institution from March 2019 to October 2023. Serum from all patients was tested for DSA with the Luminex Single Antigen Beads method. A total of 186 patients (79%) achieved myeloid engraftment within day 30 post alloSCT. Thirty-two out 236 (13%) patients engrafted after day 30 post alloSCT. The median times to neutrophil and platelet engraftment were respectively 21 days (range 11–121 days) and 19 days (range 10–203 days). PrGF occurred in 14/236 patients (6%). Twenty-nine patients (12%) were DSA positive. We found that DSA positivity correlates respectively with neutrophil and platelets engraftment failure at 30 days after allo SCT ($p = 0.01$; $p = 0.0004$). Univariate Cox analysis showed that factors, including DSAs positivity, disease type, disease status, donor type, conditioning regimen, patient's age and CD34+ were correlated with neutrophil and platelet engraftment failure at 30 days after alloSCT. Multivariate analysis confirmed the impact of DSA only for platelet engraftment, confirming the role of type and status disease, donor type, recipient age and CD34 + cell on engraftment. DSA presence has no impact on TRM, DFS and OS. The presence of DSA is not the only predictor of PrGF, which appears to have a multifactorial pathogenesis. DSA may play a role in given transplant platforms. Thus, patient screening may be helpful to choose the best donor and transplant strategy.

P67 | Impact of HLA mismatches at the molecular level on the clinical evolution of haploidentical hematopoietic stem cell transplantation with post-transplant cyclophosphamide

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Just 25% of the patients who receive an allogeneic hematopoietic stem cell transplantation (allo-HSCT) from a related donor are HLA identical. Thus, new alternatives like haploidentical HSCT (haplo-HSCT) with post-transplant cyclophosphamide (PTCy) are increasing in clinical practice. The aim of this study is to evaluate the immunogenicity of the HLA mismatches in haplo-HSCT with PTCy accordingly to relapse and GRFS. A cohort of 145 haplo-HSCT patients and donors were retrospectively analyzed. HLA-A, -B, -C, -DRB1, -DQB1 and -DPB1 typing of the pairs was performed by PCR-SSOP and/or NGS, when appropriate. HLA compatibility was evaluated by the Predicted Indirectly Recognizable HLA-Epitopes (PIRCHE) algorithm and PIRCHE Score (PS). The median age of the patients was 55 years, enriched in males (55.9%). Acute Myeloid Leukemia (AML) and Myelodysplastic syndromes (MDS) were the main indication for haplo-HSCT (52%). The followed up was 36 months. The PIRCHE algorithm showed that patients with relapse presented an augmented PS in host versus graft (HvG) direction (66, IQR: 50–85 vs. 44, IQR: 29–69; $p = 0.002$). ROC curve analysis showed that patients with PS >50 presented relapses earlier (HR: 4.66, $p = 0.001$). The same data was observed analyzing incompatible ABO haplo-HSCT (HR: 2.66, $p = 0.014$). In a multivariate analysis both parameters remain as independent risk factors for relapse. The analysis of PS and GRFS showed that patients with events included in GRFS presented an augmented PS HvG (60, IQR 36–84 vs. 43, IQR: 29–56; $p = 0.001$). Patients with PS > 49 (calculated by ROC) presented a reduced GRFS (HR: 2.19, $p = 0.001$). Similar results were obtained examining the comorbidity index (HCT-CI) > 3 (HR: 1.84, $p < 0.001$). The multivariate analysis demonstrated that both parameters were independent risk factors for a diminished GRFS. The PIRCHE algorithm could contribute to the proper selection of donors in haplo-HSCT, since it may be used as a prognostic biomarker for relapse and GRFS.

P68 | Inferior survival is observed among adult HSCT recipients having pre-transplant anti-HLA antibodies even though they are not directed against the donor

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Recent advances in allogeneic hematopoietic stem cell transplantation (HSCT) have enabled successful outcomes using HLA-mismatched donors, including haploidentical related and unrelated donors. The presence of anti-HLA antibodies (Abs), both donor-specific (DSA) and non-donor specific (non-DSA), is emerging as a crucial factor influencing post-HSCT outcomes. While the adverse impact of DSA is well-established, the significance of non-DSA Abs remains less explored. We here analyzed the impact of patients' pre-HSCT anti-HLA antibodies, particularly non-DSA Abs, on clinical outcomes after HLA-mismatched HSCT. Data from adult patients undergoing their first HLA-mismatched HSCT from January 2014 to June 2022 at a single center were collected. Donors were HLA-mismatched unrelated (i.e., 9/10) or family haploidentical. Anti-HLA Abs screening and identification were performed using Luminex-based assays. Clinical outcomes included neutrophil and platelet engraftment, GvHD, overall survival (OS). Causes of death were collected. Among $n = 64$ HSCT recipients, $n = 20$ (31%) presented anti-HLA Abs antibodies, of whom $n = 15$ and $n = 5$ had non-DSA and DSA respectively. The presence of non-DSA Abs was associated with female recipients ($p = 0.04$). While engraftment and acute or chronic GvHD did not significantly differ among groups, a significant inferior survival was observed in both DSA and non-DSA Abs groups. In particular, the mortality incidence rate ratio was 3.31 times higher in non-DSA Abs group compared to anti-HLA negative patients ($p = 0.01$). Although on very few numbers, analysis of death causes revealed more MOF, graft failure and GvHD in the Non-DSA Abs group compared with anti-HLA negative. Our findings suggest that Non-DSA Abs may influence mortality risk after HLA-mismatched HSCT. The study underscores the need for larger cohorts and further comprehensive research to elucidate the role of these Abs on mortality causes.

P69 | Management of ABO-incompatible HSCT: 2021–2023 data analysis

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Mismatching of the ABO system is not an impediment to HSCT transplantation, but it requires an immunohematological protocol to monitor patient and provide an appropriate transfusion support. The adoption of this protocol at ASUFC Blood Bank since 2021 has allowed the standardization of immunohematology management of transplanted patients, improving transfusion efficacy and accuracy. During 2021–2023, 194 PB allogeneic transplantations were performed, 84 related (31 HLA identical, 53 HLA-haplo) and 110 unrelated (85 HLA 10/10, HLA 9/8 and 15 HLA 7/8). An ABO mismatch was present in 45.5% of cases: 36 Major MM, 45 minor MM and 7 bidirectional MM. Donor/recipient evaluation based on HLA typing, anti-HLA screening, blood group typing, IHA titration, DAT and IAT was performed before transplantation. Blood group typing was repeated 60/90 days post-transplant both with serological and molecular methods. Blood type conversion was recorded on IT software. At the first transfusion request, blood typing (forward and reverse group) was repeated to ensure the right transfusion support. Complete blood type conversion occurred in 63.9% of major MM and in 71.4% of bidirectional MM. Genotype conversion was recorded in 86.7% of minor MM. IHA clearance was not calculated, as samples were sent at irregular times; when 30–60–90 day-monitoring was done, clearance occurred in 2.6 months. In 6 patients with pre-transplant titer >1:256, anti-donor IHAs are detectable 6 months post-transplant. As regards to the outcome, 19.4% Major MM, 15.6% minor MM and 14% bidirectional MM patients died (46.1% from early relapse, 13% for relapse, 6.6% for graft failure, 33.3% from multiple concurrent causes). Among these transplants, 6 were 10/10 HLA matched, 2 were 9/10, 3 were 7/8, and 4 were haploidentical. Our target to manage ABO-incompatible HSCTs in terms of transfusion support was achieved. Although HLA compatibility is the first choice for transplantation, blood type conversion has a relationship with prognosis and should be considered.

P70 | Maternal uniparental disomy of chromosome 6 (upd(6) mat) in a patient affected by acute myelogenous leukemia awaiting HSC transplant

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Maternal or paternal uniparental disomy of a chromosome is a rare condition in which offspring inherit both copies of a chromosome from a single parent. We analyzed an adult male patient with AML, with apparently normal phenotype, evaluated for HSC transplant. Despite the complete remission of the pathology, we observed homozygosity in all HLA loci both with NGS and SSO on a peripheral blood sample. To verify the possible allelic loss, we studied HLA haplotypes of the family (father, mother, 2 brothers) demonstrating the absence of paternal HLA haplotype. The subject's genotype was confirmed by HLA typing on tissues other than peripheral blood reconfirming the homozygosity of HLA region. STR analysis highlighted the real descent from the natural father on all the loci considered (p of paternity = 99.99999; LR = $1.7e^{11}$) and confirm the correct offspring. Analyses revealed a maternal uniparental disomy of chromosome 6, confirmed by the anamnesis: placental anomalies during pregnancy, low birth weight, characteristic face and skeletal anomalies during growth. In the patient's clinical history, the onset of multiple nodular skin lesions suggestive of a hematological neoplasm on biopsy is described. This neoplasm was characterized both on blood and bone marrow as AML with NUP98 rearrangement as a result of a translocation t(7;11)(p15;p15) in FISH. The patient's unique haplotype shows a maximum frequency of 0.087% in Caucasian individuals and there is no indication for activating a MUD search. A sister, who shares the maternal haplotype, is being studied for the donation of HSC from a haploidentical donor with full HLA compatibility in GvHD direction. The study of the inheritance of haplotypes is necessary, especially in leukemic subjects who show homozygosity for

HLA haplotype, just as HLA reconfirmation on material other than peripheral or medullary blood. Further studies need to evaluate complete/partial maternal isodisomy of chromosome 6 using SNP array technology.

P71 | Post-transplant chimerism monitoring—Real-time qPCR or STR?

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Analysis of chimerism following allogeneic HSCT plays a crucial role in monitoring engraftment and assessing the risk of relapse, emphasizing the critical need for sensitivity and accuracy in the methods employed. We aimed to evaluate the informativeness and practical utility of the real-time quantitative PCR (qPCR) method for analyzing chimerism levels. We conducted our analysis using three sets of samples: donor-recipient pairs who were routinely monitored following allo-HSCT, samples from EPT schemes, and artificially created chimerism samples covering 0.01%–50% chimerism. The qPCR analysis was performed using GenDx's KMR kits. The samples were also tested by STR (AmpFLSTR Identifiler Plus, Thermo Fisher). Additionally, for the EPT samples, the qPCR results were compared with the EPT consensus results (both overall and separated by the method used). Statistical analysis was performed with SPSSv26 (IBM). Both methods identified at least one informative marker for each donor-recipient pair. The STR showed greater consistency in results across the entire range in both artificial chimerism samples and EPT samples. However, a strong correlation ($r > 0.97$) was observed between the results obtained from the two methods for any of the sample sets, albeit with slight discrepancies within the range of 0%–9.3%. A higher level of discrepancy was found in samples with more than 10% mixed genetic profiles (median 5.9%, range 1.3%–9.3%) compared to samples with less than 10% (median 0.7%, range 0%–2.6%). In 2 samples determined as 100% donor chimerism by the STR, qPCR detected recipient cells—0.04% and 0.07%, respectively. In conclusion, our preliminary results highlight the sensitivity and informativeness of real-time qPCR-based chimerism analysis. Notably, higher discrepancies were observed in samples characterized by significant mixtures of donor and recipient DNA. However, the qPCR appears to be very consistent, limiting the clinical implications of these deviations.

P72 | Donor telomere length and telomerase reverse transcriptase gene polymorphism may affect the outcome of allogeneic hematopoietic stem cell transplantation in children—A preliminary study

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Telomerase reverse transcriptase (TERT) is a catalytic subunit of the telomerase that maintains genome integrity. Its variability may play an important role in leukemogenesis, including hematological malignancies. Telomere length (TL) and two polymorphisms located in the TERT gene were analyzed in 100 pediatric recipients of allogeneic hematopoietic stem cell transplantation (HSCT) and their donors. Patients below the median age (7 years) had longer TL than older transplant recipients ($p = 0.058$). In the group of donor-recipient pairs with negative CMV status before transplantation longer TLs were detected than in other donor-recipient settings ($p = 0.032$). Furthermore, donor TL was found to be associated with the incidence and severity of acute graft-versus-host disease (aGvHD) in recipients who achieved complete chimerism (CC) 100 days after transplantation. Longer donor TLs were associated with milder aGvHD (I grade) than with severe disease (grade II to IV; $p = 0.057$). Additionally, patients and donors were found to significantly differ in the distribution of the TERT rs2853669 alleles. The C allele was more common in HSCT recipients (OR = 1.854, $p = 0.053$). Moreover, the C allele was more frequent in patients who developed aGvHD than in those without any complications after HSCT ($p = 0.002$). The rs2736100 polymorphism was found to be associated with the CC status at day +100 after transplantation. It was more frequently achieved in

patients transplanted with donors carrying the rs2736100 G allele as compared to those transplanted with donors lacking this genetic variant ($p = 0.035$). Our study showed that TL and TERT genetic variation analyzed in donors could be considered as biomarkers associated with the development and severity of aGvHD and the achievement of complete chimerism in children after HSCT. This work was supported by National Science Centre (Poland) project No. 2018/31/B/NZ2/03065.

P73 | Genetic predisposition to hematologic malignancies in patients undergoing allogeneic hematopoietic stem cell transplantation: Case reports

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In recent years the identification of many genes involved in germline predispositions to myeloid neoplasms (MNs) has opened a new scenario in clinical management of patients in terms of prognosis, therapy and allo-HSCT options. We present three cases of HSCT with a positive family history for MNs. Indication for HSCT was determined according to the usual criteria. NGS testing was performed by using a target custom panel for germline conditions predisposing to MNs. GM: M, 36 years, 46, XY, severe aplastic anemia started with thrombocytopenia at 20 years; mother with bone marrow failure, recent diagnosis of pulmonary fibrosis; maternal aunt and grandmother affected by pulmonary fibrosis. Searching for a family donor has identified one HLA-haploidentical brother and 3 maternal HLA-incompatible cousins. NGS showed the germline probably pathogenic variant c.2529C > G p.(Ser843Arg) in exon 9 of TERT gene (OMIM*187270) in heterozygous condition, inherited from the mother and present in HLA-haploidentical brother. From the donor registry two matched unrelated donors (MUD-10/10 HLA) were found. LC: F, 58yo, 46, XX, AML. NGS showed the germline probably pathogenic variant c.175G > T p.(Glu59*) in exon 1 of CEBPA gene (OMIM*620560), in heterozygous condition. The only HLA-haploidentical familiar donor was the daughter, negative for CEBPA variant. Antibody screening of patient showed child-to-mother anti-HLA antibodies. From the donor registry no MUDs were found. DA: M, 52 years, 47, XY, +21/46, XY, AML. Familiar donor HLA typing showed two brothers with a mismatch (MM) in HLA-A for a supposed crossing-over event between HLA-A and C loci in one patient's haplotype. Only one was negative for the germline variant c.544C > T p.(Gln182*) in exon 1 of CEBPA gene (OMIM* 620560) identified in the patient, in heterozygous condition. In conclusion, in presence of family history for MNs, genetic counseling and testing should be recommended for any compatible candidate donors.

P74 | Standardization of evaluation of cytokine gene polymorphisms by next generation sequencing

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The success of hematopoietic stem cell transplantation is marred by conditions such as graft vs host disease, relapse and transplant related mortality. Recently, various cytokine gene polymorphisms have been implicated in predicting HSCT outcomes. Here we sought to standardize a next generation sequencing method to evaluate polymorphisms in cytokine genes. A customized next generation sequencing panel was designed to explore polymorphisms in 13 cytokine related genes using the Illumina Design studio. 40 ng genomic DNA from 90 leukemic patients and unrelated healthy donors was amplified using the custom NGS panel, followed by library preparation (AmpliSeq, Illumina, USA). 1 nanomolar final library was loaded on the MiniSeq system using the MiniSeq Mid output kit (Illumina, USA). FastQ files generated using the DNA Amplicon module were uploaded on BaseSpace variant interpreter (Illumina, USA). The VCF files were used for further analysis on the DRAGEN Amplicon App Version 4.2.4. Complete exonic regions for all the genes studied could be amplified and data was generated for all samples included. The inclusions for the analysis were only the SNPs present in the dbSNP and those present in COSMIC database were excluded. The numbers of variants observed for every gene are as follows: IL-6: 2, TGF-Beta: 3, IL-17A: 2, IL-1A: 6, IL-13: 4, IL-15: 6, IL-12A: 7, CTLA-4: 1, IL-10: 2, IL-2: 2, IFNG-1. No variants were identified in TNF-alpha and IL-4

genes. Further correlation with the role of these genes in conferring susceptibility/protection from various leukemias and correlation with transplant outcomes is yet to be accomplished. We have successfully designed and standardized a customized next generation sequencing panel for the evaluation of cytokine gene polymorphisms. A complete understanding of variants in these genes will help in population based studies and to predict transplant outcomes.

P75 | Improved haploidentical donor matching including low expression HLA loci in the immunogenetic analysis using next-generation sequencing

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Low expression HLA loci (LEL), HLA-DRB3/4/5, HLA-DQA1, HLA-DPA1 are being more commonly typed thanks to development of methods such as Next-Generation Sequencing (NGS). Typing LEL could be helpful to determine the haplotype segregation in difficult cases (no family members available). Additionally, LEL typing by NGS could increase the probability of determining the hereditary haplotypes in haploidentical recipient-donor pairs. In our study, we examined 11 HLA loci (NGSgo®-MX11-3; GenDx) in 6 donor-recipient pairs with acute myeloid leukemias (AML) that underwent a haploidentical hematopoietic stem cell transplantation (haplo-HSCT) between 2018 and 2023. We conducted a retrospective immunogenetic analysis in which we enlarged the analyzed HLA loci from 5 up to 11. Thanks to NGS we determined the (mis)matches up to the 4th field reporting for HLA typing. Permissive HLA-DPB1 mismatch was defined in all analyzed haploidentical pairs. Thanks to the HLA results we obtained a greater range of HLA matching, including LEL, therefore we identified a bigger number of the introduced mismatches. To demonstrate a correlation between LEL mismatches and the haploidentical transplant outcome more research needs to be conducted. Establishing the permissive HLA-DPB1 mismatch is crucial for a greater haploidentical transplant outcome which we concluded in ultra-high resolution by NGS. Overall typing HLA by NGS gives us ultra-high typing resolution compared to other HLA typing methods. The immunogenetic analysis is more extensive thanks to the wider range of the analyzed HLA loci including analysis of donor specific antibodies (DSA) against LEL. Furthermore, we can determine the inherited haplotypes in haploidentical donors with greater accuracy. In conclusion, typing 11 HLA loci by NGS, including LEL, should become a standard for haploidentical donor matching.

P76 | Detection and monitoring of donor-specific antibodies in a haploidentical stem cell transplant recipient: a case report

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Allogeneic hematopoietic stem cell transplantation (alloHSCT) is a potentially curative treatment in patients with myelodysplastic syndrome (MDS). For those patients who lack an HLA-identical sibling or matched unrelated donor, the use of alternative donor strategies, including mismatched unrelated donors or haploidentical family members, has significantly increased the possibility of alloHSCT. However, this may cause donor-specific antibodies (DSAs), IgG alloantibodies against mismatched donor HLA molecules, to occur and to induce graft failure. The purpose of this report is to present our first laboratory experience with DSA monitoring as a hallmark of desensitization strategy in a haploidentical HSCT recipient. A 49-year-old woman with MDS was diagnosed in November 2020. In March 2023, she received 4 cycles of Azacitidine (75 mg/m^2). In December 2023, the patient underwent haplo-HSCT. The donor was a daughter mismatched for B*27:02, DRB1*16:01 and DQB1*05:02 HLA alleles. The following sensitization events were described in the patient: 2 pregnancies and multiple transfusions. DSA was expressed as mean fluorescence intensity (MFI) and was detected using Luminex-based technology. The detected pre-transplant specific antibodies were anti-HLA-B27 (MFI = 14,253),

anti-HLA-DR16 (5784) and anti-HLA-DQ5 (15003). The patient underwent desensitization with pre-transplant plasmapheresis and rituximab 357 mg/m^2 with IVIG 1 g/kg bw. We found significant benefits only in reduction of anti-HLA-B27 (8115), anti-HLA-DR16 (3849) DSAs. Post-transplant DSA analysis showed increased mean MFI values of these antibodies. Because of the lack of hematological reconstitution, the patient is currently being prepared for another haplo-HSCT from her son as a donor. Laboratory tests did not reveal any presence of DSA against mismatched donor HLA alleles. Haploidentical HSCT in DSA-positive patients increases the risk of graft failure.

P77 | Analysis of the significance of donor chimerism at +14 days after haploidentical hematopoietic stem cell transplantation

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Chimerism analysis is performed to monitor graft engraftment or rejection. Tracking the status of engraftment in the early stages after transplantation will allow timely prediction of transplant failure in the post-transplant period and significantly reduce the risks to life. The purpose of this study is to assess the significance of donor chimerism at +14 days in haplo-HSCT. The studies were conducted with the participation of 20 patients with oncohematological diseases after haplo-HSCT at the Research and Production Center of Transfusiology. Chimerism was determined by PCR-STR in peripheral blood on the 14th and 28th days after HSCT. AmpFlSTR™ Identifiler™ Plus PCR Amplification Kit was used for amplification of STR markers, the separation of PCR products was performed using capillary electrophoresis on a 3500 Genetic Analyzer. Identification of alleles was carried out in the Chimer Marker software. Informative alleles were identified by STR-PCR for all donor/recipient pairs. The method showed 100 percent informative value. The median indicators of donor chimerism on day 14 was 86% (18%–98%), whereas on day 28 this indicator was 98% (85%–100%). All 20 couples had mixed chimerism on day +14 (5%–99%). On the 28th day, 100% donor chimerism was noted in 2 (10%) cases. Complete donor chimerism on day +28 (99%–100%) was observed in 6 (30%) of 20 haplo-HSCT. Temporary mixed chimerism was observed in 12 (60%) cases. Increasing in 2 (10%) cases. In this study, the determination of the level of chimerism on day +14 did not show significance in haplo-HSCT. The data from these studies are individual and depend on the characteristics of the patient and the donor. Solving the issue of 100% graft survival is the main goal of today's medicine.

P78 | Changes of the immunogenic profile of an acute myeloid leukemia relapsed patient with partial loss of heterozygosity in HLA genes after haploidentical transplantation of hematopoietic stem cells

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Loss of heterozygosity (HLA-LOH) is considered as one of the causes of leukemia relapse after haploidentical alloHSCT. Impact of donor-specific anti-HLA (DSA) antibodies in graft rejection in solid organ transplantation is well known, nevertheless, interconnection between anti-HLA antibodies in alloHSCT remains poorly studied. A female patient, 36 years, was diagnosed with acute myeloid leukemia in 2022 and received alloHSCT from a haploidentical related donor. After 1 year of transplantation the patient relapsed and after repeated high resolution HLA-typing we indicated partial LOH of HLA class I and II in peripheral blood, bone marrow and a blast subpopulation. In October 2023 the patient received second haploidentical related alloHSCT, but without matching between HLA type of the blast clone of peripheral blood/bone marrow and HLA type of donor. At the same time, we provided detection of anti-HLA antibodies after a second alloHSCT. On day +42 we detected the presence of anti-HLA allele specific antibodies to the graft in both HLA classes: A*24:02 (MFI = 701), B*52:01 (MFI = 500), DRB1*12:01 (MFI = 260), DRB1*13:03 (MFI = 218) and DQB1*03:01 (MFI = 8317). It should be noticed, that presented above haplotype, except B*52:01, was detected by HLA typing before first haploidentical HSCT as patient's own. On day +54 we observed disappearance of anti-B

and DRB1 antibodies, and partial reduction of anti A*24:02 (MFI = 180) and DQB1*03:01 (MFI = 1145). Moreover, on +89-day anti-DQB1*03:01 antibodies were not detected, while MFI of anti-A*24:02 increased to 401. Taking into account the LOH established before the second transplantation, we cannot say with certainty that identified antibodies are DSA or self-specific antibodies. At the same time, the presence of these antibodies in the patient's bloodstream can potentially trigger the development of GVHD, since they have specificity for HLA presented in patient's peripheral organs.

P79 | Partial loss of heterozygosity in HLA genes in patient with relapsed acute lymphoblastic leukemia

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Loss of heterozygosity in HLA genes (LOH) provides cancer cells with an effective immune evasion mechanism. Such changes cause a blast disruption of the maturation and proliferation, which leads to a blast/normal cells imbalance in the bloodstream and promotes the bone marrow cells' differentiation and associated diseases. Partial loss of heterozygosity in both HLA classes was detected in a patient with relapsed acute lymphoblastic leukemia and a high level of blasts in peripheral blood (more than 80%). The study was carried out using high-resolution HLA molecular typing (NGS) AllTypetm FASTplex, which included sequencing of polymorphic regions of HLA genes. After analyzing the results, only a limited number of heterozygotes were found in the alleles that correspond to the mother's haplotype. About 10% of diploidy was determined in exons 2 and 3 of HLA-A, in exons 3 and 5 of HLA-B and in exons 2 and 3 of HLA-DRB1. At the same time, the father's haplotype remained unchanged. The clinical case clearly demonstrates the need for careful verification of the results of molecular genetic studies in recipients, in particular with bone marrow diseases. This is explained by the fact that during a blast crisis, the overwhelming majority of cells are found with deviations in the genotype, which significantly complicates the determination of the patient's haplotype. To obtain accurate genotyping results, it is necessary to the patient into the stable remission and conduct additional studies using somatic cells, such as buccal epithelium. Note that even when we use the most modern approaches, there are some limitations associated both with the technical characteristics of analytical methods, and with the condition of the patient himself as long as the specifics of treatment of a particular pathology. Incorrect haplotype determination may cause further complications or evoke a dramatically negative reaction to the graft.

P80 | Mismatched unrelated donors for hematopoietic stem cell transplantation in children with inborn errors of immunity: selection criteria and the Newcastle experience

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Hematopoietic stem cell transplantation (HSCT) is standard-of-care for children with many inborn errors of immunity (IEI). One of two UK centers designated to perform these transplants is in the Great North Children's Hospital, Newcastle upon Tyne. HLA typing of patients, relatives and unrelated donors (UD) and selection of UD for verification typing are performed in H&I Newcastle. Donors can be HLA matched family donors (MFD) or volunteer unrelated donors (VUD) who are a 10/10 match for the patient but approximately one third of patients do not have a suitably matched donor. In the last 8 years, there has been increased use of parents as haploidentical donors. PBSC are T-depleted using a CD3+ T cell receptor (TCR) αβ/CD19+ protocol to minimize the risk of graft versus host disease (GvHD). Outcomes are equivalent to those with an MFD or 10/10 VUD in children aged under 5 years. In some cases, parents are not suitable donors, for instance due to carrier status, infections or other medical conditions. In these cases, a mismatched UD (MMUD) is sought. Donors who may share an HLA haplotype with the patient are preferred, to mimic the situation with a parental haploidentical donor. A transplant in which mismatches are in the host versus graft direction only is performed without T depletion. When GvH or bidirectional mismatches are present at HLA-A, -B, -C, -DRB1 or -DQB1, CD3 + αβ/CD19+

depletion is used. We report a series of 12 transplants in 11 patients aged 1–14 years performed from November 2015. Eleven transplants were T depleted and one had HVG only mismatches. There were mismatches at 1–3 HLA loci (A, B, C, DRB1, DQB1). One patient died following a transplant due to infections and pre-existing organ damage. Selection of a suitable MMUD, combined with an effective T depletion technique, can result in successful transplant outcomes for children with IEI and no MFD or 10/10 VUD.

IMMUNOGENETICS IN ORGAN TRANSPLANTATION

P81 | On the road to epitope matches and mismatches—with epiTOol

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Following transplantation, donor-specific HLA antibodies might be produced eventually causing humoral rejection and early graft loss. We already demonstrated the benefit of high resolution HLA typing for a detailed determination of these antibodies. Here we present the potential use of 'epiTOol', an interactive analytics tool for all-in-one processing of HLA typing and antibody data. Functionality is presented elsewhere in the same meeting. We analyzed a cohort of 108 living transplants regarding their antibody verified epitope matches (EM) as well as mismatches (EMM). The recipients were 59% related and out of them 43% 1st degree related to the donor. For HLA class I, we found unique epitopes for HLA-A ($n = 33$), -B ($n = 28$) and -C ($n = 20$), with 144KR being the most frequently mismatched epitope ($n = 29$) in our cohort. For HLA class II, the highest number of different epitopes is observed for HLA-DR ($n = 37$), followed by -DQ ($n = 28$) and -DP ($n = 13$). The number of antibody verified total EMM ranged from 0 (6 pairs) to 50 (1 pair). The largest number of EMM was observed for the DR locus ($n = 522$), followed by A ($n = 440$), DQ ($n = 418$), B ($n = 323$), C ($n = 217$) and DP ($n = 213$). The number of EM is not proportional to the number of EMM. The DR locus has the most matched epitopes $n = 1736$, followed by DQ ($n = 1307$), A ($n = 1183$), B ($n = 888$), C ($n = 798$) and DP ($n = 663$). Finally, via epiTOol we mapped the "antibody epitopes" (donor specific epitopes). Interestingly, 33% ($n = 36$) of the recipients have not formed anti-HLA antibodies following kidney transplantation although up to 40 epitope mismatches were present. In contrast, recipients with only nine EMM had produced antibodies towards the donor. Defining epitopes is helpful for understanding the humoral response but it is not a matter of numbers but a matter of quality and could be a useful tool for donor selection in the future.

P82 | Clinical relevance of isolated preformed HLA-DP donor specific antibodies on the outcome of kidney transplantation

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Donor specific HLA alloantibodies (DSA) are a well-known cause of antibody mediated rejection (AMR) in kidney transplantation. However, the clinical relevance of HLA-DP donor specific antibodies (DP-DSA) is controversial due to conflicting reports. We retrospectively evaluated the outcome of patients with isolated preformed DP-DSA receiving a kidney transplant between January 2019 and December 2023 in our center. LIFECODES LifeScreen DeLuxe and LSATM (Immucor) were used for screening and identification of anti-HLA antibodies, respectively. Crossmatches by CDC with T and B cells were negative in all cases. Recipient and donor HLA-DP genotyping was performed retrospectively. Isolated preformed DP-DSA with no other DSA were detected in 12 (1.5%) out of 804 recipients. Nine patients (77%) had received a previous transplant and three women were allosensitized during pregnancy. AMR according to Banff criteria was demonstrated in 3 patients and C4d positive staining without histological evidence of humoral rejection was observed in 2 other patients, accounting for 42% of all patients. All of them were retransplants, showed decline of graft function and were early treated according to local protocol with pulsed methylprednisolone, plasmapheresis, rituximab and intravenous immunoglobulin. Median fluorescence intensity (MFI) of preformed DP-DSA was generally high but, interestingly, two patients with pretransplant low DP-DSA levels experienced a robust recall memory response after transplantation with subsequent generation of complement binding DP-DSA.

Several highly exposed eplets were involved in DP-DSA formation. In conclusion, although the prevalence of isolated DP-DSA is low, there is a significant risk of developing AMR in retransplant patients. Thus, repeated eplet mismatch should be considered as a risk factor for AMR in previously alloimmunized patients and a close monitoring after transplantation should be recommended.

P83 | Interference of cold agglutinins and/or cryoglobulins in antibody diagnostics for kidney transplantation: A case study

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A 59-year old female with anti-glomerular basement membrane induced renal failure was admitted to our kidney transplant waitlist. Despite few immunizing events, no class I and II anti- HLA immunoglobulins (IgG and IgM) were detected in sera from this patient (LABScreen Mixed and Single Antigens). Surprisingly, these sera induced strong lytic reactions in the complement-dependent-cytotoxicity-screening assay (CDC-screening) using a variable panel of HLA-typed healthy donor PBMC's (PRA 100% −DTT and 95% +DTT). To investigate the potential interference of autoantibodies, we performed autologous CDC-crossmatch assays (CDC-XMs), which were positive without DTT, but negative with DTT. In allogenic IgG-detecting flow cytometry-based crossmatch assays (FC-XMs) with different PBMC's and patient sera, positive scores (>3) were obtained for B cells. Allogenic IgM-detecting FC-XMs were positive for B and T cells. In the patient's medical records, a history of cold agglutinins (CAs) and monoclonal IgM-kappa cryoglobulins (<0.04 g/L) were observed. CAs are mostly IgM molecules that bind to antigens present on red blood cells (RBCs) under hypothermic conditions and cause hemolytic anemia. Cryoglobulins are antibodies that precipitate under hypothermia and can, among other things, cause vasculitis. Therefore, we speculated that these cold-sensitive antibodies may contribute to our laboratory findings, for which allogenic CDC-XMs were repeated at 20°C (standard) and 37°C. In favor of this hypothesis, CDC-XM results remained positive at 20°C and turned negative at 37°C (DTT-independent). When performing these tests using cryoglobulin-only sera, no consistent interference was observed. We conclude that transplant immunologists should be alert when dealing with a patient that has CAs, as these autoantibodies may interfere with the interpretation of CDC- and FC-XMs. This interference can possibly be overcome by performing these assays at 37°C.

P84 | High levels of BCMA transcript expression prior to transplantation, increased plasmablast, lymphocyte B cell class-switched levels and viral loading are associated with early CMV reactivation in renal recipients

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CMV infection is the most frequent infection episode in kidney transplant (KT) recipients. Reactivation usually occurs in the first 3 months after transplantation and is associated with higher cellular and/or antibody-mediated rejection rates and poorer graft performance. CMV induces the expression of BAFF (a cytokine involved in the homeostasis of B cells), which communicates signals for survival and growth to B cells and virus-specific plasma cells via the R-BAFF, TACI, and BCMA receptors. These molecules of the BAFF system have also been suggested as biomarkers for the development of alloantibodies and graft dysfunction. This prospective study included 30 CMV-IgG seropositive KT recipients. The expression levels of the genes BAFF-R, transmembrane activator and CAML interactor (TACI), and B cell maturation antigen (BCMA) in peripheral blood leukocytes (PBL) pre-KT were determined using qPCR. qPCR was also used to monitor CMV reactivation in the first 3 months following KT. The remainder of the KT recipients were classified as CMV- reactivation, and those with more than 500 copies/mL in at least one sample were classified as CMV+ reactivation. There were no discernible variations in the BAFF-R and TACI transcript expression levels. In the CMV+ group, we examined the relationship between the transcript levels and peak viremia. Peak viremia levels and BCMA transcript levels showed a strong correlation. BAFF-R and TACI

expressions showed no measurable differences. In patients with early CMV reactivation, high BCMA receptor expression was associated with increased plasmablast, lymphocyte B cell class-switched levels (LBCS), and viral load. Our findings demonstrate that pre-KT BCMA transcript levels increased in KT recipients with early CMV reactivation. These transcript levels positively correlate with peak viremia and weakly with plasmablast and LBCS levels in PBLs.

P85 | Imlifidase for Kidney Transplantation of Highly Sensitized Patients with a Positive Crossmatch: The French Consensus Guidelines

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Imlifidase recently received early access authorization for highly sensitized adult kidney transplant candidates with a positive crossmatch against an ABO-compatible deceased donor. The French Society of Transplantation (SFT), the French-speaking Society of Nephrology, Dialysis and Transplantation (SFNDT) and the French-speaking Society of Histocompatibility and Immunogenetics (SFHI) convened an expert working group of 12 transplant nephrologists and 4 immunologists to propose French consensus guidelines aimed at standardizing patient selection criteria, antibody characteristics, associated treatments, and follow-up protocols. This initiative is part of an international effort to analyze properly the benefits and tolerance of this new costly treatment in real-life. The use of Imlifidase should be reserved for highly sensitized patients unlikely to be transplanted, after exhausting all available strategies first. Eligible patients must meet specific screening criteria: cPRA ≥98%, ≤ 65-year of age, ≥ 3 years on the waiting list, and a low risk of biopsy-related complications. The final decision to use Imlifidase will hinge on two criteria. Firstly, the results of a virtual crossmatch on recent serum, which shall show an MFI for the immunodominant donor-specific antibodies (DSA) HLA-A, -B, -DRB1 or -DQB1 > 6000 yet not exceeding 5000 after 1:10 dilution. Secondly, the complement-dependent cytotoxicity crossmatch at 4 or 6 h post-Imlifidase must be negative. Patients undergoing Imlifidase treatment will receive an immunosuppressive regimen based on steroids, rATG, high dose IVIg, rituximab, tacrolimus and mycophenolic acid. Frequent post-transplant testing for DSA and systematic surveillance kidney biopsies are strongly recommended to monitor post-transplant DSA rebound and detect subclinical rejection. These French guidelines aim to provide valuable insights into refining the use and implementation of Imlifidase, a major breakthrough in kidney transplantation.

P86 | Effect of peri-transplantation circumstances on the amelioration of cellular immunity following kidney transplantation

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Adaptive immunity is highly compromised in end-stage renal disease patients. Kidney transplantation is expected to improve immune cell counts and function. The aim of this study was to determine the effect of clinical factors on the recovery of immune cell populations after successful kidney transplantation. We evaluated counts of total, CD4+, CD8+, regulatory T cells (Tregs) and natural killer (NK) cells in 112 patients before and 12 months after successful kidney transplantation. Changes in cell populations were associated with recipient age, hemodialysis vintage (HDV) and cold ischemia time (CIT). Recipient age at transplantation negatively affected recovery of total T cells one-year post-transplantation ($r = -0.3$, $p = 0.002$). CD4+ T cells and Tregs were significantly affected, ($r = -0.34$, $p < 0.001$, $r = -0.31$,

$p = 0.001$ for CD4+ T cells and Tregs, respectively) but not CD8+ T cells or NK. The effect of both CIT and HDV on the count of T cells was negative ($r = -0.22$, $p = 0.02$ and $p = -0.29$, $r = 0.002$ for CIT and HDV, respectively) 1 year post-transplantation, yet only CD4+ T cells were negatively correlated with both factors ($r = -0.26$, $p = 0.009$ and $r = -0.32$, $p = 0.001$), whereas CD8+ T cells were only affected by CIT ($r = -0.19$, $p = 0.04$) and Tregs and NK were not affected by CIT and HDV. In multivariate analysis, age was the only factor that significantly predicted total T cell and CD4+ T cell counts. Finally, differences in T cell subsets were observed between recipients from living or deceased donors 1 year post-transplantation [T cells: 2300 (1600–2900) versus 1644 (1300–2100) cells/μL, $p = 0.001$, CD4+ T cells: 997 (815–1493) versus 719 (527–961) cells/μL, $p = 0.001$, CD8+ T cells 650 (463–947) versus 476 (366–659) cells/μL $p = 0.007$, Tregs 42 (24–58) versus 31 (21–41) cells/μL, $p = 0.04$]. Recipient age, CIT and HDV have significant effects on the recovery of lymphocyte populations 1 year after successful kidney transplantation. Living donor transplant recipients show better recovery of all lymphocyte populations.

P87 | Detection of donor-derived cell-free DNA in sequential kidney transplanted patients

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Many transplant centers worldwide are adopting routine donor-derived cell-free DNA (dd-cfDNA) testing to monitor graft damage in kidney transplant patients. However, the interpretation becomes complex in the context of multiple sequential kidney transplantations, posing methodological challenges in accurately assigning the detected dd-cfDNA to an individual donor. We explored the precision of a novel NGS based dd-cfDNA assay (One Lambda Devyser Accept cfDNA) in correctly pinpointing the origin of detected dd-cfDNA in patients with more than one transplanted kidney. This investigation involved both artificially generated samples and clinical samples from 31 patients who had undergone two sequential kidney transplantations. The assay demonstrated excellent accuracy in quantification and precise assignment of dd-cfDNA in our artificially generated chimeric sample experiments, covering a clinically relevant quantitative spectrum. In clinical samples, %dd-cfDNA from the initial transplanted (non-functioning) graft was detected in 20% of analyzed patients. The detected dd-cfDNA from the first graft consistently ranged from 0.1% to 0.6% and exhibited temporal fluctuations in patients with sequential sample analyses. This marks the first study employing an assay to identify the origin of dd-cfDNA in the context of multiple kidney transplants. Our findings reveal that a noteworthy proportion of transplant recipients exhibit detectable dd-cfDNA from the initial donor graft. Moreover, the detected levels fall within a range that could impact clinical decision-making.

P88 | Role of HLA matching and donor specific antibody development in long-term survival, acute rejection and cardiac allograft vasculopathy

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Although there are different data supporting benefits of HLA matching in kidney transplantation, its role in heart transplantation is still unclear. HLA mismatch (MM) between donor and recipient can lead to the development of donor-specific antibodies (DSA) which produces negative events on the outcome of heart transplantation. Moreover, DSAs are involved in the development of antibody-mediated rejection (AMR) and are associated with an increase in cardiac allograft vasculopathy (CAV). In this study we retrospectively analyzed the influence of HLA matching and anti-HLA antibodies on overall survival, AMR and CAV in heart transplantation. For this retrospective study we recruited heart transplanted patients at the Cardiac Transplantation Centre of Naples between 2000 and 2019. Among the 155 heart

transplant patients, the mean number of HLA-A, -B, -DR MM (0–6) between donor and recipient was 4.5 ± 1.1. The results show a negative association between MM HLA-DR and survival ($p = 0.01$). Comparison of patients with 0–1 MM at each locus to all others with 2 MM, for both HLA class I and class II, has not showed significant differences in the development of CAV. Our analysis detected DSA in 38.1% of patients. The production of de novo DSA reveals that there is not an influence on survival ($p = 0.72$) and/or AMR ($p = 0.39$). Instead, there is an association between the production of DSA class II and the probability of CAV development ($p = 0.03$). Mean fluorescence intensity (MFI) values were significantly higher in CAV-positive patients that CAV-negative patients ($p = 0.02$). Prospective studies are needed to evaluate HLA class II matching as an additional parameter for heart allocation, especially considering the increment of waiting list time.

P89 | Correlation between anti-angiotensin II receptor 1 and anti-endothelin type A receptor 1 in kidney transplanted pediatric patients

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Donor-specific anti-HLA antibodies (anti-HLA DSA) are a significant risk factor for kidney transplant failure. However, recent studies have shown that non-HLA antibodies, such as anti-angiotensin II receptor 1 (anti-AT1R) and anti-endothelin type A receptor 1 (anti-ETAR1), might induce an antibody-mediated rejection. In particular, interaction between endothelin type A receptor 1 (ETRA-1) and anti-ETAR-1 is responsible for vasoconstriction, cell proliferation and inflammatory processes, potentially leading to hypertension and graft failure. In the present study, we determined the presence of anti-ETAR1 and anti-AT1R antibodies in 40 patients with kidney transplantation at the Bambino Gesù Children's Hospital, in Rome. Results showed anti-ETAR1 in 21/40 (52%), anti-AT1R in 18/40 (45%) and anti-HLA DSA in 14/40 (35%) of patients. We observed an anti-ETAR1–anti-AT1R concordance in 37/40 patients (92.5%); only 3 patients (7.5%) showed negativity for anti-AT1R and positivity for anti-ETAR1. Five patients (13%) were positive for both class I DSA and anti-ETAR1, whereas 10 (25%) patients were positive for both class II DSA and anti-ETAR1. No significant correlation between anti-ETAR1 and acute rejection emerged. Both groups, anti-ETAR1 positive and negative, presented 13 acute rejections. No difference in the number of hypertensive patients was reported in the two groups. These preliminary data, indicate that more than 50% of pediatric transplanted patients are positive for anti-ETAR1 and anti-AT1R antibodies and that there is a high concordance between anti-ETAR and anti-AT1R antibodies. Conversely, little correlation between anti-ETAR and anti-HLA DSA was observed. In conclusion, anti-ETAR1 and anti-AT1R antibodies could have a pathogenetic role in humoral rejection anti-HLA negative and their detection in the follow up may be relevant.

P90 | Acute allograft dysfunction and glomerular microangiopathy in kidney transplant recipients in absence of donor specific anti-HLA antibodies: a case series

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The presence of antibodies to self-antigens (non-HLA antibodies) may be associated with antibody-mediated rejection (AMR) and allograft dysfunction. In this study we examined the association between non-HLA antibodies and acute allograft injury in kidney transplant recipients (KTR). We present a case series of six KTR who developed acute allograft dysfunction and histologic features of glomerular microangiopathic changes in absence of donor specific anti-HLA antibodies (DSA). Non-HLA antibodies were identified against third laminin-like globular (LG3), vimentin, collagen IV, fibronectin, angiotensinogen, agrin, tubulin alpha (TUBA1B), protein tyrosine phosphatase-like N (PTPRN), eukaryotic translation initiation factor 2A (EIF2A), protein kinase C zeta type (PRKCZ), and lamin B1. Non-HLA antibodies may mediate acute allograft injury through activation of complement cascade, endothelial cell activation, and recruitment of inflammatory cells. Our findings suggest a complex interplay, emphasizing the need for future trials and standardization of therapies to elucidate the consequences of these antibodies.

P91 | Biomarkers of Innate Immunity and Immunological Susceptibility to Viral Infection in Patients with Alcoholic Cirrhosis

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The harmful effect of alcohol on the immune system may be due to both a direct action of the alcohol or its metabolites on immune cells as an indirect action modifying the different mechanisms of intercellular interaction. The interplay between stimulatory (aKIR) and inhibitory (iKIR) natural killer (NK) cell receptors and their corresponding HLA ligands influences the outcome of virus infection. The aim was to analyze the influence of the KIR/HLA pair genetic profile in male alcoholic cirrhosis (AC) patients with and without viral infections to find susceptibility biomarkers that can help establish the risks and prevent viral infections. A total of 281 male AC patients were analyzed. The sociodemographic characteristics, viral hepatitis C (HCV), hepatitis B (HBV), and cytomegalovirus (CMV) infections were analyzed. Genomic DNA was extracted, and genetic the KIR/HLA profiles were investigated. A total of 6 KIR genes and their corresponding ligands (HLA-C) were analyzed. Patients were grouped into two groups: with and without associated viral infection. A statistically significant increase in the combination of KIR2DL2 +/C1C1 was observed in male AC patients with viral infection compared to those without viral infection (45.9% vs 24.5%, $p = 0.021$). The analysis of KIR2DL3+/C1+ showed a high frequency comparing healthy controls and male AC patients without virus infection (85% vs 76.4%; $p = 0.026$). The analysis of KIR2DL3+/C2C2 frequency showed a statistically significant increase comparing male AC patients without viral infection and healthy controls (23.6% vs 15%; $p = 0.026$). The genetic KIR2DL2+/C2C2 profiles may play a significant role in determining the vulnerability of male AC patients to viral infections, providing valuable insights for future research and potential therapeutic interventions.

P92 | Non-HLA antibodies in highly sensitized recipients on the kidney waiting list

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Non-HLA antibodies, whether they act independently or in synergy with HLA antibodies, are associated with antibody mediated rejection and poor graft survival. Detection of non-HLA antibodies before transplantation additionally points to recipients with increased immunologic risk for acute rejection and recipients who should be treated either prior to transplantation or in post-transplant period to avoid graft injury. The aim of this study was to assess the incidence of non-HLA antibodies among HLA sensitized recipients. The study group consisted of 33 highly sensitized (vPRA>95%) recipients on the waiting list for kidney transplantation. Using one of the two currently available non-HLA antibody assays, which allows the direct simultaneous detection of IgG antibodies to 65 non-HLA antigens (LIFECODES, Immucor), we analyzed the presence and specificities of non-HLA antibodies in pre-transplant sera samples. Out of 33 recipients, 27 were positive for HLA class I and class II antibodies, 3 patients for HLA class I antibodies only and 3 for HLA class II antibodies only. The presence of at least one of non-HLA antibodies was detected in 31/33 (93.9%) recipients. Ten sera samples were positive for 6 to 22 different non-HLA antibodies, 23 sera were positive for 1 to 5 non-HLA antibodies. The mean MFI value for positive reactions was 8513 (range 1949–17,836). A total of 43 different non-HLA antibody specificities were detected. The positivity was most frequently observed for ENO1 (25.6%) and STAT6 (16.3%) followed by GSTT1, IFNG, PLA2R1 and PRKCZ (13.9%, each).In conclusion, the presence of non-HLA antibodies was observed at a very high frequency in HLA highly sensitized recipients. Therefore, it could be of benefit for their post-transplant follow up to introduce non-HLA antibody screening prior to transplantation and as protocolar monitoring test in the post-transplant period.

P93 | Deceased kidney donor virtual crossmatch introduction in north Italian transplant program (NITp) area

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Solid phase Luminex xMAP technology based tests allow sera reactivity studies against individual HLA antigens, alleles and epitopes at the same time. We now can perform complete HLA typing in a time compatible with organ allocation in an emergency. This kind of evolution has stimulated many laboratories to take the path of virtual crossmatch (vxm). In the NITp laboratory from 2017 donor typing is performed for all HLA class I and II HLA loci and from 2020 the immunological profile of patients is fully studied with Luminex xMAP technology. We retrospectively find that in about 2.5% of prospective crossmatches an unexpected positivity was obtained in patients with PRA Luminex 0%. DTT negativized all of these XMs, showing the presence of non HLA IGM antibodies interfering with XM result. Based on these premises, since January 2022 we applied a new policy of XM that includes vxm for all patients at first transplant, studied in Luminex on at least two sera, the most recent of which are no older than 3 months and with vPRA 0%, paying the utmost attention to any immunizing event of recent months. In these cases, the organ is assigned by omitting the prospective real XM, replacing the retrospective crossmatch with the Luminex study of the day of transplant serum. From January 2022 to June 2023, over 5000 XM kidney transplants were performed, 40% of which were performed exclusively in virtual mode. This activity resulted in 1147 kidney transplants. No unexpected positivity was found in the transplant basal sera studied after negative vxm. From this data we conclude that vxm applied to patients non-immunized, at the first transplant and with at least two recent sera studied is safe and allows to reduce the time of immunological evaluation of the donor/patient couple with potential savings of time of kidney cold ischemia. In parallel with this, there is a considerable saving of laboratory tests allowing greater attention to the most immunologically complicated cases.

P94 | A personalized delisting strategy enables successful kidney transplantation in highly sensitized patients with preformed donor-specific anti-HLA antibodies

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This study explores kidney transplant outcomes in highly sensitized patients who have undergone transplantation after a personalized delisting strategy. The aim of this strategy was to facilitate transplantation with preformed donor-specific antibodies (preDSA) while minimizing acute antibody-mediated rejection (aAMR) risk. Fifty-three highly sensitized recipients with preDSA underwent a kidney transplant after delisting prohibited HLA antigens. Our stepwise delisting strategy consisted initially in allowing low-intensity antibodies (<5000 MFI) against class I HLA. If deemed insufficient, higher MFI antibodies and/or anti-HLA class II were allowed. Complement-fixing antibodies (C1q+) were consistently prohibited. No pre- or immediate post-transplant desensitization therapy was carried out. Comparison cohorts included 53 sensitized recipients without DSA (SwoDSA) and 53 non-sensitized (NS) patients. Rejection rates were similar among preDSA, SwoDSA, and NS groups (16%, 8% and 11%, respectively; $p = 0.46$). However, aAMR was higher in preDSA group (12%, 4%, and 2%, respectively; $p = 0.073$), especially increased in patients transplanted after high-risk delisting, with DSA of MFI > 5000 and directed against HLA class II. The highest MFI DSA were against HLA-DP (Median: 10796 MFI), with 50% of preDSA aAMR cases due to anti-DP antibodies ($n = 3$). Graft survival rates at 1, 3, and 5 years in preDSA group were 94%, 85%, and 61%, comparable to SwoDSA 94%, 75%, and 70% ($p = 0.69$), being significantly higher in the NS group (0.002). Five-year recipient survival rate was 89%, comparable to SwoDSA and NS groups ($p = 0.79$). In conclusion, a personalized delisting strategy enables safe kidney transplant in highly sensitized patients with preDSA, with a slight increase in aAMR and comparable graft and patient survivals to non-DSA cohorts.

P95 | Imlifidase desensitization in highly-HLA sensitized patients with positive cross-match: First experience in Parma

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Imlifidase is a novel enzyme that cleaves all human subclasses of IgG and has therapeutic potential for desensitization of highly-HLA sensitized patients; Imlifidase treatment allows kidney transplantation from a cadaveric donor in patients with DSAs and positive cross-match. In the Parma kidney transplant center, the second Italian transplant was performed in highly sensitized (cPRA >99%, class I) patients after treatment with Imlifidase. After HLA antibody delisting (by hierarchical clustering/K-means clustering analysis and sera dilution) a 46-year-old deceased donor against whom the patient had immunodominant DSA B*38:01 with an MFI value of 6000 was selected (Single Antigen, OneLambda). Historical and current sera were analyzed by cytotoxic (CDC-XM) and flow cytometric (FC-XM) cross-matching. CDC-XM was negative on T lymphocytes and weak positive on B lymphocytes only on peak historical serum, FC-XM was positive on both T and B lymphocytes with all sera (respectively 567 and 524 median channel shift in current serum). The FC-XM performed between 2 and 4 h after Imlifidase Infusion has become negative (on T and B cells) and all HLA antibody (including immunodominant DSA) have reached MFI values below 500. After kidney transplant the DSA levels were monitored daily and antibody rebound was observed on day 7 after transplant (peaked at day 10 with DSA MFI 21000). On the 24th day MFI values of HLA antibody decreased until they returned to pre-transplant levels (MFI < 7500). The patient was discharged on day 30 with stable creatinine 1.6 mg/dL but with positive C4d subclinical rejection. The use of Imlifidase in positive crossmatch transplantation requires the support of a histocompatibility laboratory with high experience in FC-XM and in pre- and post-transplant antibody monitoring.

P96 | Case report: simultaneous combined liver-kidney transplant. Focus on immunological assessment

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The protective effect of the liver on the kidney in combined allograft in HLA sensitized recipients is well known, but some immunological aspects are still controversial, namely antibody titer, HLA class I vs II and DSA vs non-DSA. We present a case of successful simultaneous combined liver-kidney transplant in a highly immunized patient, with strong DSA vs donor. A 46 year old woman was a candidate to a combined allograft for end-stage liver and kidney polycystic disease. Immunological profile showed HLA-I cPRA 99% with immunodominant anti HLA-A2 (MFI > 25,000 in neat and 1:16 diluted serum) due to 2 pregnancies. The epitope analysis was consistent with A2 as immunogenic allele, with positivity for all high (A68, A69, B57, B58: MFI > 10,000) and low cross-reactive antigens (extensive HLA-A MFI > 3000), sharing one or more epitopes. HLA-II was completely negative. The donor offered was a 50 year old woman typed as A*02:01 (DSA MFI 25000),*24:02 (DSA MFI 2500). The CDC and FCXM were strong positive. A multidisciplinary team decided to delay kidney transplant with the use of mechanical perfusion and immunological reassessment after liver revascularization. Six-hour post liver reperfusion, serum was tested and both SAB assay and CDC/FC-XM converted to negative. The patient underwent kidney transplant with standard inductive and maintenance immunosuppression. Antibody monitoring at +4 and +12 day was negative. At 3 months the function of allografts is stable with no evidence of rejection. This model gives some immunological suggestions: i. simultaneous combined liver-kidney allograft is a safe option, even for patient with high HLA-I DSAs (clearance of HLA-II DSAs is more controversial) ii. antibody removal is effective for DSAs in terms of shared epitopes iii. the use of mechanical perfusion allows to delay kidney transplant for an accurate immunological reassessment after liver reperfusion to avoid desensitization and additional immunosuppression which could impact on patient's survival.

P97 | Desensitization-resistant Eplet-specific HLA antibodies

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In living donor kidney transplantation, it is important to know beforehand which HLA antibodies are more prone and which are more resistant to cost-intensive desensitization therapy. We investigated the impact of desensitization on the clearance of antibodies against eplets from different HLA loci. All adult patients who received a living-donor kidney transplant at Koç University Hospital between December 2018 and June 2023 after desensitization therapy due to donor-specific HLA antibodies with ≥500 MFI in single antigen testing were considered for the study. Disappearance (<500 MFI) and persistence (≥500 MFI) of eplet-specific antibodies against different HLA loci were analyzed before and after desensitization using the HLAMatchmaker algorithm. Before desensitization, HLA antibodies against 92 different eplets were identified in 33 patients. Antibodies against 39 (42.4%) eplets were eliminated in all positive cases after desensitization therapy whereas antibodies against 15 (16.3%) eplets persisted in all positive cases. Antibodies against the remaining 38 (41.3%) eplets exhibited variable behavior; in some cases, they were eliminated and in others not. 100% of HLA-C, 87.5% of HLA-B, and 72.2% of HLA-DP eplet-specific antibodies could successfully be removed by desensitization. The highest rates of desensitization persistence were observed with 35.7% in HLA-DQ and with 20% in HLA-A eplet-specific antibodies. Desensitization therapy appear to exhibit independent effects on eplet-specific antibodies and may not be successful for antibodies directed especially against certain eplets from HLA-DQ and -A loci, whereas antibodies against HLA-C, -B, and -DP eplets appear to be more prone to elimination by desensitization. However, these findings need to be evaluated in a further larger series of desensitized patients.

P98 | Differences of the subpopulations of T-lymphocytes between long-term and recent kidney transplant recipients

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The aim of this study was the evaluation of differences in the activation of acquired cellular immunity between long-term and recent kidney transplant recipients. Long-term patients had received a transplant 17+ years ago, while recently transplanted patients 1 year ago. Total lymphocytes, CD4+, CD8+, Natural Killers, CD4 + CD28null and CD8 + CD28null lymphocytes were measured by flow cytometry. The population consisted of 20 long-term and 82 recently transplanted patients. The two groups did not differ regarding patient age ($p = 0.171$), creatinine levels ($p = 0.944$), eGFR ($p = 0.851$), sex ($p = 0.355$), pre-emptive transplantations and dialysis modality before transplantation ($p = 0.139$), rejection episodes ($p = 0.982$), DGF episodes ($p = 0.507$) and percentage of patients with DSAs ($p = 0.118$). However, living donor transplants percentage (11/82 vs 12/20, $p < 0,001$), basiliximab therapy (77/80 vs 12/20, $p < 0.001$) and percentage of patients with CMV infection (2/80 vs. 3/20, $p = 0.020$) were different. Long-term recipients exhibited a greater count of total lymphocytes (2300 vs 1600/μL, $p < 0.001$), CD4+ (1105 vs 762/μL, $p = 0.001$), CD8+ (700 vs 470/μL, $p = 0.036$), CD4 + CD28null (181 vs 40/μL, $p < 0.001$), and CD8 + CD28null (448 vs 200/μL, $p < 0.001$) lymphocytes. Long-term kidney transplant recipients show an amelioration of the lymphocytic series, while also exhibiting a significant increase in subpopulations with an immunosenescent phenotype like CD4 + CD28null, CD8 + CD28null lymphocytes.

P99 | The reduction of T and B regulatory lymphocytes in long-term kidney transplant recipients

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The aim of this study was the estimation of the effect of long-term kidney transplantation on T and B regulatory lymphocytes. Patients who had received a transplant 17+ years ago (group A) and 1 year ago (group B) took part in the study. The subpopulations of CD4 + CD25 + FOXP3 (Tregs), CD19 + CD24++CD38++ (Bregs1), CD19 + CD27 + CD24++ (Bregs2) and naïve, switched, non-switched memory and double negative (DN) B-lymphocytes were measured by flow cytometry. Patients of group A (N = 20, M/F = 11/9) did not differ with patients of group B (N = 40, M/F = 23/17) regarding age (57 vs 56 years, $p = 0.065$) and eGFR (63.5 ± 19.2 vs 63.6 ± 17, 1 mL/min/1,73 m2, $p = 0.132$). Significant differences between groups were observed in respect of the percentage of Tregs (2.55 vs 4.7%, $p < 0.001$), the percentage and number of Bregs1 (1.6 vs 0.0%, $p < 0.001$ and 0.53 vs 0.0/μL, $p = 0.001$) and Bregs2 (0.0 vs 2.55%, $p < 0.001$ and 0.0 vs 2.0/μL, $p = 0.001$). Group A also exhibited a significant reduction of B-lymphocytes (55 vs 99/μL, $p = 0.034$), which mainly referred to naïve (18 vs 41/μL, $p = 0.005$), switched (7.26 vs 14/μL, $p = 0.005$), and non-switched memory (1.22 vs 13/μL, $p < 0.001$) B-cells, with a simultaneous increase of DN cells (22 vs 11/μL, $p = 0.028$). Long-term kidney transplant recipients show a reduction of the percentage of Tregs, a rise of Bregs1 and a decline of Bregs2 as well as significant alterations of B-lymphocyte subpopulations.

P100 | Pre-transplant flow cytometric crossmatch in patients undergoing Rituximab treatment: employment of Pronase and anti-CD20

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A limitation in the application of cytometric cross-match (FCXM) pertains to patients highly immunized against HLA antigens, undergoing desensitization protocols with Rituximab, an α-CD20 chimeric monoclonal antibody. It has been demonstrated that in the context of pre-transplant cross-match, the presence of Rituximab in patients' serum can lead to false-positive results for B lymphocytes, making data interpretation challenging. Specifically, the drug in the recipient's serum binds itself to the CD20 on the donor's B lymphocytes and is detected by the secondary α-Human IgG Ab. In this case, a positive result for B lymphocytes could occur even in the absence of donor-specific Ab. The development of a protocol involving the treatment of donor cells with the enzyme Pronase and subsequent incubation with α-CD20 Ab (not detectable by α-human IgG Ab) has aimed to saturate CD20 receptors and prevent binding with Rituximab. In this context, sera from 24 patients undergoing Rituximab treatment were collected to be cross-matched with cells from 15 healthy donors with known HLA typing. At first, the drug bioavailability has been assessed excluding 5 sera. The remaining 19 sera underwent a search for anti-HLA Ab using SAB. Subsequently, the titration of α-CD20 Ab was performed and the standard deviation was calculated to determine the cut-off. During the study, 42 FCXM tests were conducted: out of the 13 expected positive cross-matches due to the presence of donor-specific Ab, 9 (69%) remained positive with B lymphocytes even after treatment. The remaining 4 yielded negative results: the likely cause is attributed to the low expression on the cell surface of the involved antigens and the low Ab's MFI values. Out of the remaining 29 cross-matches with SAB negative sera, 28 (97%) yielded negative results as expected. In conclusion, the collected data demonstrate the effectiveness of the studied protocol without compromising the sensitivity and specificity of the test.

P101 | Deciphering HLA antibody reactivity patterns: A cluster-based analysis of SAB assay data

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This study provides a nuanced exploration of anti-HLA antibody reactivity profiles using Single Antigen Bead (SAB) assay data. Employing k-means clustering, we

segmented a comprehensive dataset into 10 distinct clusters, each characterizing unique immunological patterns. Our analysis, visualized through a detailed heatmap, revealed significant variations in mean fluorescence intensities (MFIs) across these clusters, indicative of diverse antibody sensitization profiles. Notably, cluster 2 emerged as a focal point due to its unexpected reactivity pattern. While generally exhibiting low MFIs (average MFI < 1500), a pronounced spike was observed against specific alleles like A*29:01 (MFI: 24,000) and B*27:05 (MFI: 22,500), suggesting a targeted immune response. In contrast, cluster 7 displayed consistently high reactivities across a range of HLA alleles, with MFIs often surpassing 10,000, indicative of a broad or polyclonal sensitization. Intriguingly, cluster 5, with moderate overall reactivity (average MFI: 3000–4000), showed selective heightened responses to alleles such as B*15:10 (MFI: 17,000), hinting at specific immunological events or exposures. Conversely, cluster 9 presented minimal reactivity across all HLA alleles, with most MFIs below 500, possibly reflecting a low level of sensitization or antibody presence. This comprehensive analysis sheds light on the intricate landscape of anti-HLA antibody responses patterns, revealing how specific clusters may correspond to different immunological histories or sensitization patterns. The distinct reactivity profiles underscore the potential for using such data in understanding patient-specific immune responses, which could be pivotal in fields like transplant immunology and personalized medicine.

P102 | Can Molecular HLA mismatch scores predict antibody-mediated rejection in desensitized kidney transplant recipients?

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We investigated in desensitized kidney transplant recipients whether high HLA mismatch epitope scores and high donor-specific HLA antibody (DSA) levels pre- or post-desensitization are associated with development of antibody-mediated rejection (AMR). Thirty-three adult patients who received a living-donor kidney transplant at our center between December 2018, and March 2023, after desensitization therapy due to DSA ≥1000 MFI were analyzed. Patients with and without biopsy-proven AMR during the first three post-transplant months were compared for the four different molecular HLA mismatch scores (Predicted Indirectly ReCognizable HLA Epitopes (PIRCHE), HLA-Matchmaker, Amino Acid Mismatch Score and Electrostatic Mismatch Score 3D), demographics, and immunological risk factors. Twelve patients (36.3%) experienced AMR at a median of 11 days post-transplantation (IQR: 6–43). AMR attributable to existing DSA before desensitization occurred in 11 patients (91.7%); specifically, 6 cases (50%) could be linked to pregnancy-related antibodies against paternal HLA mismatches and 5 cases (41.7%) to DSA originating from prior blood transfusions. While no differences were noted between groups regarding the number of HLA mismatches, pregnancies, pre-transplant blood transfusions, and previous transplants, the strength of dominant class I DSA before desensitization was higher in patients with AMR than in patients without AMR ($P = 0.034$) (Table 2). PIRCHE and HLA Matchmaker scores were slightly higher in AMR patients without reaching clinical significance. Kidney transplant recipients who are pre-sensitized with DSA continue to face a higher risk of AMR, even after the disappearance of antibodies through desensitization. The risk of AMR is pronounced in patients with high levels of class I DSA. Larger-scale studies are necessary to demonstrate whether the PIRCHE and HLA Matchmaker scores are valuable in assessing the AMR risk in desensitized patients.

P103 | Clinical utility of 1:16 serum dilution as a predictor of response to therapeutic plasma exchange for HLA antibody-mediated rejection treatment and overall survival in lung transplant recipients: A two center study

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Antibody-Mediated Rejection (AMR) due to HLA donor-specific antibodies (DSA) is associated with poor outcomes in lung transplant recipients (LTR). Successful AMR treatment using therapeutic plasma exchange (TPE) improves clinical outcomes in LTR. The objective of this study was to assess the clinical utility of 1:16 serum dilution HLA antibody test results as a predictor of response to TPE for AMR treatment in LTR. A retrospective analysis of 21 LTR diagnosed with AMR due to de novo HLA DSA (dnDSA) and successfully treated with TPE was performed at Mayo Clinic ($n = 7$) and Temple University Hospital ($n = 14$). HLA antibodies were detected by Luminex single antigen beads assay. Mean Fluorescence Intensity (MFI) levels were measured before the 1st and after the 5th TPE session using undiluted and 1:16 diluted sera. Statistical analysis was performed using IBM® SPSS® Statistics (v26; Armonk, NY). Of 21 patients, 9 and 12 patients were diagnosed with early (<3 months post-transplant) and late (6 months–3 years post-transplant) AMR respectively. All patients had HLA class II dnDSA. In addition, 55% and 16% of LTR with early AMR and late AMR, respectively also had class I dnDSA. The MFI for all positive dnDSA in 1:16 diluted sera collected before 1st TPE demonstrated a significant correlation with MFI in undiluted sera collected 1 day after 5th TPE in both early ($R^2 = 0.8786$) and late ($R^2 = 0.9045$) AMR post-transplant. In addition, reduction in MFI of dnDSA in 1:16 diluted sera correlated with better overall LTR survival following TPE ($p = 0.01$). The MFI of 1:16 serum dilution before 1st TPE may be utilized as a surrogate to predict response to TPE for AMR treatment and overall survival in LTR.

P104 | Characterization of sHLA-AlexaFluor647-conjugates for accurate determination of serum HLA antibody concentration and affinity

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The sero-affinity and concentration assay (SAffCon) for the simultaneous determination of antibody concentration and affinity using microfluidic diffusional sizing (MDS) technology is a promising method to determine the amount of HLA antibodies and their binding strength in patient sera. Our study provides initial data on the production, quality control and binding characteristics of fluorochrome-conjugated soluble HLA antibodies required for MDS testing. We conjugated Alexa Fluor™ 647 (AF647) to accessible primary amines on five soluble HLA (sHLA) molecules from PureProtein (A*02:01, A*03:01, B*07:02, B*08:01 and DRB1*07:01/DRA1*01:01), purified them chromatographically and determined the degree of labeling (DOL) by spectroscopy. In addition, we measured their average molecular size via determination of the hydrodynamic radius (Rh) by MDS and performed SAffCon assays. Obtained data sets for the sHLA-AF647 conjugates demonstrated a good correlation to the expected molecular sizes of HLA class I (expected mass/Rh = 47 kDa/3.11 nm; measured Rh: 2.89–3.16 nm) or class II molecules (61 kDa/3.39 nm; measured Rh: 3.49 nm) and remained stable (size loss <10%; negligible impact on affinity results) for an average period of 4.5 weeks. Determined DOLs were between 1.17 and 2.89. Conjugates tested with higher DOLs did not increase the sensitivity of the MDS assay; on the contrary we observed a distinct fluorescence self-quenching effect. SAffCon assays with HLA-specific W6/32 or DR7_B monoclonal antibodies spiked into negative serum accurately confirmed expected antibody concentrations and provided comparable affinity results (Kd values HLA class I conjugates between 1.11 and 2.65 nM; DR7 conjugate: 8.4 nM). Soluble sHLA-AF647 conjugates are ideal tools for precise quantification and affinity assessment of HLA antibodies in patient sera if the reagent is properly conditioned as described for MDS applications.

P105 | High levels of complement-binding donor-specific anti-HLA antibodies: Are they always pathogenic?

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Donor-specific anti-HLA antibodies (DSA) are associated with antibody-mediated rejection (AMR) and poor kidney allograft outcomes. However, there are reports describing the absence of AMR in the presence of high DSA levels after kidney transplantation. We describe herein a kidney transplant recipient who developed a strong HLA-DQ DSA without any evidence of AMR or graft dysfunction. A 52-year-old male patient with end-stage kidney disease (ESKD) received a living unrelated kidney transplant. At the time of the transplant, he displayed weak DSA levels (DQ7, 641 MFI; DQ8, 1818 MFI) and a negative flow cytometric crossmatch. He was induced with alemtuzumab (anti-CD52) and initiated on tacrolimus, mycophenolate mofetil, and rapid steroid withdrawal. On POD6, he displayed a significant increase in DSA levels (DQ7, 4157 MFI; DQ8, 6386 MFI). On POD13, he displayed high DSA levels (DQ7, 14236 MFI; DQ8, 18633 MFI). The presence of the DQ7/DQ8 DSA was corroborated by phenotype (PRA) bead analysis and a different vendor's single antigen bead assay. In addition, positive C1q binding was also detected by the DQ7/DQ8 DSA. Interestingly, allograft biopsy and donor-derived cell-free DNA levels were unremarkable. The decision was made not to treat his DSA and to add prednisone (5.0 mg) to his immunosuppression. It was determined that the DSA was directed against the 55PP eplet present on HLA-DQ7, DQ8, and DQ9 (https://www.epregistry.com.br/). Repeat allograft biopsy at POD20 continued to be unremarkable despite high DSA levels (DQ7, 11768 MFI; DQ8, 16431 MFI). The patient had immediate graft function, and throughout, his creatinine has been stable at around 1.3 mg/dL including POD85 with persistent high DSA levels (DQ7, 9380 MFI; DQ8, 14182 MFI). High levels of complement-binding DSA are not always associated with AMR. Our understanding of the pathogenicity and significance of DSA continues to evolve and requires further investigation.

P106 | Effect of HLA-B -21 dimorphism on cellular response after COVID-19 vaccination in patients with renal disorders

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The HLA-B protein plays an essential role in T cell and natural killer (NK) cell immunity. Antigen presentation of HLA-B to T cells is the basis for host defense against infectious agents and graft acceptance. HLA-B contains a dimorphism encoding either Methionine (-21M, giving rise to functional HLA-E binding peptides) or Threonine (-21T, results in peptides not binding effectively to HLA-E). Haplotypes with -21M are biased towards NKG2A NK cell education, and are associated with superior NK function, while -21T promote KIR mediated education. Pathogenesis of COVID-19 involves both humoral and cellular responses. It is supposed that COVID-19 vaccines also elicited effective cell immune response, specifically IFNγ secretion by SARS-CoV-2-specific T cells. In the present study, we investigated whether the HLA-B -21 dimorphism leads to differences in cellular response after COVID-19 vaccination. One hundred and sixty-two patients with chronic kidney disease (including terminal kidney failure), and 225 kidney transplant recipients, all vaccinated with BNT162b2 mRNA COVID-19 vaccine (Comirnaty), were investigated. SARS-CoV-2-reactive T cell responses were evaluated using a SARS-CoV-2 IFNγ release assay (IGRA, EUROIMMUN). The TNFα level was determined by ELISA. HLA genotyping was performed using a Next Generation Sequencing (NGS) method. Based on HLA-B -21 dimorphism, the subjects were divided into three groups: MM, MT and TT. Genotype distribution was similar in both studied groups. However, among patients with -21M haplotype higher IFNγ release was detected for patients with renal disorders then in kidney transplant recipients ($p = 0.005$). Similar results were observed with respect to TNFα levels ($p < 0.0001$). The data show that immunosuppressive treatment may diminished SARS-CoV-2-reactive T cell activity in patients after kidney transplantation while HLA-B dimorphism at the -21 position seems not to play a significant role. Supported by: SZPITALE-JEDNOIMIENNE/30/2020.

P107 | Could donor-derived cell-free DNA level be considered a potential biomarker of subclinical graft rejection or early organ damage in kidney transplant recipients?

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Circulating cell-free DNA (cfDNA) is an emerging biomarker that could be used to detect subclinical graft rejection with enough sensibility and specificity. cfDNA consists of degraded DNA fragments released from damaged cells into the blood and other body fluids. When these fragments come from the allograft, they are called donor-derived cell-free DNA (dd-cfDNA). Concentrations of dd-cfDNA >1% after the first week post-transplantation could be related to adverse events. Plasma samples from kidney transplant recipients were collected (Streck tubes—Cell-Free DNA BCT®) in our hospital from 2020 to 2023: pre-transplant, 15 days, 3, 6 and 9 months post-transplant. dd-cfDNA levels were determined using the AlloSeq-cfDNA (CareDx) protocol and the MiSeq™ System platform (Illumina). dd-cfDNA was determined in 30 kidney transplant recipients divided into two groups based on their clinical course post-transplantation: Group 1 ($N = 21$): patients with no signs of graft rejection. Levels of dd-cfDNA≤1% were observed in 13 patients. The rest of them exhibited dd-cfDNA levels >1%, which can be explained by other pathologies/biological processes. Group 2 ($n = 3$): patients who had borderline graft rejection, with levels of dd-cfDNA≤1%. Group 3 ($n = 6$): patients with graft rejection. Patients with a diagnosis of rejection after 4 months (66%) showed dd-cfDNA levels >1%, anticipating at least 1 month the graft rejection diagnosis. Patients with dd-cfDNA≤1% suffered early rejection between 5 and 20 days post-transplant. dd-cfDNA could be considered a novel biomarker of graft rejection after first term post-transplant up to several months before its clinical presentation. However, it appears to have no predictive value for early graft rejection or borderline rejection. Moreover, other pathologies/biological processes can also affect dd-cfDNA levels.

P108 | A critical retrospective analysis of flow cytometry crossmatch in the emergency context in order to optimize the available resources

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The number of multi-organ donors in the CST-Porto has increased in the last few years. This medical/laboratory activity requires high level coordination between different teams and expert analysis in a quicker turnaround time. Moreover, laboratory work often occurs overnight, a period of time in which human and non-technical factors should not be underestimated. The crossmatch test is essential prior to organ transplantation, to detect donor specific antibodies (DSA) which are important risk factors for development of antibody mediated rejection and poor graft survival. With heart and lung exception, the presence of DSAs for loci HLA-A, -B and -DRB1 are not considered for CDC and FCXM in peri-transplant. Given the increasing number of multi-organ donors, the limited number of cells available and the time consuming nature, we decided to analyze the FCXM activity carried out in emergency context. In the last 18 months, 83 deceased donors were considered and 479 FCXM were performed. These data correspond to 349, 97, 27 and 4 FCXM to allocate 162 kidney, 26 lung, 11 heart, and 2 kidney-pancreas organs, respectively. Of the 479 FCXM, 206 were done with sera characterized by SAB assay, with 121 having DSA and 85 non-DSA, which resulted in 68 and 34 T and/or B positive FCXM respectively. In the remaining 273 FCXM, sera were not characterized by SAB, but 223 historical sera had DSAs, what resulted in 99 T or/and B FCXM positivity. Overall, our results showed that 28% of FCXM were performed with sera without DSAs, in emergency or historical sera, while 72% were done with sera having DSAs (25% emergency and 47% historical). The latter had different reactivity against T and B cells which is associated with the low expression antigens and DSA < 5000 MFI. Our analysis points out the need to review the selection algorithm for those transplant candidates to do FCXM. With a different strategy we may be able to streamline laboratory work and to optimize the scarcity of human and economic resources available.

P109 | Positive crossmatch in ABO-incompatible neonatal heart transplant due to transplacental transfer of maternal HLA antibodies

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ABO-incompatible heart transplants recently show equivalent long term survival compared to compatible ones, but transplantation with a positive crossmatch is still a serious challenge. Donor-specific antibodies (DSA) of maternal origin are often present in neonates, due to transplacental transfer. Here we described a neonatal ABO-incompatible heart transplant with positive crossmatch, due to maternal-derived HLA antibodies. The study of patient serum demonstrated the presence of anti-HLA class I antibodies (vPRA 43%), including the DSA A1 (MFI = 10,500) against the donor. The serum of the mother showed the presence of high titer of anti-HLA class I and II antibodies (vPRA 75% and 88%, respectively), including anti-A*01:01 antibodies (MFI = 26,000). The HLA-A of the father is A*01:01, *01:02; the patient HLA-A is A*01:02, *03:01. High resolution typing performed after transplant identified the locus A*01:01 also in the donor. Since this was the fourth pregnancy for the mother, our hypothesis is that she developed antibodies against the locus HLA-A*01:01 of the father and passed them to the fetus, reacting with the same HLA antigen on the surface of the new heart. The patient was positively treated only with intraoperative plasma exchange, followed by standard immunosuppressive therapies. We tested the presence of DSA in the sera 3 and 12 days and 1 month after the transplant, showing a decrease of the DSA at day 12 and almost complete disappearance 1 month after, with an immunologic event-free follow-up. To better understand the titer of this antibody, we tested all sera against peripheral blood mononuclear cells expressing A*01:01 (dilution 1:8). We observed a strong decrease of antibody titer already after 3 days, demonstrating the efficacy in plasma exchange to remove antibodies. After 1 year, the patient never developed acute heart rejection, DSA or isohemagglutinin anti-B, graft function is good and the patient is in quite good clinical conditions.

P110 | Comparison of results of crossmatch tests from peripheral blood cells with crossmatch tests from secondary lymphoid organs cells in kidney transplantation

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Classic crossmatch tests before transplantation are performed with donor cells derived from secondary lymphoid organs (SLO) (spleen or lymph node). This results in delay and potentially prolonged cold ischemia time. The purpose of the study was to compare the results of crossmatch tests with donor cells derived from deceased donor peripheral blood (DDPB) in relation to cells from SLO. Complement-dependent cytotoxicity crossmatch (CDC-crossmatch) and flow-cytometry crossmatch (flow-crossmatch) were performed in 65 candidate kidney transplant recipients with 34 deceased donors, both with SLO and DDPB cells. In two donors, no DDPB CDC-crossmatch and in another 10 no DDPB flow-crossmatch were performed. CDC-crossmatch from DDPB exhibited a high rate of non-diagnostic results in 50.7% (32/63) of cases, compared to the corresponding method from SLO cells (diagnostic result 100%). In contrast, in flow-crossmatch the percentage of non-diagnostic results from DDPB cells was significantly lower 3.6% (2/55), $\chi^2 = 31.8$, $p < 0.001$, with 100% diagnostic result in the corresponding method with SLO cells. The two non-diagnostic results from DDPB Flow-crossmatch concerned B-lymphocyte crossmatch (owing to B-lymphocyte deficiency). Donors with a non-diagnostic result in DDPB cell methods did not differ significantly in age and ICU hospitalization duration, compared to donors with a diagnostic result [age: 57 (45–65) vs 63 (58–69) years, $p = 0.11$ for CDC-crossmatch, 59 (47–72) vs 62 (53–67) years, $p = 0.93$ for flow-crossmatch, ICU days: 7(4–12) versus 7 (5–10), $p = 0.8$ for CDC-crossmatch, 13 (8–49) vs 11 (6–15), $p = 0.34$ for flow-crossmatch]. CDC-crossmatch with DDPB cells has high rates of inconclusive results compared to the corresponding DDPB flow-crossmatch. Flow-crossmatch by DDPB is therefore reliable, while CDC-crossmatch by DDPB carries a high risk of false results. Possible factors influencing the diagnostic result of CDC-crossmatch from DDPB need further investigation.

P111 | Donor derived cell-free DNA: Clinical utility in surveillance strategy for heart transplant

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Heart transplantation represents the gold standard in advanced heart failure treatment. Acute rejection and Cardiac Allograft Vasculopathy result in graft dysfunction, hemodynamic compromise, chronic graft failure and mortality: accurate and timely diagnosis is crucial. Current reference standards for diagnosis of rejection/injury are represented by endomyocardial biopsy (EMB), echocardiographic, Cardiac CT scan, Angiography, HLA donor-specific antibodies (DSA) with different level of sensitivity and invasiveness. Dd-cfDNA is a new available- sensitive blood biomarker able to detect donor-derived DNA released in the recipient circulation for early diagnosis of heart reject/injury. The aim of this pilot study was to evaluate the utility of the dd-cfDNA to early detecting acute myocardial injury in adult and pediatric patients at different time from transplant (de novo, 1–5–10 years) closely linked with clinical aspect. Ten patients were analyzed with EMBs, echocardiographic assays, DSA, measurement of immunosuppression drug-level, cardiac CT scan and dd-cfDNA fraction evaluation. Eight patients were dd-cfDNA negative (<0.2%), in accord to EMB, echocardiographic and DSA while two patients resulted positive (0.33% and 12.19%). Patient with low % dd-cfDNA resulted negative for EMB and echocardiography with low titer DSA while patient with high dd-cfDNA % showed high titer DSA (MFI 15354) and EBM positive with echocardiographic aspect of strain and diastolic dysfunction. The strong link between lab results and clinical assessment become crucial for tailoring therapeutic strategy. Preliminary study confirms the correlation between high levels of dd-cfDNA and EMB, Echo, DSA, CAV profiles. The results can guide clinicians to early identify graft at high risk and apply different strategy of therapy monitoring to preserve graft function. Further studies will need to confirm this evidence.

P112 | Diagnosis and treatment of humoral rejection in heart-transplant patients: Single center experience

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Heart transplantation is the gold standard treatment for advanced heart failure. Antibody-mediated rejection (AMR) is a major cause of medium- and long-term morbidity and mortality. Success, therefore, is closely linked to the ability to modulate the immune response in the recipient through innovative diagnostic approaches and immunosuppressive therapy tailoring. Since 2019, San Camillo Hospital's Heart Transplant Center in Rome has adopted an integrated protocol for the early diagnosis and treatment of humoral rejection, consisting of clinical, instrumental and laboratory monitoring by solid-phase techniques (Luminex-Single Antigen assay-LSA) used also to identify donor-specific anti-HLA fixing or no-fixing complement antibodies (LSA-C1q DSAs). A total of 113 transplanted patients with negative prospective cross-match were studied. Post-transplant DSA production was monitored according to ISHLT guidelines in 1, 3, 6, and 12 months-time and afterwards once a year or after clinical symptoms evidence or echocardiographic signs of graft disfunction, in association with biopsy. During the monitored period, 32 patients showed post-transplant DSA: 2 patients (6%) class I, 16 (50%) class II and 14 (44%) both class I and class II DSA. DSA complement fixation ability was assessed in 19 patients and 13 cases (68%) resulted LSA-C1q positive with a 23% of AMR incidence, associated to clinical-functional deterioration sign and echocardiographic and biopsy changes. The use of apheresis and remodulation of immunosuppressive therapy (FK, MMF, or Everolimus) resulted in a major reduction of DSA, clinical recovery, and echocardiographic improvement. The adopted protocol for monitoring and diagnosis of humoral rejection resulted in early

identification of patients at risk of graft failure. This leads to specific diagnostic and therapeutic strategies to improve long-term outcome.

P113 | Correlation between DSA and kidney transplant prognosis

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Organ transplantation is a medical procedure that replaces damaged organs that cannot be repaired with current treatments with healthy organs in terminally ill patients. The success rate and prognosis of the transplant patient are greatly affected by rejection reactions that may arise as a result of the transplant patient's immune system reacting to the transplanted organ. In our study, we aimed to study the effect of DSA on transplant rejection. Two hundred and fifty-one patients who went through renal transplantation were included. Diagnosis, gender, age, transplant date, HLA typing (donor, recipient), patient panel reactive antibody (PRA) result, donor-specific antibody (DSA) level, presence or absence of transplant rejection, date of transplant rejection, etc. were investigated. Of the 251 patients, 30 experienced an antibody-mediated rejection (AMR) episode, and 221 did not experience an AMR episode. In terms of DSA, the AMR-positive patient group had 76.7% total DSA, of which 33.3% and 46.7% came from preform and de novo DSA, respectively. Total DSA was found to be 23.1%, preformed DSA to be 14.9%, and de novo DSA to be 10.9% in the AMR-negative patient group. Four patients (13.3%) in the AMR-positive patient group and 24 patients (10.9%) in the AMR-negative patient group had acute cellular rejection (ACR) episodes. After comparing the two groups' median rejection-free days, the AMR-positive patient group had a value of 261.5 $\pm$ 208.7, while the AMR-negative patient group had a value of 1569 $\pm$ 113.6. Out of these, preformed DSA and de novo DSA exhibited a significant relationship with AMR, whereas there was no significant correlation found with ACR, total DSA, or the presence of rejection. An analysis of Kaplan–Meier survival was done comparing total, preformed, de novo DSA to AMR. When compared to preformed DSA and total DSA, there was a discernible correlation between AMR and de novo DSA. The chance of developing AMR rose when de novo DSA was present.

P114 | Luminex single antigen assay on 1:10 diluted serum predicts the drop in anti-HLA antibodies before desensitization

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Many patients have limited access to kidney transplantation because of their immunization against the HLA system. In the Rouen transplantation center, desensitization protocol (DP) is a therapeutic option to improve access to transplantation for hyper-immunized patients. One part of the DP treatment is done by performing Immunoadsorption. In pre-transplant situations, we perform 10 plasma exchange sessions in 2 weeks and then three sessions a week until the patient gets a transplant. In order to include patients in DP, the Agence de la Biomédecine requires that the diluted sera (1/10) before desensitization shows a major decrease in anti-HLA antibodies identified by Luminex assay as compared to the undiluted sera. However, to our knowledge, the relevance of this dilution has not been studied so far. In our retrospective study, we tested with a Luminex Single antigen test (One Lambda Labscreen, EDTA pretreated) the pure and 1/10th diluted serum of 11 patients awaiting a renal transplantation before HLA desensitization, and the pure serum after 10 sessions of immunoadsoprtion (IA), that is to say the evolution of 1067 Luminex beads in HLA class I and 855 in class II. The objective is to determine if testing 1/10 diluted sera before starting DP is reliable to predict DP efficacy regarding the intensity of anti HLA antibodies. We report a high variability of MFI between the pure serum and the diluted serum on Day 0 of desensitization. There was a strong correlation between the antibody intensity on the 1:10 diluted serum before and on the pure serum after 10 sessions of IA in class 1 ($r = 0.99$, 95% CI: 0.98–0.99; $p < 0.0001$) and class 2 ($r = 0.98$, 95% CI: 0.97–0.98; $p < 0.0001$). Diluted serum before desensitization predicts the decline in HLA antibodies better than pure serum.

P115 | A comprehensive comparative assessment of mean fluorescence intensity of Luminex single antigen bead tests between laboratories and commercial platforms; A report from the Italian histocompatibility network

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The Luminex Single-Antigen Bead (SAB) test is the relevant assay for HLA antibody detection and MFI value is commonly used to list non-permissive DSA and stratify immunological risk in organ transplant. The Italian Society for Histocompatibility and Immunogenetics (AIBT), in cooperation with the Italian National Transplant Centre (CNT) established a working group to assess variability in MFI values between the labs of the network and to compare the results obtained with different commercial platforms in use. The design of the project included 10 high reactive sera selected from previous CNT External Proficiency Testing rounds, 20 labs participating with the platform in use for routine clinical care and standard protocols according to the manufacturers' recommendations. To assess variability between labs, median coefficient of variability (%CV) for each bead (around 1000 observations) was calculated and stratified for bead specificity, HLA loci and MFI value ranges for the two platforms separately. To evaluate performance of the two kits, beads with the same specificity were aligned and median MFI values across all users compared. The comparison was performed according to Clinical and Laboratory Standard Institute in terms of correlation (Passing-Bablok linear model and non-linear model), agreement and bias of measurements (Bland–Altman Plot) and best fitting cut-offs of positivity and permissiveness (ROC Curves). The results of this explorative cohort are summarized as follows. Variability between labs: median CV between 15% and 20% for both vendors, namely in clinical most informative MFI ranges around positivity and permissiveness; no significant difference between HLA loci and a few bead outliers. Comparison between vendors: non-linear correlation between MFI values for the two vendors, strong bias towards MFI measured and definition of indicative best fitting clinical cut-offs for the two kits. The results are now being validated in an independent cohort of sera.

P116 | Exploring false positive reactions in anti-HLA antibody identification via single antigen testing

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The Single Antigen tests offer high sensitivity for detecting anti-HLA antibodies, making them valuable tools for organ transplantation. However, this high sensitivity can also lead to false positives. Our goal is to identify falsely positive reactions and determine their frequencies. We included 195 patients in our study who reported no immunizing events and had negative results for anti-HLA antibodies (using the LabScreen Mixed Kit—One Lambda). Subsequently, we analyzed the results of anti-HLA antibody identification (using the Single Antigen Kit—One Lambda) in these patients. We found positivity in 53 patients (27.17%) for class I and 56 (28.71%) for class II. The median number of targets was 2 [1–4] for both HLA classes. The most frequent targets in HLA class I were C*12:03, representing 5.19% of positive targets, followed by B*37:01 and C*15:02 (4.55%), A*11:02, C*05:01, C*06:02, and C*17:01 (3.9%). One patient exhibited a "Pan-C" profile. Regarding HLA class II, the most frequent targets were DPB1*01:01 (12.96%), DPB1*06:01 (9.26%), DQB1*03:01, DQB1*03:19, and DQB1*06:03 (4.94%). As for the MFI, most were on the lower range with a median of 2061 [1401–3416] for class I and 1544 [1197–1845] for class II, but some exhibited a very high MFI reaching a maximum value of 15,394 for class I and 13,662 for class II. Determining falsely positive reactivities of single antigen tests is crucial in organ transplantation. By identifying interactions that could be erroneously interpreted as anti-HLA antibodies, it becomes possible to avoid inappropriate decisions that could compromise the transplant process. Therefore, it is imperative to study these reactivities, analyze them in conjunction with other tests such as flow cytometry crossmatch (CXM) and screening assays, as well as taking into account other clinical data to make informed decisions to ensure the best possible outcome for transplant patients.

P117 | Urine biomarker CXCL10 bead-based detection assay for monitoring clinical transplant rejection

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International multicenter validation of CXCL10 or IP-10 in the urine of transplant patients establishes the importance of this biomarker in early detection of tissue injury in relation to T-cell mediated inflammation. The IP-10 bead-based assay is a 1 h assay consisting of 3 separate incubation steps and a final wash step per test run. The IP-10 assay platform can be set as a single- or multiple-well test, a flexible alternative to other IP-10 based assays that come as fixed strip, half-, or full-tray for a single time point. The IP-10 standard assay displayed a lower limit of detection, or sensitivity, in the 1–3 pg/mL range, availing post-transplant immunologic monitoring and rejection surveillance. Detection and analytical recovery of the exogenous 50 pg/mL of IP-10 analytes in 8 urine control samples across 6 different batch lots were assessed at various dilution factors. The optimal coefficient of variation (%CV < 20) occurs at dilutions of 1:6 and 1:8. When selected patient urine samples from an HLA diagnostics lab in Basel were used to assess the accuracy of the assay, results from 1:6 and 1:8 urine dilutions showed that the quantitative percent of IP-10 recovery can be assessed within the subclinical range (<50 pg/mL, 6/10 patient samples) and clinically relevant range (>50 pg/mL, 4/10 patient samples). The IP-10 biomarker assessment tool, with its wide range of coverage, can be used across different clinical laboratories for routine transplant rejection surveillance management. Overall, the non-invasive IP-10 bead-based detection assay is a cost effective, fast, sensitive, and accurate assay that can be performed repetitively in lieu of multiple biopsies on post-transplant patient urine samples for detection of possible transplant rejection.

P118 | Evaluation of serotype representation in different Luminex single antigen kits regarding the serotype frequencies in 2021 deceased donors

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Accurate assignment of HLA antibodies is fundamental for a reliable virtual crossmatch. Thus, this study aimed to determine the antigen coverage of the Luminex single antigen (LSA) kits from One Lambda (OL) and Immucor (Imm) regarding the HLA antigens present in deceased donors (DD) from São Paulo State, Brazil. HLA typing (11-loci, PCR-SSO) of 2021 DD and the antigens in OL (Standard (Std) and Explex LSA) and Imm LSA kits were classified according to the 18th IHIWS serotype proposal. Each HLA locus was separately analyzed, and lack of coverage was defined as the presence of a serotype in the DD population and absence in the LSA kit. The OL Std and the Imm kits showed full coverage for HLA-DRB3/4/5, -DQA1, -DQB1, and -DPA1 serotypes. The percentages of lack of coverage for HLA-A, -B, -C, -DRB1, and -DPB1 with the OL Std kit were 3.7%, 3.0%, 19.3%, 2.9%, and 0.7%, respectively, and with the Imm kit, were 4.9%, 3.1%, 1.6%, 4.0%, and 3.3%, respectively. The low coverage for HLA-C in the OL Std kit is due to the absence of C*07:01 and C*08:02 serotypes. The Explex kit cannot be considered a panel since its average coverage is only 43%. However, combined with the OL Std kit, it considerably reduces the lack of coverage, especially for HLA-C, which dropped from 19.3% to 0.1%. The percentages of lack of coverage for HLA-A, -B, -C, -DRB1 and -DPB1 with the combination of OL Std and Imm kits were 1.0%, 3.0%, 1.6%, 2.2%, and 0.7%, respectively. Of note, serotypes A*02*11, B*15:08, B39*10, and DRB1*11:02, with population frequencies of 0.8%, 0.4%, 0.3%, and 1.7%, are the main serotypes not represented by any of the investigated LSA kits. Considering cost–benefit, Explex is used for special cases, and we alternate the use of OL Std and Imm LSA kits for every 3-month serum sample from DD waitlist patients. This policy greatly improved the serotype coverage, especially for HLA-C and HLA-A.

P119 | Strategy for delisting prohibited HLA antigens to increase the possibilities of kidney transplantation of hypersensitized patients

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Highly sensitized patients have very limited access to kidney transplantation, resulting in a long waiting time on dialysis. Delisting of prohibited HLA antigens should allow the performance of DSA-positive, crossmatch-negative transplants. We describe the experience of four Spanish transplant centers after delisting prohibited HLA antigens to reduce cPRA below 99%. Delisting was gradually performed, allowing HLA antigens with MFI < 5000, avoiding repeated antigens from previous transplants. If the cPRA did not decrease, a more aggressive HLA delisting was performed up to a maximum MFI of 10,000 except for DP. In some cases, the capacity to activate complement (C3d or C1q) and the 1/16 dilution were performed to individually decide the delisting. Forty-eight patients underwent delisting from May 2022 to August 2023, with total time on the waiting list of 5.6 [3.3–9.1] years and time on dialysis of 9.8 [5.7–13.5] years. Baseline cPRA was 100.0 [99.9–100.0]%. After delisting, it dropped to 98.3 [96.0–99.0]%. Thirty patients obtained an offer within the Spanish Highly Sensitized Program (PATHI) after a period of 98 [52–154] days of which 18 had a negative CDC and flow cytometry crossmatch and underwent kidney transplant. The number of DSAs at the time of transplant was 2 [1–4], with MFI of the dominant DSA of 8036 [3857–20,955]. 55.6% of recipients received post-transplant desensitization. Rejection developed in 7 patients (38.9%), in all cases humoral and in 2 cases (11.1%) mixed, after 43 [13–91] days post-transplant. In only two cases rejection could not be controlled with treatment and, in one case, it progressed to chronic antibody-mediated rejection. All grafts, except one, are functional at 186 [67–384] days post-transplant and, in 7 patients, at 1 year of follow-up. A delisting strategy can be considered for hypersensitized patients who have no other options to find a compatible donor on the waiting list.

P120 | Immunogenetic profiling in living donor kidney transplantation: Insights from DSAs and pronase-treated flow cytometry crossmatch

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Ensuring a precise evaluation of immunological risks is essential for the success of kidney transplantation (KT). The presence and characteristics of Donor-Specific Antibodies (DSAs) are key in forecasting the likelihood of graft rejection. Enhanced detection methods for DSAs significantly contribute to predicting the outcomes of grafts. The application of pronase in Flow Cytometry Crossmatch (FCXM) procedures augments the identification of antibodies that are clinically significant, offering a comprehensive insight into potential immunological risk. The study covered 77 living donor kidney pairs, analyzing Donor-Specific Antibodies (DSAs) and FCXM (T and B cells). DSAs were classified into HLA class I and II, with their baseline Mean Fluorescence Intensity (MFI) measured. FCXM results were categorized as positive or negative. Among these, the DSAs demonstrated an average count of 0.57 per patient, (present in 18 patients). For class I, DSAs averaged around 7335 (range 1712–14,067), while class II DSAs showed an average MFI of approximately 9947 (range 1197–22,847). FCXM results were also pivotal in our assessment. Among the 77 patients, around 13% exhibited positive FCXM results, 3 positive for T cells, 3 positive for B cells, and 7 positive for both T and B cells. Among the patients, 9 had class I DSAs, and 14 had class II DSAs, reflecting the varied immunological challenges in the cohort. In 2 patients T cell positivity was observed in 2 patients, 1 ABO incompatible and the other with DSAs near the cut-off. The study highlights the role of critical DSAs and FCXM in KT candidates, addressing potential discrimination and emphasizing nuanced post-transplant monitoring. This balances thorough immunogenetic evaluation with fair patient care, aiming to improve graft survival and outcomes in living donor KT.

P121 | Optimizing kidney re-transplantation outcomes: Validation of a highly sensitive assay for monitoring of donor-derived cell-free DNA

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Every year 100,000 patients in the US with End Stage Renal Disease (ESRD) are awaiting a kidney transplant, either from a Living Donor (LD) or a Deceased Donor (DD). Only 30% of these individuals are transplanted annually, leaving 70% of the patients with alternative treatments such as dialysis. The median kidney graft survival ranges between 15 and 17 years, and therefore it is not uncommon for kidney transplant patients to require re-transplantation for proper kidney function. Lost grafts

or parts thereof frequently remain in situ within the patient during re-transplantation, potentially being a source of donor-derived cell-free DNA (dd-cfDNA) which is released into the circulation. Release of dd-cfDNA from the first graft, could pose challenges for the accurate quantification of dd-cfDNA from the newly transplanted graft. Therefore, addressing this issue is crucial for optimizing the success of sequential kidney transplantation procedures and improving overall patient care. One Lambda Devyser Accept cfDNA is a highly sensitive NGS-based assay for monitoring of dd-cfDNA in transplanted patients. The purpose of the study was to evaluate and validate the test for use in situations with retransplanted patients, that is, samples containing three different genotypes (Patient, Donor 1 and Donor 2). The validation included a comprehensive assessment of the assay's analytical performance, including the determination of Limit of Blank (LoB), Limit of Detection (LoD), Limit of Quantification (LoQ), linearity, and accuracy of measurement (trueness and precision). Artificial samples were generated to represent samples containing three different genotypes in the range of 0.2%–30% dd-cfDNA. All samples were sequenced on Illumina MiSeq instruments and the fastq files were analyzed using Advyser Solid organs software. Validation data demonstrate a robust and sensitive assay suitable for monitoring of dd-cfDNA in retransplanted patients.

P122 | HLA-DQA1*03:02-DQB1*03:03 is the dominant immunogenic heterodimer for post-transplant HLA-DQ de novo DSA development in a cohort of Chinese kidney transplant patients

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Eplets, acting as specificity determinants on donor HLA antigen molecules, may induce the formation of de novo donor-specific antibodies (dnDSA) by the recipient's B cells post-transplantation. HLA-DQ DSA are the most common type of DSA that target mismatched donor HLA-DQ antigens. The HLA-DQ protein exists in form of heterodimer consisting of HLA-DQA1 and HLA-DQB1 molecules. The specific risk each mismatched HLA-DQ dimer contributing to dnDSA formation remains underexplored. This study constitutes the first analysis of the specific risks associated with HLA-DQ dimer mismatches in the emergence of de novo donor-specific antibodies (dnDSA) following kidney transplantation in China. This research is distinct in its ability to differentiate between high and low-risk mismatched HLA-DQ dimers for the development of DQ dnDSA within a kidney transplant cohort, providing a deeper understanding of the mechanisms that regulate the immunogenicity of different HLA-DQ dimers. In this study, we provide evidence that the formation of dnDSA against HLA-DQ is primarily correlated with DRB1 eplet load and less so with the mismatched HLA-DQB1 eplet load. The HLA-DQA1*03:02-DQB1*03:03 dimer appears to be the most immunogenic in inducing dnDSA. The 55PP and 76V acted as the most immunogenic eplets in a cohort of 434 kidney transplant patients in China.

P123 | Cross-platform optimization of (d)dPCR assays intended for donor-derived cell-free DNA monitoring

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Donor derived cell-free (cf)DNA monitoring is widely evaluated as a biomarker for graft injury and/or rejection in solid organ transplantation (SOT). cfDNA monitoring could confirm and even substitute invasive, expensive and subjective biopsies. Methods need to operate on low quantities of cfDNA and be sensitive, scalable and fast. Digital PCR meets these requirements and the assay presented here eliminates the need for donor pre-transplant DNA, using informative markers based on HLA typing. The assay was initially developed for the QX200 platform, which is technically limited by wastage, variability and fragility of the oil suspension PCR as well as number of channels available for multiplexing. The Absolute Q, applying a microfluidic array, avoids these limitations. Here, we illustrate the challenges in transferring a dPCR based assay between platforms. To test assay performance, cell line gDNA was mixed and then sheared to cfDNA size. Multiplexed pre-amplification was performed, followed by a 2-color allele-specific multiplexed FAM/HEX reaction on the Absolute Q dPCR. FAM represents the 'donor' HLA and HEX the 'recipient' HLA. Assays were loaded onto MAP16 plates together with MasterMix, pre-amplified cfDNA-analog and buffer. Absolute Q software and Excel were used for data analysis. Initial results showed overloading of the array, inhibiting analysis. Reduction of pre-PCR cycle number and

an intermediate dilution resolved this issue. Further, assay specific, issues involved secondary clouds and rain, inhibiting the placement of a correct threshold. Adjusting temperature, concentration and in some cases investigation of secondary clouds by NGS resolved these issues or allowed classification of the secondary cloud as target, allowing for linear performance of the assay over a range of artificial cfDNA chimeric levels. In summary, results of the optimized assay on QX200 and Absolute Q show inter-system employability and high reproducibility.

P124 | Playing hide and seek with HLA-DRB3/4/5 data in kidney transplantation reports in Argentina

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Organ transplantation's success relies on intricate compatibility assessments involving HLA and antibodies. This study delves into the underreported role of HLA-DRB3, DRB4, and DRB5 gene products (DR51, DR52, and DR53) in transplant practices. In Argentina, the widely used HLA Typing Kit includes these genes. However, the absence of specific legislation dictating which loci should be analyzed and reported in deceased donors for transplantation introduces uncertainty into the HLA typing process and reports. Examining 25 HLA typings and 141 flow cytometry crossmatch (FC-XM) reports over 3 months, the study uncovers a significant presence of DR51, DR52, and DR53 antigens. Within this analysis, 3 FC-XM cases show "T cell negative/B cell positive" results with anti-HLA-DR51/52/53 antibodies that could have been avoided by virtual crossmatching if DR51/52/53 antigens would have been included in the donors HLA typing. The study concludes by urging further research on the clinical impact of anti-HLA-DR51/52/53 antibodies in solid organ transplantation. It emphasizes the need for inclusive reporting practices, echoing a "ready or not, here we come" moment in transplantation protocols in Argentina: an imperative to uncover hidden complexities and ensure precise organ allocation. By integrating DRB3, DRB4, and DRB5 data into HLA typing reports, we leverage existing technology without necessitating reagent modifications. This integration aligns with current capabilities, offering insights into the potential roles of these antigens in rejection episodes. Moreover, this research highlights a pivotal aspect of transplantation, advocating for an inclusive reporting approach that seamlessly aligns with advancing knowledge and technological capabilities at virtually no additional cost.

P125 | Analysis of the influence of IgM on the results of CDC-XM in patients with chronic kidney disease

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Complement-dependent CDC-XM is routinely performed before solid organ transplantation to identify DSA that may lead to graft rejection or dysfunction. At the same time, the blood of patients may contain lymphocytotoxic antibodies of the IgM class, which do not affect the engraftment of the graft. In the Republic of Kazakhstan, this analysis is mandatory and is performed before each solid organ transplantation. If the result is positive, a standard XM test is performed with serum treated with the reagent DTT. We aimed to determine the effectiveness of using serum treated with DTT in a cross-match analysis to reduce the proportion of false positive results. The study included 628 patients, a total of 1036 CDC-XM tests were performed, given that several donors were examined for each patient. All patients initially underwent standard CDC-XM; if the test was positive, patients were re-examined using serum treated with DTT. To isolate purer and live populations of lymphocytes, cell isolation technology with negative selection was used using RosetteSep kits (StemCell technologies, Canada). The results of the primary CDC-XM test were as follows: 346 CDC-XM tests were positive (33.4%) and 690 were negative (66.6%). All 346 patient samples that tested positive were retested with serum treated with DTT. Of these, only 106 samples in the new study tested positive (30.6%) and 240 samples tested negative (69.4%). As a result, the use of DTT increased the number of negative CDC-XMs from 66.6% to 89.7% of the total number of studied samples. Mandatory use of DTT testing is advisable because it increases the chance of receiving a kidney transplant for patients in need of a donor organ. In addition, it is important to take into account the patient's medical history, factors leading to a false positive result: the presence of diabetes mellitus, autoimmune diseases and the use of immunosuppressive drugs such as rituximab, etc.

P126 | Kinetics of donor-derived cell-free DNA in the early kidney post-transplantation phase

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In solid organ transplantation donor cell-free DNA is released upon allograft injury and can be quantified as a donor-derived fraction (dd-cfDNA%). Association between increase of dd-cfDNA% and risk of allograft rejection were reported. Thus, persistently low dd-cfDNA % suggest absence of allograft injury and low risk of rejection. The dd-cfDNA% from 8 kidney recipients, from day 3 post-transplant was quantified by next generation sequencing (Devyser Accept cfDNA), simultaneously with DSA research by Luminex single-antigen (Immucor). Recipients showed different dd-cfDNA kinetics. Five patients showed exponential kinetics, comparable to literature data for stable transplantation, with dd-cfDNA% decrease, after an initial peak (mean of 0.7% day 10), and good renal function. No DSA where detected. Three patients presented abnormal kinetics. One presented, after initial improvement in eGFR and creatinine, a worsening of renal function with thrombocytopenia. The dd-cfDNA% on day 5 was 9.8%,then decreased to 0.6% on day 36 and 0.1% on day 124. Biopsy showed evidence of thrombotic microangiopathy, with no rejection. No DSA where detected. A second patient presented increase of dd-cfDNA% on day 10 (7.4%) and worsening of renal function markers, followed by a decline to 2.3% at day 17. Urinary tract infection was excluded. Luminex showed incremented MFI of preformed DSA DR7. Biopsy showed evidence of microvascular inflammations with no rejection. A third patient presented a good recovery of renal function, but a slight increase of dd-cfDNA% at day 11 (2.7%) with CRP peak and leukocytosis. Infectious tests showed a *K. pneumoniae* infection, then resolved. On day 36 dd-cfDNA% was 0.3%. Abnormal kinetics of dd-cfDNA% suggest a clinical or subclinical allograft injury, in line with literature data. The dd-cfDNA% may be also an early biomarker of rejection and de novo DSA development. Early graft injury detection may provide clinicians with opportunities for therapeutic interventions.

P127 | HLA mismatch and circulating donor-specific antibodies predict long term graft loss after kidney transplantation: a retrospective study from Campania region, Italy

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Donor-specific antibodies (DSA) are an established biomarker predicting antibody-mediated rejection, as the leading cause of graft loss after kidney transplantation. Furthermore, HLA matching offers a more precise assessment of donor-recipient HLA compatibility and may prevent more effectively, sensitization against allograft tissue. Indeed, increased numbers of HLA mismatches (MM) is significantly associated with a higher risk of immunological rejection, de novo DSA development, and graft failure. Over the last decade, a comprehensive approach to optimize kidney matching and monitor transplant recipients for acute and chronic graft dysfunction was the goal for the success of the kidney transplantation. In our long-term retrospective study, we have found that pre- and post-transplantation HLA antibodies were significantly associated with de novo DSA occurrence (pre-transplant HLA class I antibodies $p = 0.039$, pre-transplant HLA class II antibodies $p = 0.011$, post-transplant HLA class I antibodies $p < 0.01$ and post-transplant HLA class II antibodies $p < 0.01$). In addition,

HLA MM at HLA-A (hazard ratio (HR), 2.44; 95% confidence interval (CI): 1.15–5.16; $p = 0.01$) and DSA class I (HR, 10.24; 95% CI: 1.44–72.62; $p = 0.02$) appeared to be significant predictors of poorer graft survival. Our investigation demonstrates the long term experience of DSA occurrence in patients with kidney transplant in Campania region, Italy.

P128 | Performance of Devyser's NGS-based assay for monitoring donor-derived cell-free DNA in kidney allografted patients

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Donor-derived cell free DNA (dd-cfDNA) has emerged as a promising biomarker for monitoring solid organ transplanted patients to discriminate between active rejection and no rejection. To explore the analytical performance of Devysers new, next generation sequencing IVDR-certified Accept cfDNA kit based on 50 indels. We assessed sonicated binary plasma mixes (BPM) to mimic cfDNA at 167 base pairs at decreasing donor and cfDNA concentrations together with cfDNA samples from kidney allograft patients in the immediate post-transplant period. BPM samples were selected to maximize number of informative markers to assess the 0.1% dd-cfDNA limit of detection (LOD) and 10 ng input concentration established by Devyser. Total cfDNA was assessed for purity by TapeStation analysis and concentration was confirmed by Qubit fluorometry. BPM samples were tested both at a constant 10 ng input cfDNA and seven dilutions between 0.01% and 10% dd-cfDNA, and at a constant 1% dd-cfDNA and six dilutions between 0.5 and 50 ng input cfDNA. With 33 informative markers identified for the BPM samples, the dd-cfDNA LOD was 0.05% dd-cfDNA and 1 ng input cfDNA. The intra-assay and inter-assay CV at 0.1% dd-cfDNA was 19% and 17%, respectively and for 10 ng input 2% and 4%, respectively. In patient samples ($n = 12$) 9–22 informative markers were identified with 1–12 being homozygous markers. As expected, dd-cfDNA in our patient cohort was higher 1–3 days post-transplant (3.3%–18.5%) compared to samples taken up to a week post-transplant (0.1%–1.5%), and total cfDNA concentration did not correlate with percent dd-cfDNA. In conclusion, assessment of BPM samples proves the Devyser kit as a sensitive and robust assay to determine the fraction of dd-cfDNA and the LOD levels as set by Devyser at 0.1% dd-cfDNA and 10 ng input cfDNA are rational given the large variability in total number of informative markers present in transplant recipients.

P129 | Effect of serum dilution in the patient with antibody-mediated rejection undergoing therapeutic plasma exchange

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Therapeutic plasma exchange (TPE) is a valuable technique to reduce the remarkable level of antibodies present in the plasma. In the period 2019–2023, 6 patients with acute kidney transplant rejection diagnosed by renal biopsy and clinical parameters, were treated with a therapeutic plasma exchange procedure. Three to five procedures were performed on alternate days with the COM.TEC®. At each procedure, one 100% balanced plasma volume was exchanged, with a blood flow rate of 30–40 mL/min, and a plasma flow speed of around 26 mL/min. 5% albumin was used as the replacement fluid; calcium gluconate (1 vial in 100 mL of saline solution) was administered to balance the ACD-induced hypocalcemia. The average duration of each procedure was 165 min. Pre- and post-procedure laboratory checks were performed (blood count, coagulation, proteinemia, calcinemia). Differences in the number of procedures were estimated by following the MFI value of the anti-HLA-DSA antibodies of the patient, evaluated through Luminex SA analysis (One Lambda: $< 10{,}000 = 3$ procedures, $> 10{,}000 = 5$ procedures). The prediction of the effectiveness of the aphaeretic treatment has been made evaluating the reduction in the value of anti-HLA-DSA MFIs in 1:16 diluted serum with AB serum, to remove interference due to the prozone phenomenon depending on the Luminex SA technology used. In all patients it was found a

reduction in anti-HLA-DSA Abs, below the 1000 threshold, with maintenance of renal function and reduction of creatinine. Our results suggest that a quick assessment of the power of the anti-HLA-DSA antibodies in 1:16 diluted sera, and not in the whole one, is certainly an effective tool to evaluate both the number of treatments to be performed and the real effectiveness of the TPE.

P130 | The interpretation of the anti-HLA-A3 appearance after treatment with anti-thymocyte globulin

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The regular monitoring of the anti-HLA antibody status of kidney transplantation candidates is of key importance as well as post-transplantation monitoring. Nevertheless, the analysis interpretation can be challenging due to the effect of various drugs that must be administered before or after such surgical procedures. One such example is anti-thymocyte globulin (ATG), which is a polyclonal rabbit antibody raised against the Jurkat human T cell line (HLA-A3,32; B7,35), that is widely used in therapy because it leads to rapid depletion of T lymphocytes from the peripheral blood. We report the case of identification of de novo anti-HLA-A3 antibody in a 47 year old male patient who underwent his second living kidney transplantation receiving an HLA-A3 graft, treated with ATG (Grafalon, Neovii Biotech GmbH). Regular testing of the patient's serum over a long period (Luminex method) established HLA class I positivity before the second kidney transplantation, after which complications occurred in the form of delayed graft function. Unlike other class I antibodies, anti-HLA-A3 was identified (Luminex SAB, Immucor SA1) for the first time on the 9th day post-transplantation, bringing doubt about whether it is "real" DSA or not. After 7 days, the next serum test was performed and the disputed antibody disappeared. It was not detected in subsequent tests either. Prospective and retrospective cross-matching was performed by complement-dependent cytotoxicity (CDC) methods and the result was negative. In the meantime, the function of the graft improved and the patient was discharged from the hospital. This kind of influence of ATG on antibody testing has been described in the literature so far and this fact should always be kept in mind when interpreting the anti-HLA antibodies results.

P131 | CDC vs Luminex in HLA class I antibody detection—12 years' experience in Tissue Typing Laboratory, Rijeka

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Given that HLA allosensitization of patients on the waiting list for kidney transplantation can cause numerous adverse effects, determining the presence and specificity of HLA antibodies and identifying unacceptable antigens sometimes represents a great challenge regardless of the development of new technologies. This retrospective analysis included 283 serum samples that were tested positive by Lifecodes LSA Class I (Immucor GTI Diagnostics, Inc) assay during regular screening of patients on the kidney transplant waiting list performed at the Tissue Typing Laboratory, Rijeka, Croatia from 2012 to the end of 2023. All samples were tested in parallel by the CDC method, and based on the results, a comparison of two groups (CDC-LUM+ and CDC + LUM+) was made. Between the two groups, the differences in the medians of the highest mean fluorescence intensity (MFI) values for each HLA class I locus were analyzed using the non-parametric Mann–Whitney U test. Antibodies in the CDC + LUM+ group for all HLA loci had statistically significantly higher MFI values ($P < 0.001$) compared to the CDC-LUM+ group. Using the ROC-curve, MFI cut-off values for each HLA locus were determined. Antibodies with an MFI value higher than the cut-off value will be detected by both methods, while antibodies with an MFI value below the cut-off value will be detected only by Luminex. The MFI cut-off value for HLA-A was 6183, for HLA-B 12,341, while for the HLA-C locus it was 10,576.The model of calculated MFI cut-off values for HLA class I loci represents the correlation between the Luminex and CDC methods. Although Luminex has a high sensitivity in the detection of HLA antibodies, CDC method still has its role in determining unacceptable antigens in patients on the waiting list. The reaction of antibodies to recombinant antigens attached to Luminex beads should be interpreted complementary to the reaction to natural HLA antigens.

P132 | Optimizing a protocol for diluting patient serum with EDTA as a pre-treatment step towards suppressing the prozone effect in the detection of anti-HLA antibodies

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The detection of alloantibodies against HLA (alloAbs) is pivotal in the provision of organs for transplantation and for the diagnosis of ABMR. Screening for pre-existing alloAbs or detection of de novo DSAs is crucial for achieving better clinical outcomes. Solid-phase assays are widely used for their assessment. The so-called prozone effect is a phenomenon whereby neat sera with high titers of alloAbs may test negative while showing positive results after pre-treatment with EDTA. The aim of this study was to validate the optimal dilution of serum with EDTA that best eliminates the prozone effect. Twenty-seven serum samples from 26 patients were assessed at two EDTA concentrations (100 mM and 25 mM). AlloAbs were detected using the LABScreen Single Antigen Kit (One Lambda). The samples were analyzed with the LABScan3D platform. The difference between the total MFI and vPRA was used as an endpoint to assess the prozone effect in the serum samples treated with 100 mM and 25 mM EDTA. The Mann Whitney U test was applied to assess the MFI and vPRA from both group of samples. A $p < 0.05$ was considered to indicate statistical significance. Out of 27 samples, 14 tested negative after treatment with 100 mM EDTA, and 13 tested positive. At 25 mM, only 1 result was a true negative. In the remaining 92.87% ($n = 13$) of the samples, HLA class I and II AlloAbs were identified in 28.6% ($n = 4$) and 71.4% ($n = 10$), respectively. Similarly, 92.31% ($n = 12$) of the previously positive samples had increased specificity; 53.85% ($n = 7$) had new class I AlloAbs, and 46.5% ($n = 6$) had class II AlloAbs. The overall MFI increased on average to 110,780.65 (range 81.08–2,719,722.79; $P < 0.00001$), and the vPRA increased on average by 32.86% (range 0.06%–90.18%; $P < 0.00008$). Only two samples remained with equal results at both dilutions. Pretreatment with 25 mM EDTA to eliminate the prozone effect in the detection of anti-HLA Abs was superior to pretreatment with 100 mM EDTA.

P133 | Comparison of two single antigen bead assays for detection of anti-HLA antibodies and assessment of their complement-binding capacity

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Two single-antigen bead (SAB) methods are available for anti-HLA antibodies (Ab) detection and reactivity estimation, based on complement binding. This study aims at comparing the two methods in terms of anti-HLA Ab specificity detection and their complement binding capacity. Sera from 97 patients, with positive Panel Reactive Antibody Test (>5%), on kidney transplantation waiting list or transplanted, were tested by two SAB methods, Immucor (IC) and One-lambda (OL), for class I and II anti-HLA Ab specificities and their ability to bind complement (C3d or C1q). OL detected more class I positive specificities per patient than IC [25 (9–34) vs 18 (7–28), $p < 0.001$]. No difference was found in class II anti-HLA Ab. With IC, 19% and 15% of examined anti-HLA class I and II specificities respectively were positive, with MFI 4195 (1995–11,272) and 6706 (2647–13,184), respectively. Similarly, with OL 24% and 17% of anti-HLA class I and II specificities respectively were positive, with MFI 6185 (2855–12,099) and 9498 (3630–17,702)] respectively. In IC assay, 26% and 36% of positive class I and II specificities, respectively, bound the C3d fraction of complement. Complement binding occurred in higher MFI [class I: 13900 (9540–17,999) vs 2991 (1657–5674), $p < 0.001$, class II: 11832 (7128–16,531) vs 4152 (1798–9534), $p < 0.001$]. In OL, 25% and 24% of class I and II specificities respectively bound the C1q fraction, with

complement binding occurring in higher MFI [class I: 15452 (9369–23,095) vs 4446 (2435–8381), $p < 0.001$, class II: 18852(14,415–24,707) vs 6575 (2851–13,412), $p < 0.001$]. OL was more sensitive in detecting class I and II anti-HLA Ab, despite there being no difference in the number of class II specificities per patient. MFI of complement binding specificities were higher in both methods. The two assays were equivalent in detecting complement-binding class I Ab, however the C3d IC assay was more sensitive in detecting complement-binding class II anti-HLA Ab.

P134 | First experiences with detection of dd-cfDNA in heart transplant patients in North Macedonia

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Heart transplantation is the preferred therapeutical option for patients with end-stage heart failure. Endomyocardial biopsy is the current gold standard for cardiac allograft monitoring. It is an invasive procedure that has to be performed regularly especially during the first year post transplant. The aim of our study is to present our first experience with the detection of donor derived-cell free DNA (dd-cfDNA) in cadaveric heart transplant patients in North Macedonia. We analyzed 7 heart transplant patients, transplanted in the period from May 2020 to July 2023 at the University Clinic for Cardiac Surgery in Skopje, North Macedonia. The first cadaveric heart transplant was performed in our country in 2020 in the middle of the COVID pandemic, followed by 6 transplants in the next 2 years. Endomyocardial biopsy was performed with the help of doctors from abroad. Samples for detection of dd-cfDNA were collected in two Streck tubes for each patient and the cfDNA was isolated using QIAamp MinElute ccfDNA kit, Qiagen, Germany. The percentage of dd-cfDNA was analyzed using Alloseq cfDNA, CareDx Inc. Four of the patients were analyzed in two time points and three in one time point. Six of the patients had dd-cfDNA less than 1%, which was in concordance with the clean biopsy. One patient had 11.10% dd-cfDNA. He had a history of acute cellular rejection grade II 1 year post transplant, that after treatment remained chronic cellular rejection grade I. We will continue to monitor these patients in the next period but dd-cfDNA is a good non-invasive marker for early detection of rejection, optimizing levels of immunosuppressive medications to prevent rejection and minimize drug toxicities.

P135 | MicroRNAs as potential graft rejection or tolerance biomarkers and their dilemma in clinical routines behaving like devilish, angelic, or frightening elements

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Currently, different studies hypothesize that microRNA (miRNA) molecules play crucial roles in various biological processes, including transplantation, and that understanding their regulatory functions can contribute to predicting and understanding graft outcomes. It is suggested that miRNAs, which regulate gene expression at the post-transcriptional level, can serve as valuable biomarkers in health and disease, particularly in organ transplantation. The aim of this systematic review was to comprehensively explore the role of microRNA (miRNA) molecules in fundamental biological functions, such as cell cycle, differentiation, proliferation, and cell death. The genesis and functionality of miRNAs were delved into. On the other hand, the potential of miRNAs as circulating biomarkers in health and disease was analyzed, emphasizing their stability and resistance to freezing and thawing cycles. We delved deeper into miRNAs in kidney allograft transplantation, aiming to reveal insights into predicting graft status. The analysis was extended to the involvement of miRNAs in B cell function, humoral rejection, and donor-specific antibody (DSA) production during transplantation. The challenges and opportunities associated with the analysis of circulating miRNAs were also evaluated and miRNA expression profiles were evaluated, considering their potential as diagnostic and prognostic markers in transplants. Finally, the main

limitations and challenges in miRNA studies were addressed.

P136 | Validation of the flow cytometry crossmatch technique with the BD FACSLyric and calculation of new cut-off values

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Flow cytometry crossmatch (FCXM) has become an essential tool in organ transplantation as it has higher sensitivity than CDC-XM. This technique is used either in pre or post-transplantation to detect reactivity in patients with increased risk of antibody mediated rejection and poor graft survival. Recently CST-Porto acquired a BD FACSLyricTM6C to replace FACSCaliburTM. In order to validate our FCXM results, we compared results obtained with both cytometers. Validation was based on statistical analysis of correlation, detection limit and uncertainty. We compared 74 and 77 samples for T and B cell FCXM respectively, which revealed a strong correlation coefficient rate (Pearson's $r = 0.814$ for FCXM-T and $r = 0.824$ for FCXM-B). More, the power regression analysis showed a highly accuracy either for T ($r^2 = 0.9515$) or B ($r^2 = 0.9843$) FCXM. Calibur FCXM sample results were based on standard deviation (SD) 2.25 and 1.8 above negative control (NC) for T and B lymphocytes, respectively. EasyROC, was also used to find which SD (2SD; 2.5SD; 3SD) had the best performance and the cut-off value that has a better correlation between sensitivity and specificity. We obtained for T lymphocytes 2.5SD, cutoff value 1882 MFI, AUC 0.85 ($p < 0.0001$), sensitivity 100%, specificity 72% and for B cells 2SD, cut-off value 3570 MFI, AUC 0.77 ($p < 0.0001$), sensitivity 60.9%, specificity 93.3%. We established 2.5SD above the negative control value for predicting a positive FCXM either for T or B lymphocytes. Our results support the use of 2.5SD above the negative NC for predicting a positive FCXM either for T or B lymphocytes and a gray zone between 2SD and 2.5SD as doubtful. In conclusion, Lyric proved to give laboratory performance results with high sensitivity and reproductivity but with a different detection limit to Calibur. It also enables standardization protocols, automation and integrated solutions to optimize laboratory workflow and secure data results.

P137 | Challenges in immunological evaluation of four sensitized patients

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In a limited resources setting, work up of highly sensitized cases poses a tough challenge with discordant results between methods and laboratories. Four sensitized female patients of which one had Systemic Lupus Erythematosus were worked up for possible living donor renal transplant. Three patients were evaluated using CDC, Flow, Luminex crossmatch and Single Antigen Bead (SAB) assay. Samples from all patients were tested in more than one laboratory. Virtual crossmatch was positive for three patients with negative final physical crossmatch. Unexpectedly, there was discordance in results between different crossmatch methods and solid phase assays and between labs. With no national program for antibody assays, wide variation is observed for tests with expected concordance and between labs for same test which confuses clients. Luminex crossmatch is still widely used in India and often the results don't correlate with Flow crossmatch. The transplant team is forced to make clinical decisions with limited evaluation. Two patients, of which one was highly sensitized were successfully transplanted while one is still awaiting transplant. The fourth patient has been ruled out in view of high degree of reactivity. There is a requirement for a mandatory national external proficiency testing program for labs and more extensive evaluation is needed for sensitized patients.

P138 | Analysis of HLA-A, -B and -DRB1 genes in living donor kidney transplantation in Montenegro

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This study examines HLA-A, -B and -DRB1 polymorphisms in 50 kidney transplant pairs from Montenegro,

enrolled in the living donor kidney transplantation program between 2006 and 2017. In the group of patients ($n = 50$), for HLA-A, 4 allele groups have a frequency of ≥10%: A*02 (30%), A*24 (15%), A*03 (10%), A*26 (10%). For HLA-B, 3 allele groups have a frequency of ≥10%: B*51 (18%), B*35 (14%), B*18 (13%). For HLA-DRB1, 4 allele groups have a frequency of ≥10%: DRB1*01 (18%), DRB1*11 (15%), DRB1*15 (14%), DRB1*03 (10%). Also, two haplotypes have a frequency of 4%, haplotype HLA-A*02∼B*35∼DRB1*15 and haplotype HLA-A*02∼B*51∼DRB1*04. The most frequent European haplotype HLA-A*01∼B*08∼DRB1*03 is in the third place (3%). In the group of living donors ($n = 50$), for HLA-A, 3 allele groups have a frequency of ≥10%: A*02 (31%), A*03 (14%), A*24 (10%). For HLA-B, 3 allele groups have a frequency of ≥10%: B*51 (19%), B*18 (15%), B*35 (10%). For HLA-DRB1, 5 allele groups have a frequency of ≥10%: DRB1*15 (17%), DRB1*01 (14%), DRB1*11 (14%), DRB1*13 (14%), DRB1*04 (10%). Haplotype HLA-A*02∼B*51∼DRB1*11 has a frequency of 6%. In second and third place are haplotypes HLA-A*01∼B*08∼DRB1*03 and HLA-A*02∼B*27∼DRB1*01 with frequency of 4%. For 50 examined transplant pairs, six transplant pairs had 0 ABDR mismatches (MM), five pairs 1 MM, 11 pairs 2 MM, 16 pairs 3 MM, six pairs 4 MM, six pairs 5 MM and one pair had 6 MM. In summary, in our studied transplant pairs, the number of MM at all three HLA loci is as follows: at the HLA-A locus, 15 MM were observed, at the HLA-B locus 17 MM and at the HLA-DRB1 locus 17 MM. This study provides the very first data on HLA gene polymorphism for a Montenegrin population which is crucial for matching analysis in kidney transplantation, but also for future population and disease association studies.

P139 | HLA-antibodies in solid organ transplantation: Eplet analysis of a paired kidney cohort

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The standard of high-resolution typing of HLA loci in the setting of solid organ transplantation allows for the assessment of the so called eplet score. Eplets are the key elements of epitopes, consisting of superficially located amino acid residues and represent the actual target structures of HLA antibodies. Artificial intelligence enables us to calculate the eplet mismatch load between donor and recipient. Various eplet mismatch thresholds have been proposed up to which a transplantation should be safely feasible but have not yet been accepted in practice. We here examine a cohort in which both kidneys of one donor were allocated to two patients studied by us, looking for a correlation between the development of de novo donor-specific antibodies (dnDSA) and the eplet score. Eplet analysis was performed using HLA Matchmaker (HLA Fusion, HLA Matchmaker, One Lambda-ThermoFisher), high-resolution typing for 11 HLA loci was carried out using NGSgo-MX11-3kit (GenDx) and MiSeq Sequencer (Illumina). Monitoring for HLA antibodies was performed by SAB assay (One Lambda-lab screen). The paired transplant patient cohort consisted of 20 patients (10 pairs, 1 female, and 19 male) transplanted at age 32–71, followed up for 6 months to 8 years. Eplet mismatch analysis displayed a mean eplet mismatch load for HLA class I (A + B + C) and class II (DR + DQ) of 11 each. Six patients developed dnDSA which did not lead to graft loss. Patients with low eplet mismatch scores also developed dnDSA. Regarding the pairs, other than expected, not only the recipients with a higher eplet mismatch score developed dnDSA. We did not find a correlation between the number of eplet mismatches and the performance of the transplant. Our findings do not show a threshold of eplet mismatches below which the risk of dnDSA occurrence was absent. Nevertheless, the consideration of eplets will increase the sensitivity of immune risk assessment, there are still challenges to overcome before implementation as a standard.

IMMUNOTHERAPY, GENE THERAPY & NK CELLS

P140 | NK cell-based adoptive immunotherapy for controlling HLA class II antibody-mediated rejection in transplantation

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Antibody-mediated rejection (ABMR) is a major complication after transplantation that is associated with poor graft prognosis. The presence of donor-specific anti-HLA antibodies (DSA) class I and II, especially class II, increases the risk of developing ABMR. Current therapies are aimed at controlling the immune response, but there is no specific therapy to selectively reduce the generation of DSA. The objective was to establish a new therapeutic tool to specifically eliminate anti-HLA-DQ7 antibody-producing B cells by generating cytotoxic lymphocytes transduced with chimeric HLA-DQ7 antibody receptors (DQB17-CHAR). This is a pre-clinical trial, using NK cells, both the NK92 cell line and primary NK cells from healthy volunteers. The design, generation and production of DQ7-CHAR, with subsequent evaluation of its cytotoxic action and cytokine production, will be carried out using an in vitro model. We have identified and cloned the genetic sequence corresponding to HLA class II, specifically HLA DQ7 (both the α DQA1*05:01 domain and the β DQB1*03:01 domain). The DQ7-CHAR construct has been generated from domain binding; leader peptide (PL), extracellular domain (HLA-DQ7) and intracellular domains (from A2-CHAR, generated in our laboratory). We produced lentiviral particles with the construct in HEK-293T cells. After isolation of NK 92 cells line and primary NK cells from a voluntary donor, transduction and production of DQ7-CHAR-NKc is performed. We are developing a new selective and specific therapy against B lymphocytes producing anti-HLA class II antibodies. With the development of this therapy, we will have a new tool for the desensitization of hypersensitized patients as well as for the control of ABMR, improving the results of graft survival, without increasing the infectious complications associated with conventional immunosuppressive therapy.

P141 | Genetic engineering of limbs during ex vivo machine perfusion to overcome the unsolved hurdle of rejection

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Ex vivo organ perfusion represents a powerful delivery route for gene therapeutic agents such as lentiviral vectors and allows for organ specificity and safety. Genetic engineering of allografts towards reduction graft immunogenicity may support graft survival. In this study, we aimed to develop a modular platform that allows genetic modification of vascularized composite allografts (VCA). Rat hind limbs were perfused ex vivo under subnormothermic conditions for 4 h with lentiviral vectors encoding for nanoluciferase. Transduction efficiencies of the different hind limb tissues were determined by measuring the bioluminescence activity (Relative Luminescence Units, RLU). Tissue integrity was investigated by histological analyses, quantification of lactate dehydrogenase, and myoglobin. Genetic modification was detected in all tissue types, including vascular, muscular, and dermal tissues. Compared to the non-transduced negative control tissues (NCT), bioluminescence of vector-perfused regions was detectable at high levels 12 days after perfusion in the artery tissue of $7.1 \times 10^5 - 6.0 \times 10^5$ RLU (NCT: $4.5 \times 10^1 - 1.8 \times 10^1$ RLU) and in skin tissues of $9.1 \times 10^5 - 10.2 \times 10^5$ RLU (NCT: $(0.8 \times 10^2 - 0.2 \times 10^2$ RLU). In culture supernatants of the muscle bioluminescence values of $8.0 \times 10^5 - 6.2 \times 10^5$ RLU and $1.6 \times 10^6 - 1.4 \times 10^6$ RLU were detected. Levels of injury markers such as lactate, myoglobin, and lactate dehydrogenase, as well as histological analyses showed that ex vivo limb perfusion with lentiviral vectors did not cause tissue damage. Accordingly, limb cytokine secretion signatures were not significantly affected. These data demonstrate that permanent genetic modification by lentiviral transduction during ex vivo limb perfusion is efficiently possible. This platform has the potential to evolve into a robust method to overcome the currently unsolved VCA rejection problem by reducing their immunogenicity and taking VCA transplantation to the next level.

P142 | Elucidating the sequence features driving KIR diversification

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Natural killer (NK) cells play a pivotal role in the early immune response against infections and tumor formation. Killer cell immunoglobulin-like receptors (KIR) are key modulators of NK cell activity, displaying activating or inhibitory potential upon recognition of MHC class I molecules. The genomic organization of KIR genes is complex, involving copy number variation that currently results in over 70 region configurations documented in

humans. This diversity is generated through meiotic recombination, which also expands the KIR gene repertoire by the formation of hybrid entities. In rhesus macaques, the KIR region configurations display even more diversity, with over 100 distinct organizations identified in a relatively small cohort. More than half of these organizations exhibit hybrid genes, indicating a more pronounced mode of fast diversification of KIR regions in macaques, potentially driven by selective factors, such as reproductive success or pathogen resistance. So far, the sequence motifs facilitating these meiotic rearrangements in the KIR region are poorly understood. Different factors correlate with recombination rates, including gene density, sequence similarity levels, the presence of repetitive elements and the distribution of PRDM9 binding motifs. This latter zinc-finger protein initiates double-stranded breaks in the DNA, a key event in meiotic recombination. Examination of these different sequence features on 22 rhesus macaque and 13 human KIR region configurations revealed Long Terminal Repeats and PRDM9 binding motifs associated with recombination hotspots. Especially the DNA recognition patterns of PRDM9 might explain the differential recombination rates documented for the KIR region in humans and macaques. Overall, the diversification of KIR region configurations contributes to the adaptability of the immune system in its response to a wide range of pathogens.

P143 | Cytomegalovirus (CMV) intra-uterine transmission: Involvement of KIR receptors-HLA ligand interactions

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Cytomegalovirus (CMV) causes the most common congenital viral infection which may lead to deafness and neurodevelopmental delay. The maternal immune response plays an important role in preventing transplacental transmission of the virus, whose risk decreases in case of previous exposure. The clinical impact is higher in the first 14 weeks, when the fetal immune system is still developing. The clinical outcome is the result of a multifactorial process including maternal, placental and fetal factors. The extravillous trophoblast expresses HLA-C, -E and -G molecules that interact with KIRs on dNK, and the maternal-fetal immune cross-talk complex creates a tolerogenic niche for normal fetal development. The role of maternal/fetal HLA has been demonstrated in pregnancy complications but not in CMV transmission. We typed 54 CMV+ mothers and 35 fetuses by PCR-SSO and PCR-SSP: 21 with transmission of the infection (T) and 33 without (NT). The KIR3DL1 + A + B-Bw4 combination characterized T mothers (75% vs 45.16%, $p = 0.04$, OR = 3.6). In particular, the absence of KIR3DL1 + B-Bw4 was more significant among NT mothers (67.74% vs 35%, $p = 0.04$ OR = 3.9). Although not significantly, Bx genotype is more frequent among NT mothers (64.44% vs 35.56%). We also noted an increase in maternal KIR2DL1 + fetal HLA-C2 among T mothers (85.7% vs 66.67%). Then, comparing two twins (1 T and 1 NT), we observed that the maternal KIR-HLA-C2 interaction characterized the twin with vertical transmission. It seems that the inhibition of maternal NK cells favors the vertical transmission of CMV, and that there is also a role for the KIR:HLA-C interaction, known to be involved in correct placentation. Therefore, it will be interesting to investigate, in a broader set, also HLA-G and -E molecules in addition to the maternal MIC-A/B receptors, already known for their involvement in infection.

P144 | Post-injection monitoring of CAR-T cells targeting CD19 in the treatment of hematologic malignancies: Quantitative PCR versus flow cytometry

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CAR-T cells are an innovative therapy currently indicated for the treatment of hematological malignancies. They are engineered from patient's T cells and genetically transduced by a viral vector carrying a chimeric antigen receptor (CAR) that specifically targets patient's tumor cells. Yescarta® and Tecartus® are CAR-T cells targeting the CD19 receptor. They are indicated for the treatment of B cell hemopathies. The post-injection

monitoring of CAR-T cells at CHU Amiens is based on flow cytometry (FCM). The aim of this study is to assess the value of monitoring by another method: quantitative PCR (qPCR). We included 22 patients from our CHU treated with CAR-T cells. The integration of CAR anti-CD19 receptor into T-cell genome is achieved using a retroviral vector (MSCV). qPCR (ΔΔCt method) specifically amplifies the MSCV target reflecting the level of CAR-T cells present in the patient. qPCR was performed on samples already analyzed by FCM (CD19 CAR Detection Reagent, Miltenyi). We compared the evolution of CAR-T cells from the day of injection to 30 days post-injection (D30) in the same sample using FCM and qPCR. D30 was chosen because after this time we can seldom detect CAR-T cells on patient samples by FCM. We observed an excellent correlation between the two methods, especially when comparing the percentage of CAR-T cells in total lymphocytes with the fold change expected by qPCR ($r = 0.84$). The results of both assays, for the whole cohort of patients up to D30, show similar kinetics, with an expansion of CAR-T cells (from D7 to D12) followed by a decrease. We also quantified the presence of CAR-T cells beyond D30 by qPCR. Although the fold change values are very low, they can still be detected in most of our patients. qPCR is a simple and accurate method for monitoring patients treated with CAR-T cells. qPCR results correlate with those of FCM and appears to be more sensitive for patients followed after D30.

P145 | Short term follow up of soluble immune checkpoints in renal transplantation

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Soluble immune checkpoint molecules (sICMs) play a crucial role in controlling immune reactions and could be key to improving outcomes in organ transplants, patient monitoring, and the development of new immunotherapies. In this study, we measured levels of 12 sICMs, including sCD25, 4-1BB, sCD27, CD86, Free Active TGF-β1, CTLA-4, PD-L1, PD-L2, PD-1, Tim-3, LAG-3, and Galectin-9, using a flow cytometry-based multiplex bead assay. We analyzed frozen serum samples from 30 kidney transplant recipients taken before transplantation, on the third post-operative day, and on the seventh post-operative day, alongside samples from 15 healthy individuals. Kidney function was assessed by measuring creatinine and glomerular filtration rate (GFR), calculated using the CKD-EPI formula, at different times through a retrospective review. We used statistical tool SPSS 27.0, employing methods such as Tukey HSD, One-way ANOVA, Kruskal Wallis Test, and Mann Whitney-U test with Bonferroni correction. Our analysis revealed significant differences in the levels of sCD25, Galectin-9, PD-L1, TIM-3, sCD27, and PD-L2 between patients at pre-transplantation and healthy individuals. Changes were also noted in the levels of these molecules from the day of transplantation to day seven, indicating a dynamic regulation of immune checkpoints in kidney transplant patients. Notably, Galectin-9 levels on day seven showed a correlation with kidney function indicators like creatinine and GFR. Additionally, Galectin-9 levels on the seventh day exhibited correlations with creatinine levels and glomerular filtration rate on the fourteenth day after transplantation. These findings highlight the fluctuation of soluble immune checkpoints in patients with chronic kidney disease versus healthy individuals and suggest their potential in adapting immune responses post-transplantation. This opens avenues for further research into their role in improving transplant outcomes through immunotherapy.

P146 | Analysis of HLA matching between deceased donors and cord blood units from a national bank network as a basis for potential platforms for chimerism-based immune tolerance after solid organ transplantation

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Obtaining immune tolerance through donor hematopoietic chimerism in solid organ transplant (SOT) recipients

is feasible, although challenging in the HLA-disparate setting such as in most SOTs from deceased donors. Despite being a "third-party" from an immunological point of view, banked cord blood units (CBUs), may theoretically represent an interesting source of hematopoietic stem cells (HSCs) for the purpose of immune tolerance after SOT. We described here the actual HLA match between a sample of $n = 1427$ deceased multi-organ donors after brain death (DBD) in Italy between Jan 2021 and July 2022 and $n = 38{,}743$ CBUs banked in the Italian Cord Blood Network. For DBD, HLA typing resolution was serological or based on nucleic acid testing (one-field) at HLA-A, -B, -C and -DRB1. HLA typing of the CBUs was heterogeneous in terms of resolution and loci typed with the majority ($n = 26{,}947$, 69.5%) typed for HLA-A, -B and -DRB1. For $n = 186$ DBD (13%) there was at least one 6/6 HLA-matched CBU available in the network (median 1, range: 1–14). Among the remaining $n = 1241$ DBD without a 6/6 HLA-matched CBU, there were $n = 824$ (58% of the total donors) with at least one 5/6 HLA-matched (median 5, range: 1–71). Overall, 71% of DBD ($n = 1010$) had at least one 6/6- or 5/6 HLA-matched CBU. For any of the $n = 186$ DBD with at least one 6/6 HLA-matched CBU there were also $n = 5074$ available 5/6 HLA-matched CBUs, with $n = 1441$, $n = 1697$ and $n = 1936$ mismatches at HLA-A, -B and -DRB1 respectively. The present collaborative analysis showed that 71% of deceased organ donors match with at least one suitable CBU (≥5/6), reducing to 13% for more stringent matching of 6/6. In addition to being a source of HSCs, CBUs provide also other cellular populations and therefore they might be used for new applications in SOT, providing that cell therapies (i.e. mesenchymal, CAR-T, Tregs) are proving to be effective in obtaining immune tolerance. Additional research is needed to understand the feasibility of this approach.

P147 | Possible role of KIR3DL2/HLA-A*11:01 interaction in SARS-CoV-2 infection severity

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The clinical course of SARS-CoV-2 varies due to differences in innate and adaptive immune response mechanisms. Natural killer (NK) lymphocytes play a crucial role in defense against viral infections, with their activity strongly regulated by Killer cell Immunoglobulin-like Receptors (KIR) expressed on NK cell surfaces. Among the inhibitory KIR receptors, KIR2DL1 recognizes HLA-C allotypes characterized by Lysine 80; KIR2DL2/L3 bind HLA-C allotypes Asparagine 80; KIR3DL1 recognizes HLA-B and HLA-A with Bw4 epitope and KIR3DL2 recognizes HLA-A*03 and HLA-A*11. The study investigates the impact of KIRs and their ligands on SARS-CoV-2. High-resolution class I HLA typing of 419 voluntary HSC donors from RRAQ01, not vaccinated and testing positive for SARS-CoV-2, was conducted. The patients were divided in 2 groups based on the severity of the disease: group A (asymptomatic or pauci-symptomatic; $N = 139$) and group B (severe symptomatic; $N = 280$). Of the 419 donors, 55 (13.1%) were HLA-A*11:01+. 8/139 (5.7%) in group A, while 47/280 (16.8%) in group B were HLA-A*11:01+. Our data indicate that KIR3DL2 + HLA*11:01 + patients [χ^2 (yates) = 8.9669, $p = 0.0027$] exhibited more severe disease outcomes. KIR3DL2, a frame gene present in almost all genotypes, encodes an inhibitory receptor which, in presence of a ligand (HLA-A*11:01) could inhibit the recognition of the infected cell, causing a lack of protection against SARS-CoV-2, leading to more severe outcomes. Since KIR3DL2/ligand interaction appears to be strongly peptide dependent, an immunopeptidome analysis could clarify the KIR3DL2 and viral peptide-presenting HLA antigens interaction. Furthermore, since 116 different KIR3DL2 allotypes are currently in the IPD-KIR Database, KIR3DL2 allelic polymorphism could provide interesting indications. More studies on other populations and functional studies are necessary to confirm the real involvement of KIR3DL2 in HLA-A*11:01 immunological response in SARS-CoV-2 infection.

P148 | Potential co-partnership in predisposition to COVID-19: KIR-HLA class I pairs control NK cells activity and T cells discriminate between HLA-C C1 and C2 groups in Antiviral Immunity

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COVID-19 caused wide morbidity and mortality on a global scale. Understanding innate and adaptive immune responses is essential to decode mechanisms of host responses. Many factors affect the clinical variability, but genetic factors may contribute to individual differences in susceptibility. HLA is a complex of highly polymorphic genes that have central functions in immune regulation. HLA-I antigens are involved in presenting foreign antigens to CD8+ T cells, and some alleles are ligands for KIRs expressed on NK cells. KIR2D molecules recognize HLA-C1 and C2 ligands, based on dimorphisms at positions 77 and 80. To identify KIR-HLA combinations involved in the outcome, we analyzed 70 hospitalized patients (February–May 2020) and 12 controls not infected during COVID-19 pandemic. KIR and HLA genotyping was performed by Luminex-PCR SSO and PCR SSP. KIR2D2L1+ C2+ (OR = 4.6, $p = 0.02$) combination was encountered at a significantly higher frequency in patients vs the control group (69.6% vs 33.33%). Notably, C1/C2 was significantly higher (46% vs 8.33%) and C1/C1 was significantly lower (27.53% vs 58.33%, $p = 0.02$) in patients vs controls. Interestingly, HLA-C2 characterized the patients (72.46% vs 41.66% $p = 0.04$ OR = 3.68). Our data seem in line with the functional model for KIR-HLA mediated hierarchy of inhibition, although the sample should be increased, mainly the control group. KIR2DL1-C2 interaction could determine a strong inhibition that is difficult to overcome by activating signals, leading to lack of target killing. On the other hand, we only found a very slight increase of KIRD2S1 + C2+ among patients (33.33% vs 25%). In addition, immunopeptidomic analysis revealed that Ser77 in C1 and Asn77 in C2 influence peptide presentation and HLA-C restricted T cell responses, where C1 favors small and C2 prefers large residues, with implications in disease. In COVID-19 infection, viral escape mutants might elude T cell recognition of C1 allotypes by increasing peptide fragments size.

P149 | KIR haplotyping using allele-level KIR typing results from NGSengine

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The killer-cell immunoglobulin-like receptor (KIR) locus is characterized by a high diversity in haplotypes, showing high levels of structural variation, copy number variation and allelic polymorphism. Allele-level KIR genotyping became feasible with the advent of Next-Generation Sequencing (NGS). However, establishing gene copy number and haplotyping KIR remains challenging. Using NGSengine® software (GenDx) and a separate KIR haplotyping tool (GenDx), haplotypes can be inferred without error prone and time-consuming manual analysis. Manual KIR haplotyping is complex, due to the relatively high frequency of various uncommon KIR haplotypes. The haplotyping tool uses KIR typing results in csv format, exported from NGSengine, and compares the genotype with a list of 92 published haplotypes. The output of the tool are two haplotypes, whereby copy numbers and any ambiguities are inferred. In total, 70 samples were sequenced for 13 KIR loci using the NGSgo-AmpX KIR (GenDx) strategy. The panel consisted of 58 Get-RM cell lines (Coriell institute) sequenced on a PacBio Sequel II and 12 UCLA proficiency samples sequenced on a MiSeq (2x150bp) for validation. In total, the inferred copy number of 85% of the samples could be linked to published haplotypes. Observed haplotype A and B group frequencies were in line with expected frequencies, although uncommon haplotypes were identified as well. The automated KIR haplotyping approach supports the interpretation of NGS-based KIR data. It reduces error rates and contributes to future refinement of KIR genotyping using NGSengine, taking the next step towards unambiguous KIR haplotyping by NGS.

P150 | TLR1 gene polymorphism in SARS-CoV-2 bilateral pneumonia

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SARS-CoV-2 contains molecular components recognized by various immune system sensors, including membrane lipoproteins that bind to the TLR1 cell receptor. The effectiveness of this binding and the triggering of subsequent innate and adaptive immune responses may, among other factors, depend on genetic polymorphisms

in the TLR1 gene, in particular the substitution of guanine with adenine at position 743 (743G > A). The objective of this study was to determine the association of the 743G > A polymorphism of the TLR1 gene with the development of bilateral pneumonia in COVID-19. The study group consisted of 99 patients with bilateral COVID-19 pneumonia of moderate ($n = 57$) and severe ($n = 42$) forms (diagnosis determined by computed tomography and confirmed by detection of SARS-CoV-2 RNA in nasopharyngeal swabs). All the individuals studied considered themselves to belong to the Russian ethnic group, and their ancestors lived in the Chelyabinsk region for three generations. The control group consisted of data on a population of European ancestry ($n = 99$) from the open database 1000 Genomes. Genotyping was performed using polymerase chain reaction with amplification created restriction sites followed up by restriction fragment length polymorphism analysis. The groups were compared using Pearson's χ^2 test; differences were considered significant at $p \leq 0.05$. As a result of the study, it was determined that the frequencies of the 743G > A polymorphism in patients with moderate (85.1%) and severe (79.8%) forms of pneumonia are not significantly different and do not differ from the frequency in the control group (84.8%; $\chi^2 = 1.31$ $p = 0.519$). It can be concluded that there is no association between the 743G > A polymorphism of the TLR1 gene and the development of bilateral pneumonia in COVID-19. The research was supported by the Russian Foundation for Basic Research, project № 20-44-740004.

P151 | Allele-level characterization of KIR gene polymorphism in healthy elderly populations from Bulgaria, Romania and Turkey

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Aging is defined as the accumulation of changes in a human body over time and is a critical aspect of human health. One of the immunological manifestations of advanced age is the decline in NK cell function. In the "Immunogenetics of Aging" component within the 18th International HLA and Immunogenetics Workshop, we pursued the hypothesis that certain polymorphisms identified within KIR genes might be associated with longevity. Unrelated healthy individuals from three populations (Bulgarian, Romanian, and Turkish) and two age groups were investigated: elderly (ages 65–99 years) and young (ages 18–35 years). Data was generated through NGS. Our findings indicated some notable variations in allelic frequency across age groups. In elderly Bulgarians, we observed a slightly increased frequency of the KIR3DL2*01001 allele ($p = 0.02$) of the inhibitory framework gene. In the Romanian population, two alleles that belong to common allotypes of the KIR2DL2/3 locus were found with highly significant increased frequency in healthy elderly: KIR2DL2*00101 ($p < 0.001$) and KIR2DL3*00201 ($p = 0.005$). KIR2DL2 and KIR2DL3 have similar specificity by binding to all C1 HLA-C allotypes and some C2 allotypes, but different in their avidity. Thus, the prevalence of KIR2DL2 and KIR2DL3 alleles would provide additional inhibitory signals to NK cells, even in the absence of C2-specific receptors. Furthermore, some inhibitory KIR3DL3 alleles were observed with increased frequency in elderly Turks: KIR3DL3*00701 ($p = 0.01$) and KIR3DL3*00206 ($p = 0.02$). In conclusion, while our study reveals some possibly beneficial roles of certain inhibitory KIR alleles in aging, it underscores the need for further investigations to understand these mechanisms and their broader implications. Focusing on specific southeast European populations, our research emphasizes the importance of studying diverse ethnic groups in understanding aging and immune senescence.

P152 | Distribution of KIR genes in a Romanian cohort

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Killer cell immunoglobulin-like receptor (KIR) represents a family of genes clustered in one of the most variable regions of the human genome. KIR receptors show the highest degree of diversity at the level of NK cells and

have a stimulatory or inhibitory effect on these cells. The main purpose of the study was to determine the KIR allele and genotype frequencies in the analyzed Romanian patients. The current study includes 266 unrelated Romanian patients which underwent genetic testing for 16 KIR genes. The genotyped KIR alleles were KIR2DL1, 2DL2, 2DL3, 2DL4, 2DL5, 2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 3DL1, 3DL2, 3DL3, 3DS1, 2DP1, 3DP1. KIR genotyping was performed by the PCR-SSO technique, using DNA extracted from whole blood collected in EDTA. The most frequent KIR genotype observed in our patients was the AB genotype (58.6%), followed by AA (28.1%), BB being the least frequent genotype (13.1%). Genotype AA was assigned for patients carrying the KIR haplotype A associated genes (KIR2DL1, KIR2DL3, KIR2DS4 and KIR3DL1). Patients lacking one of these four alleles were associated with the BB genotype. Other combinations of KIR genes are considered to be genotype AB. The results of the current study reveal the frequencies of KIR genes and genotypes found in the Romanian population. Further studies are needed for the confirmation of the results and for the future involvement in and support for the national transplantation programs.

P153 | Killer cell immunoglobulin-like receptor genes and their HLA ligands in southern Tunisia

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Killer cell immunoglobulin-like receptors (KIR) are found on the surface of natural killer (NK) cells and certain T cell subsets. These receptors play a crucial role in modulating the activity of NK cells, either by inhibiting or activating their function, and have been implicated in the development of several human diseases. HLA class I molecules serve as ligands for certain KIR. Exploring the diversity of KIR may help to identify specific KIR and HLA genotypes that influence susceptibility to infectious and autoimmune diseases, as well as transplant outcomes. The aim of this study was to investigate the distribution of KIR genes and HLA ligands in the population of southern Tunisia. Our study population consisted of 116 healthy unrelated individuals from southern Tunisia typed for HLA class I by NGS (Next Generation Sequencing). KIR typing was performed by sequence specific primer (SSP) PCR. Twenty-four KIR genotypes were found in our population. The AA haplotype group was the most frequent (27.58%). Twenty-three KIR genotypes belonged to the Bx haplotype group. KIR2DL4, KIR3DL2, KIR3DL3 and KIR3DP1 were found in 100% of the individuals. Among the ligands, HLA-C2 was more common than HLA-C1 (80.1% vs. 629%), with the majority of individuals (43.1%) expressing both C1 and C2, while 37.06% and 19.82% were typed C2/C2 and C1/C1, respectively. HLA-Bw4 and HLA-A3/A11 expression was detected in 64.6% and 18.9% of individuals, respectively. In this study we have analyzed the frequencies of KIRs and their HLA ligands in our southern Tunisian population. These results can be used as a reference for future association studies.

P154 | KIR/HLA pairs in Southern Tunisia

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Killer cell immunoglobulin-like receptors (KIR) are located on the surface of natural killer (NK) cells and specific subsets of T cells. They have a significant impact on the regulation of NK cell activity, either by inhibiting or activating their function, and can influence susceptibility to infectious and autoimmune diseases, as well as transplant outcomes. Some KIR genes are recognized by and act as ligands for HLA class I molecules. In this study, we investigated the prevalence of some KIR/HLA pairs in the South Tunisian population. Our study included 116 healthy unrelated individuals from southern Tunisia. Expression of C1/C2 and Bw4 ligands was deduced from next generation sequencing (NGS) HLA class I genotyping. KIR genotyping was performed by sequence specific primer (SSP) PCR. The receptor/ligand pairs analyzed included inhibitory KIR/HLA pairs: KIR3DL1 with Bw4, KIR2DL2/3 with HLA-C1, KIR2DL1 with HLA-C2 and KIR3DL2 with HLA-A3/A11 and activating KIR/HLA pairs: KIR2DS1 with C2, KIR2DS2 with C1 and KIR3DS1 with Bw4. The observed frequencies of

different matched inhibitory KIR/HLA pairs in descending order of prevalence were as follows: KIR2DL1 with C2 (78.44%), KIR3DL1 with Bw4 (63.79%), KIR2DL2/3 with C1 (62.93%) and KIR3DL2 with A3/11 (18.96%). The presence of three pairs was the most common (38.79%), followed by two pairs (37.93%), one pair (18.1%) and four pairs (3.45%). Two individuals (1.72%) had no inhibitory KIR/HLA pair. The observed frequencies of different matched activating KIR/HLA pairs were as follows KIR2DS1 with C2 (44.82%), KIR2DS2 with C1 (31.03%) and KIR3DS1 with Bw4 (15.51%). In conclusion, these results can be used as controls for future investigations. Further studies are needed to understand how KIR/HLA combinations might influence disease.

MHC EVOLUTION, POPULATION GENETICS

P155 | Conserved extended 8.1 ancestral haplotype—The polymorphism beyond it

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The 8.1 ancestral haplotype (AH) is a common Caucasoid haplotype carried by most people who type for HLA-A1, B8 and DR3. It has been proposed that a small number of genes within the 8.1 AH modify immune responsiveness and affect multiple immunopathological diseases. In this study we aimed to investigate whether the conserved extended 8.1 AH exists on high resolution level and what is the polymorphism beyond it. In this study 141 unrelated donors previously typed as HLA-A*01, -; B*08, -; DRB1*03, - were used. NGS typing was performed for HLA-A, -B, -C, -DRB1, -DRB3, -DQA1, -DQB1, -DPA1 and -DPB1, with full gene coverage except for HLA-DRB1, -DRB3 and -DQB1 genes where regions of exon 1 and exons 4–6 were omitted (NGSgo, GenDx). On class I loci, two different HLA-A alleles (HLA-A*01:01:01 and A*01:38), four different HLA-B alleles (HLA-B*08:01:01, B*08:01:01:02, B*08:01:01:07 and B*08:01:01:08) and four different HLA-C alleles (HLA-C*07:01:01, C*07:01:01:06, C*07:02:01 and C*07:07) were observed. Four individuals were heterozygous at HLA-DRB3 with HLA-DRB3*01:01, 02:02 typing; two individuals were heterozygous at HLA-DQA1 and DQB1 loci with HLA-DQA1*03:01:01~DQB1*03:02 and DQA1*01:02:02:01~DQB1*05:02 present in the second haplotype. At HLA-DPA1 locus a total of 10 different alleles were observed while for HLA-DPB1 locus 18 different alleles were detected. A total of 45 out of 141 individuals (31.91%) were homozygous for all nine loci and typed as: HLA-A*01:01:01~C*07:01:01~B*08:01:01~DRB1*03:01P~DRB3*01:01P~DQA1*05:01:01~DQB1*02:01P~DPA1*02:01P~DPB1*01:01:01 or A*01:01:01~B*08:01:01~C*07:01:01~DRB1*03:01P~DRB3*01:01P~DQA1*05:01:01~DQB1*02:01P~DPA1*01:03P~DPB1*04:01P ($N = 36$, 25.5% and $N = 9$, 6.4%, respectively). Eight novel alleles were observed (three on HLA-DQA1 and one allele at HLA-B, C, DRB1, DRB3 and DPA1). In the era of personalized medicine, the future studies should focus to the role and clinical relevance of the polymorphisms within 8.1 AH.

P156 | The strength of the humoral immune response to mRNA SARS-CoV-2 vaccination is influenced by HLA type of the vaccinee

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There is marked variation in individuals' immune response to vaccination, which could be attributable to genetic factors. HLA molecules have been recognized as significant influencers in adaptive immune responses to various vaccines, including hepatitis B, measles, and influenza. Recent studies revealed the influence of HLA, coded by particular alleles, on humoral and cellular immune response to vaccination against COVID-19. In the present study we sought to investigate the contribution of vaccinated individuals' HLA type to the variation in strength of humoral response to the vaccination with the mRNA SARS-Cov-2 (Comirnaty, Pfizer/BioNTech) vaccine. A group of 120 health care workers were vaccinated for the first time at the beginning of 2021. After 6–8 weeks, concentration of antibodies was determined in vaccinees' sera using Architect SARS-CoV-2 IgG II Quant reagents. Control group (CG) was composed of 147 randomly chosen subjects. Both groups were typed for HLA-A, -B, -C, -DRB1 and -DQB1 by next generation sequencing (NGSgo-GenDx). Vaccinated individuals were divided in the groups of very strong responders (>20,000 AU/mL) (VSRG), responders (50–20,000 AU/mL) (RG) and non-responders (<50 AU/mL). Statistical analysis was performed using two tailed Fischer's exact test. A highly significant increase in frequency of A*03:01 was observed

in the VSRG compared to CG ($p = 9 \times 10^{-4}$) as well as when compared to RG (1×10^{-4}). Moreover, while DQB1*06:02 was also found significantly associated with very strong response to vaccination, A*24:02 seem to have an influence on non-responsiveness. Our results confirm the influence of vaccinated individual's HLA-type on humoral immunity, especially on the strength of the response to vaccination, where A*03:01 plays a crucial role.

P157 | Allelic HLA-DPA1 ~ DPB1 haplotype analysis in a large Chinese population of 584 families

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HLA-DP is an important part of human MHC system. It consists of heterodimers of DPA1 and DPB1. The frequencies of different HLA-DPA1 and -DPB1 alleles were extensively reported based on a large number of unrelated individuals with statistical analysis tools. However, the HLA-DPA1 ~ DPB1 haplotype studies at the allelic level based on large family samples are lacking. In this study, a next generation sequencing method was applied to define 4-field allelic typing of HLA-DPA1 and -DPB1 for 1797 individuals from 584 Chinese families. HLA-DPA1 ~ DPB1 haplotypes were determined by allele segregation within a nuclear family. In total, 27 HLA-DPA1 alleles and 61 HLA-DPB1 alleles were detected and the alleles with frequencies over 10% were DPA1*02:02:02:01 (0.4670), DPB1*05:01:01:01 (0.3055), DPA1*01:03:01:01 (0.1640), and DPB1*02:01:02:01 (0.1348). The frequency distribution of HLA-DPA1 and -DPB1 alleles in this study population is similar to that of Asian Pacific Islanders and Asian Americans. A total of 104 HLA-DPA1~DPB1 haplotypes were found. HLA-DPA1~DPB1 haplotypes with near 100% linkage disequilibrium were observed, such as DPA1*02:01:01:06~DPB1*13:01:01:01 and DPA1*02:01:01:03~DPB1*17:01:01:01. The haplotype DPA1*02:01~DPB1*02:02 and DPA1*04:01~DPB1*05:01 appear to be unique in Chinese and other Asian populations. The strong linkage disequilibrium of HLA-DPA1 ~DPB1 is likely a result of geographic characteristic positive functional selection and physical closeness between DPA1 and DPB1 genes limiting their recombination.

P158 | Complement MHC Bf alleles show trans species evolution between man and chimpanzee

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HLA has been associated to many diseases, particularly autoimmunity. However, only statistical phenomenological and non-pathogenetic description has been achieved after decades of studies. This shows that MHC single locus association studies are probably of limited usefulness. Extended HLA (class I and class II genes) haplotypes should also be studied conjointly with class III or complement alleles (complotypes). Complotypes in humans are defined as alleles belonging to C2, C4 and Bf genes/proteins. In addition, we have recently remarked that the placing of MHC class I and class II genes together with complement genes from at least birds to humans points to a strong positive directional selection to maintain certain MHC class I, II and III alleles together in order to fight microbes, self-maintenance and avoid autoimmunity; this MHC chromosome stretch is usually transmitted in a block. In the present paper we show that this type of selection may also work on primate alleles since we describe two orangutan new Factor B class III alleles (Popy-Bf*A01 and Popy-Bf*A02) in two different individuals that bear a rare allelic variation identical to other found in human (rs641153) and chimpanzee (Patr-Bf*A01).Finally, the same pathway is presently being followed with non-classical class I HLA genes single alleles association to disease and thus same negative results may be achieved if same single locus methodology is followed.

P159 | Genotypic frequencies of mutations associated with Alpha-1 anti-trypsin deficiency in unrelated bone marrow donors from the Murcia Region donor registry in the Southeast of Spain

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Alpha-1 antitrypsin-AAT1 deficiency (AAT1D) is an inherited disease with an increased risk of chronic obstructive pulmonary disease (COPD), liver disease, and skin and blood vessel problems. AAT1D is caused by mutations in SERPINE1 gene. The development of COPD requires both a genetic predisposition and the contribution of an environmental factor. The aim of this study was to analyze the genotypic frequencies of mutations associated with AAT1 deficiency in the donor registry of the Region of Murcia in southeastern Spain due to the high risk of presenting with different pathologies and underdiagnosis in the population. A total of 112 DNA-healthy voluntary unrelated bone marrow donors from different parts of the Region of Murcia were analyzed retrospectively. AAT1 deficiency patient testing involved an automated biochemical screening routine. The three main variants, Pi*M, Pi*Z, and Pi*S, were analyzed in the SERPINE1 gene. Our results showed a frequency of 3.12% of the Pi*Z (K342) mutation in over 224 alleles tested in the healthy population. The frequency of Pi*S (V264) was 11.1%. The frequency of the haplotype with the most dangerous mutation, EK342 EE264, was 4.46%, and the frequency of EK342 EV264 was 1.78% in the healthy population. Frequencies of other EE342 EV264-mutated haplotypes accounted for 18.7%. As for the EE342 VV264 haplotype, 0.89% of the total healthy population presented heterozygous for the EV264 mutation and one individual presented homozygous for the VV264 mutation. In conclusion, the frequencies of Pi mutations in the healthy population of the Region of Murcia were not remarkably different from the few studies reported in Spain. The genotype and haplotype frequencies followed the usual pattern. Health authorities should be aware of this high prevalence of the Pi*S allelic variant and pathological genotypes such as Pi*MZ and Pi*SZ in the healthy population if they consider screening the smoking population.

P160 | High-throughput genotyping of HLA-E, HLA-F and HLA-G and analysis of allele frequency distributions in Croatia

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The HLA class I and class II genes have a critical role in presentation of antigen peptides, organ transplantation and autoimmune diseases. However, other functional HLA genes have also been detected, the so-called non-classical HLA class Ib genes: HLA-E, -F, -G. Their main function is the modulation of immune responses, both in physiological and pathological conditions. In this study we characterized the allelic diversity of the non-classical HLA class Ib genes in the Croatian population. High resolution genotyping of HLA-E, -F and -G of 111 cadaveric donors from Croatia was performed by next-generation sequencing (NGS) on the Isoseq platform, followed by direct counting to obtain allele frequencies. We detected a total of 11 HLA variants at the second field resolution level for the tested loci (three at HLA-E, three at HLA-F and five at HLA-G locus). Two alleles were equally distributed in HLA-E (HLA-E*01:01–55.9% and HLA-E*01:03–42.3%), while HLA-F*01:01 (85.6%) and HLA-G*01:01 (77.5%) were predominant for the other two loci. The analysis at four-field resolution level revealed 12 different alleles at HLA-E locus with HLA-E*01:01:01:01 being predominant (52.3%); 17 alleles at HLA-F locus with HLA-F*01:01:01:01, HLA-F*01:01:01:08 and HLA-F*01:01:01:09 alleles being equally distributed (20.3%, 22.1% and 18.9%, respectively); and 13 alleles at HLA-G locus, where HLA-G*01:01:01:01 and HLA-G*01:01:02:01 alleles demonstrated the highest frequencies (27.5% and 20.3%, respectively). The frequencies observed in this study cohort are similar to those reported in other studies from Europe. This study emphasizes the importance of NGS technologies in studying the allelic polymorphism and defining the diversity of non-classical HLA class Ib genes. This leads to a better understanding of their global distribution and provides necessary information for the subsequent gene-disease association and transplantation studies.

P161 | HLA association study with bipolar disorder in South Tunisian population

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Bipolar disorder (BD) is a chronic and cyclical affective disorder characterized by mood changes with recurring phases of mania and depression. It is an important public health problem, and its management still relies on clinical assessments due to the lack of reliable biomarkers to guide diagnosis, prognosis or disease course. Recent genome-wide association studies (GWAS) implicate the HLA region in BD. HLA associations with BD were investigated in different populations but not in Tunisia. Our aim was to study HLA association with BD in South Tunisia. Twenty-five BD type I patients (recruited during a period of 6 months) and 123 unrelated healthy controls were enrolled. HLA class I (HLA-A and -B) typing was performed using a microlymphocytotoxicity complement-dependent technique completed by molecular typing. Statistical analysis was performed using Excel. HLA marker polymorphism was compared between the two groups. A positive association of HLA-A9, particularly HLA-A23 split antigen was found in our patients compared to controls (56% vs 24.39%; $p < 0.01$; OR = 3.95 and 28% vs 8.13%; $p = 0.01$; RR = 4.39, respectively). On the other hand, HLA-A19 super-type (A29, A30, A31, A32, A33 and A74) was negatively associated with the disease (24% versus 47.15%; $p = 0.03$; RR = 0.35). The frequency of HLA-B53 in BD patients compared to controls was higher (20% versus 5.69%; $p = 0.05$; RR = 4.14). We have reported a positive association of the HLA-A23 and HLA-B53 antigens with BD in our South Tunisian population. However, a larger patient population size is needed to confirm the involvement of these antigens in the predisposition to this disease in our population.

P162 | HLA-B, -C, -DRB1, -DQA1, and -DQB1 alleles involved in genetic predisposition to psoriasis, and psoriasis associated with psoriatic arthritis in East Croatian patients

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Psoriasis vulgaris (PV) is a complex, chronic, immune-mediated disease. It has been shown that the major genetic determinant associated with psoriasis is located in the HLA region and that up to 40% of psoriasis patients develop psoriatic arthritis (PsA). This retrospective study aimed to investigate the association of HLA alleles and haplotypes with PV without signs of PsA, PV associated with PsA (PV + PsA), and to detect susceptibility alleles for PsA development in PV patients. The tested groups of patients from East Croatia consisted of 79 unrelated PV patients (32 male, 47 female) with a mean age of 47 ± 16 years, and 62 patients with PV + PsA (34 male, 28 female) with a mean age of 66 ± 14 years. HLA-B, -C, -DRB1, -DQA1, and -DQB1 alleles were genotyped using PCR-SSP (Olerup, CareDx) or PCR-SSO (Immucor Inc.) techniques. The results were compared with allele and haplotype distributions among the control group consisting of 120 healthy, unrelated, blood donor volunteers from East Croatia with no history of PV or PsA. The Fisher exact test and Odds ratio (95% CI) were performed to evaluate the correlation between HLA allele and haplotype frequencies in tested and control groups. The level of significance was set at $P < 0.05$. Our findings indicate a positive association of HLA-B*13 (OR 3.62, $p = 0.0089$), -B*38 (OR 3.29, $p = 0.048$), -C*06 (OR 2.79, $p = 0.003$) alleles, and HLA-DRB1*07∼DQA1*02∼DQB1*02 haplotype (OR 2.78, $p = 0.003$) with PV, as well as a positive association of the HLA-C*06 (OR 3.00, $p = 0.005$) with PV + PsA patients. A significantly lower incidence of alleles HLA-B*15, and -B*35 (OR 0.09, $p = 0.023$ and OR 0.33, $p = 0.001$, respectively) was observed in the PV group associated with PsA. A significantly higher frequency of HLA-B*27 was observed in the PV + PsA subset compared to PV (OR 3.70, $p = 0.01$). Our data are largely in agreement with previously published studies regarding the HLA risk alleles/haplotypes for PV, and PsA in patients with PV.

P163 | Genetic and anthropological relationships between Canary islands and Azores islands: The Saharo-Canarian circle

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Genetic studies in the Azores show that the modern population is composed of both a northern and southern Europe people. However, a significant Chinese input of

HLA characteristic genes has occurred. This probably happened by 1421 AD by Chinese Atlantic explorers when Zheng He sent his fleet to survey seas. HLA-B*27:07 is found in Orientals and Azoreans, but not in Europeans. A kind of Machado Joseph neuropathy (Chr 14) founder mutation is only common to Orientals and Azoreans. In addition, widely studied Carthaginian coins were found on Corvo Island (Azores) made in Spain from Barca family ruling epoch (1st century AD). This is contradictory to the official version that pre-Portuguese Azores had been virgin and inhabited. Cart-ruts in Azores indicate a (Atlantic) common culture with Canary Islands and Mediterranean. On the other hand, genetic studies on Canarians show that they present European, Iberian and Berber characteristics: North African Berbers and Iberians are difficult to genetically be distinguished. Prehistory in the Canary Islands is richly adapted to a volcanic scene. A prehistoric lunisolar megalithic calendar: Quesera of Zonzamas, pyramids similar to those found in nearby Western Sahara (90 km far). Also "Latin" or Ibero Guanche rock scripts which can be transcribed with Iberian-Tartessian signory, but not with Latin, and also found at Tim Missaw cave (Sahara, Algeria). Populated Sahara area desiccation after 5000 years BC and subsequent people migration could be the origin of Canary Island, Sahara and other Mediterranean culture traits. Thus, we have defined the Saharo-Canarian Circle as a genetic, anthropological and prehistoric culture radiation; it might have given raise to the Iberian-Tartessian signory and also to other ancient lineal Mediterranean scripts.

P164 | The influence of HLA-DRB1 and HLA-DQB1 on post-transfusion alloimmunization with red blood cell antigens in Polish population

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Many different factors influence the propensity of transfusion recipients and pregnant women to form red blood cell alloantibodies (RBCA). RBCA may cause hemolytic transfusion reactions, hemolytic disease of the fetus and newborn and may be a complication in transplantation medicine. Antigenic differences between responder and foreign erythrocytes may lead to such an immune answer, in part with suspected specific HLA class II associations. Alloimmune antibodies against red-blood-cell (RBC) antigens induced in susceptible individuals (responders) by transfusion, pregnancy or transplantation may have serious clinical consequences. The aim of this study was to investigate association of alloimmunization against selected RBC antigens with HLA class II. A total of 125 responders (71 mono-responders and 54 multi-responders) were enrolled into the study. HLA-DRB1 and HLA-DQB1 variants were determined by PCR-SSP and their frequencies compared between the 50 ethnically matched controls. Development of mono and multiple RBC antibodies was associated with HLA-DRB1-DQB1 haplotypes: anti-E and anti-C, HLA DRB1*07:01-DQB1*02:02, anti-C, anti-D, anti-K, anti-Fya, HLA DRB1*15:01-DQB1*06:02, anti-Fya, HLA-DRB1*04:01-DQB1*03:01, anti-Jka—HLA-DRB1*01:01-DQB1*05:01, and DRB1*03:01-DQB1*02:01, anti-M, HLA DRB1*11:01-DQB1*03:01. The more frequent occurrence of the HLA-DRB1*15:01 allele in people producing multi-specific antibodies to red blood cells (multi-responders). Additionally, a more frequent combined occurrence of HLA-DRB1*07:01 and DRB1*15:01 alleles was found in the group of people producing multi-specific antibodies to red blood cells (multi-responders).

P165 | HLA-G 3′UTR 14bp indel polymorphism in Southern Tunisian Bipolar Disorder patients

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Bipolar disorder (BD) is a common severe mental illness, affecting 1%–4% of the population worldwide. Although the pathogenic mechanism remains elusive, BD is recognized as a multifactorial disorder involving interactions between multiple genetic and environmental factors. Inflammatory process is known to underline disease risk and evolution. Tolerogenic molecules, such as HLA-G, mediate the modulation of such processes. The HLA-G 14 bp indel in exon 8 is associated with the stability and splicing pattern of HLA-G mRNA, which could affect HLA-G protein expression. We were interested in studying the HLA-G 3′UTR 14 bp indel polymorphism as a susceptibility factor to BD in the South Tunisian population. Twenty five BD patients (recruited during a period of 6 months) and 86 unrelated healthy controls were enrolled. For both groups, the HLA-G 3′UTR 14 bp indel

was typed using bi-directional PCR allele-specific amplification (bi-PASA) system. Statistical analysis was performed with R. The results of molecular genotyping of the HLA-G 3′UTR 14 bp indel polymorphism allowed us to determine the three genotypes (II, ID, and DD) of both patient and control populations. Analysis of genotypes frequencies distribution revealed a statistically significant difference between the two groups ($p = 0.034$; OR = 3.65). The frequencies of ID, DD and II genotypes were respectively 64%, 16% and 20% in the patients group and 39.5%, 36% and 24% in the control group. Analysis of the allele carriage distribution reveled a more frequent "I" allele in patients that in controls (84% vs 64%, p = NS). In our study, we found an association between the ID genotype polymorphism of the HLA-G 3′UTR 14 bp indel and BD in the South-Tunisian population. The "I" allele was more frequent in BD patients. On the other hand, D allele seems to have a protective role against this disease.

P166 | Managing possible new alleles in immunogenetics laboratory of the Portuguese Institute of Blood and Transplantation, Porto

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Next-Generation Sequencing (NGS) methods for HLA typing are now widely used, allowing an increase in the capability of new allele identification, which would never be possible with classic Sanger sequencing. In June 2021, the Immunogenic Laboratory (IL) of IPST Porto adopted NGS HLA genotyping for solid organ and hematopoietic stem cell transplantation and disease association. Between June 2021 and November 2023, DNA was obtained from 4619 human whole blood samples using the QIASymphony DNA mini Kit. AllType FASTplex NGS 11 Loci Flex Kit was used to amplify and sequence HLA class I and II. Genotyping was achieved with TypeStream Visual (TSV). NGS mismatches in HLA class I (exon 1, 2, 3 and 4) and HLA class II (exon 1, 2 and 3) were confirmed by SBT with HLAssure SE Kit and Accutype software. TSV software generates FASTA sequences, assigns the most similar allele and location of the mismatch. Accession number was obtained using the GenBank database and the official name was given when completing the Submission Tool in IPD-IMGT/HLA Database. From 4619, 10 new alleles were identified, estimating a rate of 0.345 per month. A*03:458Q, A*02:1086, A*31:01:52, C*08:264 and DRB1*01:144 were unique and detected for the first time; other new alleles, DRB1*01:01:41, DRB1*14:243, DRB1*04:354, DRB1*01:144, DRB1*16:75, DPA1*01:141 were already reported by other groups, but yet not present in our HLA libraries. All of the novelties were single nucleotide substitutions: 80% were missense and 20% silent mutations; 60% were located in exon 1, coding for the leader peptide; 30% occurred in exons coding for antigen-binding sites; and 10% in exon 4, coding for extracellular arm α3. NGS HLA typing in routine opened a window for the discovery of new alleles. Even though we are aware of the limited guidance on how to manage this information and its impact at clinical expectations, all of the novelties found at IL are submitted, contributing to the scientific enrichment.

P167 | Linkage disequilibrium between MICA-129Met/Val and HLA-C1/C2 in the Russian population of the Chelyabinsk region of the Russian South Urals

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The non-classical MHC class I-related chain (MIC) genes are located within the HLA class I region. Their organization, expression and products differ considerably from classical HLA class I genes. MIC proteins are considered to be markers of 'stress' in the epithelia, and act as ligands for cells expressing a common activatory natural killer-cell receptor (NKG2D). The single-nucleotide polymorphism rs1051792 causing a valine (Val) to methionine (Met) exchange at position 129 of the MICA protein is of specific interest. It separates MICA into isoforms that bind NKG2D with high (Met) and low affinities (Val). In addition, there are functional differences in the protein products of the HLA-C locus – C1 and C2. HLA-C1 epitopes provides resistance to infection but at high risk for recurrent miscarriage, pre-eclampsia and fetal growth restriction. In the current study we got haplotype frequencies and linkage disequilibrium HLA-C1/C2∼MICA-129Met/Val in the major populations of Chelyabinsk region—the Russians. A group of 100 unrelated

normal healthy Russian HSC donors living in the Chelyabinsk region (Russian south Urals) were included in the population study. Their belonging to the Russian was determined by their family history at least three generations and their language. HLA-C was sequenced on a MiSeq platform using reagent kit HLA-Expert (DNA-Technology LLC, Moscow, Russia). MICA typing used PCR-SSP. Population genetic analysis included tests for Hardy–Weinberg equilibrium (HWE), haplotype frequencies (HF) and LD using Arlequin 3.5. We obtained the following results. Haplotype HLA-C1/C2∼MICA-129Met/Val (HF, %; D′; P value): C1∼Val (45.5; 0.2173; 0.0048), C2∼Met (17.5; 0.2297; 0.0032), C1∼Met (16; -0.2297; 0.0032), C2∼Val (20.5; -0.2173; 0.0048). Thus, significant linkage was found in two haplotypes HLA-C1∼MICA129Val and HLA-C2∼MICA129Met. What may indicate the co-evolution of MHC class I molecules for NK-cell receptors.

P168 | HLA alleles and haplotypes in a Sudanese population and their relationship with Mediterraneans and East to West demic diffusion

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Migrants from the green Sahara to Mediterranean Sea had both a genetic and a cultural connection effect that has clearly been found between Western Europe and North Africa. Sudanese populations from different ethnicities have been typed for HLA-A, -B, -DRB1 and -DQB and results show that Nubians are genetically related with African Sub-Saharan populations and distant from other Sudanese tribes, who are closer to Mediterranean populations than to Sub-Saharan ones. This is concordant with our own meta-analysis data and other research. Our present work is, to our knowledge, the first and only HLA study on Sudanese people that takes into account different Sudan ethnic groups. Samples were collected before the partition between North and South Sudan. A prehistoric genetic and people exchange between Africa and the Mediterranean basin maybe observed and is supported with the results obtained in this Sudanese HLA study. However, the demic diffusion model of agriculture and other anthropological traits from Middle East to West Europe/Maghreb does not exist: a more detailed Sahel and North African countries ancient and recent admixture studies are also being carried out which may clearer explain pastoralists/agriculture innovations origins in the Eurafrican Mediterranean and Atlantic façades, in conjunction with other studies which include language, writing type and physical anthropology.

P169 | Autonomic study and influence of UV radiation on evolution

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The aim of this study was to explore the correlation between quantum mechanisms and their impact on photosynthetic biological models applied to the autonomic genetic evolution within the p53 gene of *Homo sapiens*. To achieve this, mutations were introduced into the p53 gene based on parameters of evolutionary conservation, with a particular focus on the 42 CpG points associated with mutations influenced by ultraviolet radiation. These mutated sequences were then compared with sequences of the same gene found in various primates, with the mouse serving as an "outgroup" for reference. Phylogenetic trees were constructed using the "Neighbor Joining" (NJ) method, and a correspondence analysis was conducted using the VISTA and PAUP programs. Genetic distances were calculated using MEGA software. The results indicated that the introduction of 6 mutations in the *H. sapiens* p53 gene led to genetic divergence when compared to non-mutated sequences. These findings suggest a direct association between quantum effects and biological evolution. Consequently, it can be inferred that quantum mechanisms may play a significant role in genetic evolution, particularly concerning phenomena such as the photoelectric effect, Frenkel exiton, photosynthesis, and mutations in the p53 gene. These outcomes support the hypothesis that quantum effects can influence evolutionary processes at the molecular and genetic levels. Similar results were observed with the HLA-G and MHC-G genes in primates.

P170 | Identification of HLA alleles involved in immune thrombotic thrombocytopenic purpura patients from Turkey

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Thrombotic thrombocytopenic purpura (TTP) is one of the rare group disorders classified as thrombotic microangiopathies (TMAs). Approximately 90% of TTP development is immune-mediated by the formation of antibodies against the enzyme ADAMTS-13. The exact etiology is unknown. To establish an association between HLA and autoimmune basis, as susceptibility or protection against the disease we performed a study aiming to evaluate the role of HLA in immune-mediated TTP (iTTP). Considering epidemiological factors such as age, sex, ethnicity, and geographical origins we performed the study in our country, Turkey, consisting of a very heterogeneous population. Patient data collection was retrospectively obtained from electronic database on two University hospitals with big therapeutic apheresis services. The control arm were healthy people registered as stem cell donors matched in terms of age and gender. HLA-DRB1 and -DQB1 tests of patients and healthy controls were analyzed by PCR-SSO and SSP methods. The frequency of HLA-DRB1 and HLA-DQB1 alleles between acquired TTP and the control group was compared using the Chi-squared method. Yates correction and logistic regression were performed on these results. A total of 75 iTTP patients and 150 healthy individuals enrolled to the study. When the low resolution results were analyzed, it was found that HLA-DRB1*11 and -DQB1*03 were related to the susceptibility to acquired TTP and the association of DRB1*11, -DQB1*03, -DRB1*15 and -DQB1*06 may be a risk factor for acquired TTP. On the other hand, the association of HLA-DRB1*15 and DRB1*07 with -DQB1*02 may be protective for acquired TTP. When the high resolution results were analyzed, HLA-DRB1*11:01, -DRB1*14:01 and -DRB1*13:05 may be risk factors for acquired TTP and -DRB1*01:01, -DRB1*07:01, -DRB1*13:01, -DRB1*14:54, -DQB1*05:01 and -DQB1*02:02 may be protective for acquired TTP. Our findings support an association with iTTP across a very heterogenous population in Turkey.

P171 | The frequency of HLA-B*78:01 allele in the southern Tunisian population

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The first B78 allele, B*78:01, was discovered in Moroccans in 1989. Although initially associated with African populations, B*78:02 and B*78:03 have been identified in Caucasian populations, B*78:04 has been detected in Hispanic populations, and B*78:05 in the Japanese population. Our study presents HLA typing conducted in South Tunisia between 2010 and 2023. HLA class I (A and B) typing was performed using standard microlymphocytotoxicity, supplemented with PCR-SSP or PCR-SSO (Luminex) if requested. Among a total of 10,000 individuals, B*78:01 was confirmed through DNA typing in 10 unrelated individuals. While some members of the B5 CREG group are prevalent across various population groups, others, such as the B*78 alleles, are rare. Our study found that the frequency of the B*78:01 allele is very low (0.10%). This low frequency can be attributed to confusion with the B35 antigen (Bw6), primarily identified by microlymphocytotoxicity. Comparing our results with the literature on the frequency of the B*78:01 allele, we observed that this allele is equally rare in European populations. In contrast, its frequency is notably higher in sub-Saharan populations (Senegal and Burkina Faso), ranging between 5% and 8%.

P172 | Distribution of HLA antigens among patients with acute myeloid leukemia in the Republic of Kazakhstan

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It is known that the HLA antigens of the main populations inhabiting the Earth are common. It has been established that antigens present in all races have different frequencies of phenotypes for certain ethnic groups.

These conditions allow us to draw an important conclusion for immunogenetics about the need to conduct research to study the connections between the HLA system or the HLA system and various pathological conditions within a certain population. The data obtained were used to establish a connection between HLA antigens and the development of AML. The study included 108 samples of recipients diagnosed with AML, typing of which was performed at high resolution using the NGS method for 5 HLA loci. The results of typing of 3694 potential HSC donors who entered the National HSC Register were used as a control group. The presence of statistically significant differences between groups was calculated using the Pearson's χ^2 index ($p \leq 0.05$). The most frequently occurring HLA alleles among recipients were the following (%): A*02:01(21), A*24:02(13.4), A*01:01(12), A*11:01(7.8), A*03:01(6.9); B*51:01(9.3), B*07:01(7.4), B*13:02(6.9), B*35:01(5.6), B*40:02(5.6), B*08:01 and B*18:01(5.1), C*06:02(5.7), C*07:02(10.6), C*04:01(8.8), C*07:01(8.3), C*12:03(7.9), C*03:04 and C*15:02(6), C*03:03(5); DRB1*07:01(15.3), DRB1*15:01 (12.4), DRB1*03:01(8.8), DRB1*11:01(6.9), DRB1*01:01 (5.1); DQB1*03:01(22.7), DQB1*02:01(19), DQB1*06:02 (11.6), DQB1*05:01(8.3), DQB1*03:02(6.9), DQB1*06:03 (6.5), DQB1*03:03(5.6). The following antigens were noted significantly more often in the recipient group compared to the control group: A*30:04, A*66:01, B*07:10, B*14:01, B*18:01, B*35:02, C*05:01, C*06:02, DRB1*03:01, DRB1*11:01, DRB1*13:03, DQB1*03:04, DQB1*06:01. The information obtained on the distribution of HLA antigens among recipients with AML makes it possible to make assumptions about the protective and predisposing role of the above-mentioned corresponding genes of the HLA system and is important for predicting the risk of disease.

P173 | HLA-A, -B, -C, -DRB1, and -DQB1 allele and haplotype frequencies: An analysis of umbilical cord blood units at the Sichuan Cord Blood Bank

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We analyzed HLA-A, -B, -C, -DRB1, and -DQB1 allele frequencies in 8056 cord blood units from the Sichuan Cord Blood Bank using Next-generation sequencing (NGS) and identified 72 HLA-A, 131 HLA-B, 79 HLA-C, 77 HLA-DRB1 and 44 HLA-DQB1 alleles. The most frequent alleles for each locus were A*11:01 (28.83%), B*46:01 (15.82%), C*01:02 (21.05%), DRB1*09:01 (15.42%), and DQB1*03:03 (18.5%), respectively. Haplotype frequencies were estimated using an implementation of the expectation maximization (EM) algorithm. A total of 4464 different HLA-A∼B∼C∼DRB1∼DQB1 haplotypes were identified. Of these, 132 haplotypes with frequency higher than 0.1% were made up about 46.4% of all the haplotypes. A*02:07∼B*46:01∼C*01:02∼DRB1*09:01∼DQB1*03:03 was the most frequent HLA haplotype at a frequency of 4.57%.

P174 | Identification of three novel HLA class I alleles, A*24:556N, B*55:129, and C*01:02:87, found in Chinese cord blood units

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We report here the sequence of three novel HLA class I alleles, officially named as HLA-A*24:556N, B*55:129 and C*01:02:87. Among almost 2000 Chinese cord blood donors tested in 2022, they were identified in three donors respectively, one male and the other two in females. HLA typing was performed by NGS and three novel HLA class I alleles were found. The novel alleles were confirmed by allele-specific amplification and sequence-based typing. In order to identify the haplotype carrying the novel allele, we extended the analysis to the mothers of the donors. The sequence of HLA-A*24:556N differed from HLA-A*24:01:01:01 by a frame shift mutation, a deletion at gDNA position 288 to 291, which had a premature stop codon in exon 2. The sequence of HLA-B*55:129 differed from B*55:02:01G by a nucleotide change in exon 2 at position 266 where A was replaced by G (codon 65 CAG → CGG), which resulted in an amino acid change from glutamine to arginine. The sequence of HLA-C*01:02:87 differed from C*01:02:01 by one nucleotide change in exon 4 at position 705 where G was replaced by A (codon 211 GCG → GCA), which did not result in an amino acid change. Combined with the mothers' results, the novel B*55:129 allele

segregated with the haplotype HLA-A*24:02, C*08:01, DRB1*12:01:01G, and DQB1*03:01:01G. The second haplotype was determined as A*11:01, B*15:02, C*08:01, DRB1*12:02, and DQB1*03:01:01G. The novel C*01:02:87 allele segregated with the haplotype HLA-A*02:07, B*46:01, DRB1*09:01,and DQB1*03:01.The second haplotype was determined as A*11:01, B*15:01, C*04:01, DRB1*11:01, and DQB1*03:03:01G. We were unable to deduce the HLA haplotype in association with HLA-A*24:556N based solely on the mother's result because both of the cord blood donor and his mother share the same DRB1 typing of DRB1*11:01, 15:01. The novel alleles HLA-A*24:556N and C*01:02:87 were maternal, and B*55:129 was paternal.

P175 | Detection of the HLA A*02:275 allele, which was included in the HLA nomenclature in September 2022, in only one patient scheduled for hematopoietic stem cell transplantation: A single center experience

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Hematopoietic stem cell transplantation (HSCT) is a proven therapy for the treatment of malignant and benign blood disorders. The success of HSCT can be determined by the relationship between HLA matching and the stability of the graft in the host after transplantation. Recently, thanks to the availability of high-resolution tissue typing methods, donor and recipient compatibility for HLA alleles has been shown to be an important determinant of transplant outcome. Tissue groups of pediatric patients admitted to our center were examined by a next generation sequencing (NGS) method. HLA-A*01:01, HLA-A*02:01, HLA-A*03:01, HLA-A*03:02, 11:01, HLA-A*24:02, HLA-A*26:01 and -A*32:01 alleles were found most frequently in our patients whose tissue groups were analyzed. The HLA-A*02:275 allele, which was added to the HLA Nomenclature in September 2022, was detected for the first time in only one patient planned for hematopoietic stem cell transplantation in our center. With the advancement of technology, the detection of HLA alleles will help in understanding population genetics.

NEW TECHNOLOGIES IN IMMUNOGENETICS

P176 | Revolutionizing high resolution HLA genotyping for transplantation assessment: validation, implementation and challenges of Oxford Nanopore Technologies' Q20+ sequencing

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The advent of third-generation sequencing (TGS) represents a significant shift in the field of genetic sequencing, enabling long-read single-molecule sequencing to overcome limitations of preceding short-read NGS platforms. Several studies have assessed the utilization of TGS platforms in HLA genotyping, though many of these studies have described the high error rate as a major limitation to achieving a robust and highly accurate HLA typing assay. In 2021, Oxford Nanopore Technologies (ONT) released the high-accuracy sequencing Kit 14 and the MinION flow cell model R10.4.1, which were reported to achieve sequencing accuracies up to 99%. The aim of this study was to validate this novel high-accuracy sequencing kit for HLA genotyping coupled with an in-house full-gene HLA PCR assay. Comparison with historical data obtained using legacy flow cell models such as R9.4, R10.3 and R10.4 was also done to assess progressive improvement in sequencing performance with each sequential release. The workflow was validated based on data throughput, sequence quality and accuracy, and HLA genotyping resolution. An initial validation was performed using an internal reference panel of 42 samples representing all known allele groups, followed by data obtained from 111 routine sequencing batch runs since the implementation, to assess assay performance and further improvements. Furthermore, challenges arising due to data storage, assessment of barcode contamination and the utilization for different HLA genotyping applications are discussed. The findings of this study highlight advantages of ONT sequencing kit 14/R10.4.1 for HLA genotyping, implementation requirements and the challenges faced by the routine diagnostic HLA laboratory.

P177 | The development of full gene multiplex PCR based assay for the non-classical HLA-E, HLA-F, HLA-G, HLA-H, MICA, and MICB genes

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Matching of haplotypes in HLA-identical sibling transplants confers a higher degree of similarity across the MHC region compared to allele matching of individual HLA loci. HLA matching of HLA genes in unrelated hematopoietic cell transplants (HCT) utilizes less than 5% of the entire MHC region. This confers a high risk of undetected polymorphisms between donors and patients, whereby the individual haplotypes are not known. The presence of conserved haplotype blocks in the MHC region and the co-segregation of classical and non-classical HLA loci within these blocks results in a high degree of linkage disequilibrium between these loci. This study aimed to develop and validate multiplex full-gene PCR based assays targeting HLA-E, HLA-F, HLA-G, HLA-H, MICA, and MICB that could be used to identify novel markers in extended haplotypes outside of the classical HLA loci that could impact transplant outcomes. Validation of the assay was performed using a panel of 94 samples derived from the International Histocompatibility Working Group (IHWG), including a consanguineous panel of 48 samples. A total of 564 alleles were sequenced across 94 individual samples, full-gene sequencing results were obtained for 512 (90.8%) non-classical HLA alleles. Of the 512 sequencing results obtained, 114 reported an improved resolution and 202 were concordant with reference results. A total of 163 alleles were unconfirmed due to the absence of reference results, and 33 alleles were reported as conflicting. Additionally, 55 novel alleles were identified, 4 of which were novel synonymous polymorphisms within exonic regions, and 51 alleles had novel intronic polymorphisms. Extended haplotypes identified in family segregation data analysis will be presented. This study provides the tools to investigate the role of HLA haplotype matching in the outcomes of HCT.

P178 | Exploring ABO-histocompatibility: Luminex assay allows detection and characterization of endothelial-targeted ABO antibodies

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Naturally occurring antibodies to ABO-histo-antigens cause ABO-O and -B individuals to wait longer for compatible donors for transplantation. Equitable access can be improved by undertaking ABO-incompatible (ABOi) transplantation. Anti-glycan antibodies to ABO-A and -B targets must be measured to assess eligibility for ABOi transplantation but agglutination assays have many limitations. For children, the development of ABO antibodies in early life is not well understood. Our goal was to measure the status of IgG and IgM ABO antibodies across a pediatric population, compare ABO antibody levels between ABO groups and to endothelial (subtype II) glycans specifically. Samples were obtained from non-transplant patients at cardiac catheterization ($n = 130$; ABO-O, $n = 66$; ABO-A, $n = 47$; ABO-B, $n = 17$). Patients were between the ages of 2 and 18 years; infants were excluded to rule out passively acquired maternal antibodies. Transplant candidates were excluded due to likelihood of passive ABO antibodies from transfusion. Sera were incubated with Luminex beads coupled to ABO-A and -B subtype I-VI glycans; ABO antibodies bound to beads were detected using fluorochrome-labeled anti-human IgG and IgM secondary antibodies with output measured as mean fluorescence intensity (MFI). Each blood group had comparable representation of age. ABO-O individuals had significantly higher levels of IgG anti-A-II ($p = 0.02$) and anti-B-II ($p < 0.0001$) ABO antibodies than ABO-B and -A individuals, respectively. Similar differences were not observed for IgM levels. The bead-based assay allows accurate characterization of both IgM and IgG isotype ABO antibodies with specificity for A-II and B-II glycans, which are the sole endothelial cell targets. These data suggest important differences in ABO antibody profiles between blood groups. Use of tools such as this bead-based assay will enable studies that investigate the roles of endothelial-specific ABO antibodies.

P179 | Comparative assessment of cytometry by time-of-flight and full spectral flow cytometry based on a 33-color antibody panel

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Mass cytometry and full spectrum flow cytometry have recently emerged as new promising single cell proteomic analysis tools that can be exploited to decipher the extensive diversity of immune cell repertoires and their implication in human diseases. In this study, we evaluated the performance of mass cytometry against full spectrum flow cytometry using an identical 33-color antibody panel on four healthy individuals. Our data revealed an overall high concordance in the quantification of major immune cell populations between the two platforms using a semi-automated clustering approach. We further showed a strong correlation of cluster assignment when comparing manual and automated clustering. Both comparisons revealed minor disagreements in the quantification and assignment of rare cell sub-populations. Our study showed that both single cell proteomic technologies generate highly overlapping results and substantiate that the choice of technology is not a primary factor for successful biological assessment of cell profiles but must be considered in a broader design framework of clinical studies.

P180 | Rapid HLA high-resolution genotyping utilizing nanopore DNA sequencing technology for allocation of organs from deceased donors

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HLA epitopes within the donor HLA system are recognized as pivotal determinants for the success of solid organ transplantation. Nevertheless, the definition of HLA epitopes necessitates high-resolution (HR) genotyping, often not attainable before the allocation of deceased donors. Nanopore DNA Sequencing technology is a 3rd generation approach, emerging as a promising avenue for rapid HLA HR typing. This study evaluates Nanopore sequencing for urgent HLA HR typing of cadaveric donors. It introduces a rapid HLA typing method employing the Nanotype 24/11 v2 Ruo assay (Omixon Inc. Budapest) using 11 loci (HLA-A, -B, -C, -DRB1, -DRB3/4/5, -DQA1, -DQB1, -DPA1 and -DPB1) from 30 samples, paving the way for on-call deceased donor allocation. This assay employs multiplexed long-range PCR with library preparation within 90 min. Data, generated on a MinION sequencing device using a MinION flow cell type R9.4, undergoes high-accuracy base calling, followed by analysis in Nanotyper software, restricted only in exons. In comparing results with pre-typed data (AlloSeq Tx17 kit, CareDx Inc. Stockholm) and sequencing on Illumina MiSeq, the Nanopore method yielded 100% concordance for 330 loci with the current NGS method, maintaining a minimum 2-field typing resolution. The method also provided accurate data at all HLA loci in approximately 4 h, without prolonging allocation time. The average read length was 3150 bp, with an average minimum coverage for key exons of 1630 for all HLA loci. Notably, key exon allelic imbalance for heterozygous samples at most HLA loci was over 0.6. In conclusion, conducting HR typing across all HLA loci for deceased organ donor allocation has notable clinical benefits. The implementation of Nanopore HLA typing for deceased donors before transplantation, paired with antibody screening and identification, enhances virtual cross-match accuracy, especially for hypersensitized recipients, within a time-efficient framework.

P181 | NGS-Pronto: High throughput, high resolution HLA typing using nanopore sequencing

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Routine diagnostic HLA typing by Next-Generation Sequencing (NGS) can be time-consuming and involves high costs. Oxford Nanopore Technologies (ONT) nanopore sequencing and NGS-Turbo® enable HLA typing of one sample by NGS within 3 h. Furthermore, the investment costs of nanopore sequencing are relatively low, allowing broad access to this technology. Here we demonstrate NGS-Pronto®, a fast, high-resolution HLA typing method for routine diagnostic use. To test the performance of NGS-Pronto, DNA from the Genetic Testing Reference Material Coordination Program (GeT-RM) HLA58 panel was used. Amplicons were generated with NGSgo-ProntoAmp®, consisting of 3 premixes containing primers able to generate whole gene amplicons for 11 HLA loci. Library preparation was performed with a novel method (NGS-ProntoPrep®), that allows a minimum of 4 and a maximum of 96 samples per library. Through this flexibility, NGS-Pronto can be applied in clinical labs with varying sample turnover. Depending on

the number of samples included, library preparation will take 60–120 min. Amplification and library preparation of 58 samples was performed within 4 hours total. Data was generated on a GridION sequencing device, using super accuracy basecalling. Sequencing took ~10 h (10 min per sample) to obtain 10,000 reads per sample. Subsequently, data was analyzed with NGSengine®-Turbo software (GenDx) optimized for ONT data. NGSengine®-Turbo obtained 100% concordant three-field resolution typing results with 5 minutes analysis time per sample. In summary, the NGS-Pronto workflow is designed to be flexible, convenient and fast, allowing for HLA typing of up to 96 samples to be at three-field resolution.

P182 | Validation of NGSTurbo typing with nanopore sequencing for rapid and accurate HLA typing in deceased donor organ transplantation

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The evolution of long-read and rapid sequencing technologies presents a pivotal influence on HLA typing for deceased donor organ transplantation. In this study, we aim to validate the NGSTurbo typing method (GenDx) by employing Nanopore sequencing as a rapid procedure for deceased donor typing, using the results obtained with the Flongle and MinION devices. DNA samples were prepared for sequencing using the NGSTurbo kit, followed by sequencing with the Flongle or MinION using the MinKNOWN software; subsequently, the generated reads were basecalled with Dorado and analyzed using NGSEngine-Turbo software. The validation procedure involved samples from peripheral blood or spleen, with known high-resolution HLA typing. The MinION device demonstrated accurate high-resolution HLA typing for 10/10 samples. Simplex basecalling of 10,000 reads resulted in 100% exon coverage, a read depth surpassing 150 reads per locus, a median background signal below 5%, and a minor allele frequency exceeding 35%. The hands-on time was approximately 55 min, with 10 min dedicated to analysis, resulting in a total processing time of 3 h and 10 min. Additionally, Duplex basecalling revealed approximately 10% of reads compared to Simplex, with improved noise levels below 1%. However, maintaining sufficient read depth, especially with fewer than 20,000 sequencing reads, posed challenges. The Flongle device achieved correct high-resolution typing results in 4/8 runs, but technical issues with the flow cells impeded success in the remaining runs. Additionally, the noise signal in the Flongle data was notably higher compared to the MinION. This study emphasizes the reliability and efficiency of the NGSTurbo typing method when used with MinION Nanopore sequencing. These findings demonstrate that rapid high-resolution typing is possible, paving the way for implementation in diagnostics for deceased donor HLA typing.

P183 | High-resolution HLA genotyping using nanopores—A multicenter study

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Molecular techniques for HLA typing have become an integral part of everyday laboratory work. While real-time polymerase chain reaction delivers fast results in low-resolution, high-resolution HLA typing is generated using next-generation-sequencing, essential for stem cell transplantations. High-resolution typing will become increasingly important in organ transplantation in the future, however the minimal time for deceased donor HLA typing is challenging. HLA typing using nanopores has undergone enormous development in recent years and shows the potential for high resolution HLA typing of 11 loci within a few hours. The aim of this multicenter study was to investigate the nanopore sequencing in terms of concordance with NGS HLA typing results and practicability for diagnostic laboratories. A total of 381 samples were sequenced in four European laboratories with NanoTYPE™ (Omixon Biocomputing Ltd.) on a MinION (Oxford Nanopore Technologies). The results show a concordance of 99.58% for the HLA loci, HLA-A, -B, -C, -DRB1, -DRB3, -DRB4, -DRB5, -DQA1, -DQB1, -DPA1 and -DPB1. The fastq files analyzed with

NanoTyper software (Omixon Biocomputing Ltd.), whereby quality data generated for each sample. A total of 34,926 (97.15%) quality control values were issued as "passed." Only 2.4% of the QC values were assessed by the software as "inspect" and 0.45% as "failed". Our results show that correct HLA typing results were achieved despite software warnings. The advantages of nanopore sequencing are shown in simple implementation in the laboratory, fewer ambiguities and cost efficiency, which is demonstrated by the reusability of the flow cells. The current limitation with regard to exon coverage will be resolved surely in the next few years, so that this technology can use in diagnostics for high resolution HLA typing for deceased donor HLA typing. This could offer opportunities for routine epitope determinations in the future.

P184 | Contribution of circulating donor-derived DNA in the prediction of kidney transplant rejection

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Donor derived cell free DNA (dd-cfDNA) dosage is a predictive marker of organ rejection currently under study. We evaluated its potential for implementation at the HLA laboratory of Lille. First, we extracted total circulating free DNA (cfDNA) from patients' plasma. Then used Single Nucleotide Polymorphisms (SNPs) differing between donor and recipient to evaluate with NGS (Illumina MiSeq) the ratio of cfDNA originating from the donor (Alloseq kit, CareDX). We included 21 patients who had undergone kidney transplant biopsy close to the dd-cfDNA analysis. Among these patients, 9 underwent a biopsy on clinical suspicion, and 12 routinely, 3 months after the transplant. We then compared the absence or presence of Donor-Specific-Antibodies (DSA) to the ratio of dd-cfDNA. The results obtained support the use of dd-cfDNA as a screening marker for graft injury, especially in the absence of point of call. Levels were significantly higher in patients whose transplanted kidney showed any kind of injury than in patients whose transplanted kidney was lesion-free. This result was even more significant when antibody-mediated rejection was compared with all other biopsy results. We established a positivity threshold by ROC curves, allowing the evaluation of sensitivity and specificity for different values. The optimal cut-off value was chosen as combining 100% sensitivity with acceptable specificity (67%), to enable dd-cfDNA measurement to be performed as a first-line alternative to routine 3 months biopsy. In conclusion, although the results are promising, an additional prospective study focusing on the correlation between third month systematic biopsy and dd-cfDNA will be necessary to refine thresholds, sensitivity and specificity.

P185 | Overcoming challenges in KIR gene typing: A third generation nanopore sequencing approach

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Killer cell immunoglobulin-like receptor (KIR) genes regulate NK cell activity, interacting with MHC class I molecules to distinguish healthy from infected/malignant cells. KIR variability impacts immune responses, affecting disease susceptibility and transplantation outcomes. Our laboratory employs Next Generation Sequencing (NGS) for KIR gene typing, enabling determination of gene presence/absence but lacking allelic resolution. We aimed to evaluate various library preparation chemistries from Third Generation Oxford Nanopore technology on samples previously typed using Illumina Technology and comparing their typing concordance. We compared the performance of two library preparation chemistries from Oxford Nanopore on 22 previously NGS-typed samples. Twelve samples were sequenced using both the old (EXP-NBD104/14-SQK-LSK109) and new (SQK-NBD114.24) chemistries, while the remaining 12, with existing allelic data, were analyzed solely with the new chemistry. The old chemistry displayed concerning discrepancies, falsely identifying KIR2DL5 and KIR2DP1 genes, highlighting its limitations. On the other hand, the new chemistry achieved a 40% accuracy rate using the fast basecalling and 100% accuracy using the high accuracy basecalling. Regarding the last 12 samples, an analysis workflow using CLC Genomics software established 100% allelic concordance for KIR2DL1, KIR2DL3, KIR2DL4, and KIR2DL5A in the samples with known alleles. Attaining allele-level resolution of the KIR genes has posed significant challenges with short-read sequencing technology, largely attributed to the exceptionally high homology of

these genes. For this reason, high allele-level resolution has posed significant challenges with short-read sequencing technologies. In this study, we have illustrated how nanopore technology not only offers information at the level of presence/absence but also overcomes this challenge, enabling analysis of KIR genes at high resolution level.

P186 | HLA class I (HLA-A, -B, -C) single reaction, full-gene, unambiguous genotyping by Oxford Nanopore sequencing

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HLA class I genes are located in the highly polymorphic Human Major Histocompatibility Complex (MHC) at 6p21.3 and HLA matching is the key for the successful stem cell transplant due to its ability to evoke antigen-mediated immune response. In the past decade, short-read next generation sequencing has become the standard of HLA genotyping due to its improved resolution and enhanced throughput although due to the polymorphism homology between the HLA class I genes phasing ambiguities can result in ambiguous HLA genotyping. Long-read sequencing by Oxford Nanopore Technologies has been evaluated for high throughput and unambiguous HLA 3rd and 4th field genotyping in last few years. Despite having advantages over short-read sequencing including low start-up cost and improved phasing, lower genotyping sequencing quality was observed when compared to short-read sequencing. However, studies have shown continuous improvement in the sequencing quality of as long-read chemistry and bioinformatic tools have developed. An HLA class I genotyping assay on the most recent R10.4.1 flow cells has been developed. Blood samples with a range of common and rare HLA class I alleles were tested. Full length gene amplification primers (5′–3′ inclusive) were designed and multiplexed into a single reaction with balanced coverage for HLA-A, HLA-B and HLA-C. The result interpretation confirmed concordance of all samples up to 4th field to their historical genotypes by short-read sequencing. The recent flow cell with improved chemistry has provided >99.9% raw read accuracy and 50% allele balance for heterozygous samples. In conclusion, evaluation of the R10.4.1 flow cell turned out as a great asset for the development of HLA class I genotyping assay for desired number of samples processing in shorter turnaround time and leaves a bright opportunity to develop an unambiguous, high throughput assay for HLA class II genotyping.

P187 | Assessing the performance of NG-Mix, a novel next generation sequencing option developed by the reagent production unit of the French Blood Center

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High-resolution genotyping of HLA using next-generation sequencing technology is now essential in our laboratories. The Reagent Production Unit of the French Blood Center (Etablissement Français du Sang) developed the NG-Mix kit for the typing of 11 HLA loci. This NG-Mix kit comprises in-house developed primers and indexes combined with commercial reagents and a long-range DNA polymerase. It utilizes five amplification reagents containing specific primer mixes. Sequencing is done on a MiSeq (Illumina) platform. Data analysis is performed using ThermoFisher Type Stream Visual software, modified to be compatible with this new kit. The NG-Mix kit was assessed by histocompatibility laboratories in Grenoble, Lyon and Saint-Etienne (France) using blood and saliva samples. The data obtained were analyzed in comparison with those generated with the previous technique used in these laboratories. Analysis of 574 samples revealed overall consistency exceeding 95% between the two methods. Identified challenges included cross or preferential amplification of some alleles, drop-outs particularly for HLA-B and -DQB1 alleles, and low sequencing depth leading to ambiguities for mostly DRB1, DPA1 and DPB1 genes. Additionally, we noticed difficulties in identifying new alleles with insertions/deletions, managing contaminated samples and insufficient null allele reporting clarity. These issues, which could be

partially attributable to the use of a unique algorithm by the software, necessitated re-analysis with other techniques such as PCR-SSO or PCR-SSP. Future technical developments will focus on optimizing reagents and software adaptation. With the validation of the NG-Mix kit, and after running over 6000 typings in our region, we emphasize the need for third-generation sequencing techniques to meet both performance and emergency requirements.

P188 | Assessment of HLA typing and post-transplant monitoring combined on a single NGS run

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There has been a widespread adoption of NGS-based methods for HLA typing and a growing interest and capability to utilize NGS for post-transplant monitoring. This presents a potential challenge. Is it possible to combine various library types, with different fragment lengths and read targets on a single NGS run? The aim of this study was to assess the feasibility of combining NGS HLA typing and chimerism testing on a single run and to optimize the approach. Twelve previously tested chimerism samples were selected, including mixed chimerism samples ranging from 0.06% to 49.75% minor contributor. NGS-HLA libraries were prepared utilizing AlloSeq Tx17 and chimerism libraries were prepared with AlloSeq HCT. Libraries were first normalized based on fragment length as assessed by TapeStation analysis and final library concentration determined by fluorometric readings. The libraries were then pooled in varying ratios between 1:4 and 3:1 (Tx17:HCT). 2× 150 bp sequencing was performed using the Illumina v2 (300 cycle) standard kit. Target coverage was >250,000 reads for AlloSeq Tx™ and >800,000 reads for AlloSeq HCT™, with a total of 24 samples combined per sequencing run. Alloseq Tx™ fastq files were analyzed using AlloSeq™ Assign v1.0.3. AlloSeq HCT™ fastq files were analyzed with AlloSeq HCT™ v2.2 software. HLA genotyping accuracy was 100% concordant and chimerism testing results were consistent with expected values. There was no evidence of contamination, and sequencing noise was consistent between the types of runs. The combining of AlloSeq Tx17™ and AlloSeq HCT™ libraries is possible through the appropriate pooling ratios of each library type. This can lead to a reduction of sequencing costs and maximize sample throughput.

P189 | Validation of AlloSeq cfDNA to detect Donor derived cell-free DNA (dd-cfDNA) from renal transplant recipient (RTR) blood samples extracted using the Promega-Maxwell® RSC ccfDNA LV Plasma Kit

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The AlloSeq assay assesses graft injury and potential allograft rejection by measuring dd-cfDNA without the need for genotyping the donor or recipient. CareDx recommends using the Qiagen QIAseq cfDNA kit to isolate cfDNA from plasma, followed by quantification using the Qubit 4 Fluorometer. Results are then generated utilizing Next Generation Sequencing (NGS) to analyze 202 Single Nucleotide Polymorphisms (SNPs) to determine the percentage of dd-cfDNA. In our laboratory, we routinely use Promega kits for DNA extraction, so the aim of this work was to validate the use of the Promega Maxwell® RSC ccfDNA LV plasma kit with the AlloSeq assay, comparing our results to the recommended Qiagen cfDNA extraction kit. We used the Qubit dsDNA HS, Promega ProNex® and Agilent HS large fragment 50 kb kits for DNA quantification and sizing of our samples. Sixty-three plasma samples from 24 RTRs were prepared from blood samples using Streck Cell-Free DNA BCT™ tubes. From these samples, 26 were extracted using both the Qiagen and Promega DNA extraction kits and 11 were extracted using only the Promega kit. The 3 DNA quantification methods gave comparable DNA concentration profiles, independently confirming that Qubit quantification is fit for purpose. These samples were amplified and sequenced using the AlloSeq assay on Illumina MiSeq.

The sequencing data was analyzed using the AlloSeq cfDNA Software where the dd-cfDNA fraction (analyzed as a percentage of total cfDNA) was quantified. Donor derived cfDNA results were obtained for 89.2% (33/37) of Promega extracted samples, in contrast to 61.5% (16/26) of samples extracted using the Qiagen kit. We were able to directly compare the dd cfDNA results of 15 samples extracted on both kits, showing concordance across the kits (2/15 = positive (>1% dd-cfDNA); 13/15 = negative). This study validates the Promega extraction protocol and Qubit quantification, for the AlloSeq assay and demonstrates that this kit accurately detects dd-cfDNA in our recipient cohort.

P190 | High-resolution full gene HLA-DRB1 genotyping using Oxford nanopore long read sequencing technology

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Full gene high-resolution HLA typing (at third field) improves accurate assessment of donor-recipient compatibility in clinical transplantation. DRB1 genotyping is challenging due to the extreme degree of HLA polymorphism and the presence of closely related DRB3, 4 and 5 genes and pseudogenes. This diversity complicates the identification of a universal primer binding site that can effectively target all known DRB1 alleles. Currently, there are no commercially available PCR amplification kits with primers located outside of the 5′ or 3′ untranslated regions (UTRs) of DRB1 that can generate 5′ to 3′ UTR inclusive amplicons. Additionally, some protocols require two separate PCR amplification reactions to cover the entire length of DRB1. Long-read Oxford Nanopore Technologies (ONT) sequencing methodologies has recently enhanced its modal raw reading sequencing accuracy to a claimed 99% on the latest R10.4.1 flow cells, becoming a robust platform for unambiguous DRB1 typing. The reusable feature of flow cells also provides a flexible throughput solution, facilitating faster turnaround time for small batches of samples. A novel set of DRB1 primers was designed to enable the production of DRB1 amplicons covering the 5′ UTR, all introns, exons and the 3′ UTR in a single PCR. Forward and reverse primers for DRB1 alleles were designed using consensus sequences obtained from aligning the upstream 5′ UTR and downstream 3′ UTR regions of available representatives from all known DRB1 allele groups (first field). DRB1 primer concentrations were adjusted to ensure allele balance. A large cohort of representatives of common and low frequency DRB1 types were tested. The results demonstrate a high concordance rate when compared to the typing results generated by short-read sequencing. In conclusion, the assessment of this novel set of DRB1 primers indicates their capability to perform unambiguous third field DRB1 typing when using ONT R10.4.1 flow cells.

P191 | MICA & MICB—Are your samples up to the challenge?

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The MHC class I chain-related genes A (MICA) and B (MICB) are located on chromosome 6 and act as ligands for the activating receptor NKG2D expressed on the surface of NK cells and specific T lymphocytes. Anti-MICA antibodies have been associated with acute and chronic rejection in solid organ transplantation. There is also evidence that MICA mismatching increases the incidence of acute and chronic graft-versus-host disease in Hematopoietic Stem Cell Transplantation (HSCT). Our laboratory utilizes GenDx singleplex NGSgo® as its principal HLA typing method for 11 classical HLA loci. Recently, we have increased the flexibility of our NGS workflow allowing combinations of classical HLA loci, together with other genes important in transplantation. The aim of this project was to verify and optimize the GenDx NGSgo-AmpX® MICA and MICB process to add to our existing bespoke Next Generation Sequencing (NGS) pipeline. Seventy-two samples were amplified, and of these 56 samples, with known MICA and/or MICB types,

were sequenced using Illumina MiSeq. The assay was optimized using 6–62 ng/μL DNA derived from blood, cell lines and spleen. DNA amplicon visualization by gel electrophoresis, revealed that samples <15 ng/μL amplified poorly, translating to low amplicon concentration by Qubit quantification. Increasing DNA volume for low concentration samples did not significantly improve amplification success. All samples sequenced were concordant with previous MICA and/or MICB types. A 9.8% test failure rate (8/56 samples (11/112 tests): 8 MICA, 3 MICB) was observed. Of the 8 sample failures with DNA concentration > 15 ng/μL, 7 samples exhibited DNA fragmentation. Sequencing was also optimized by increasing the extension time from 6 to 9 mins and doubling the pooling of MICA compared to MICB. This study has demonstrated that sufficient and intact DNA template is critical to ensure successful amplification and sequencing when using the GenDx NGSgo-AmpX® MICA and MICB kit for routine testing.

P192 | High resolution HLA typing comparative analysis of blood stem cell and solid organ donors and recipients with next generation sequencing and real time PCR

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High-resolution (HR) HLA typing improves the precision of evaluating donor-recipient compatibility. A variety of molecular methods are available, with their use depending on the cost and the laboratory's rapid turnaround time. HoloTYPE, NanoTYPE Next Generation Sequencing (NGS) and Real Time PCR are methods examined herein for HR HLA typing. These were assessed in the aspect of Bone Marrow and Solid Organ histocompatibility analysis. DNA samples were evaluated for High-resolution HLA typing using the three different methods at 7 HLA loci (A, B, C, DRB1, DQB1, DQA1 and DPB1). For each sample, the DNA concentration was measured using the Qubit instrument and the library for each method was prepared according to the manufacturer instructions. The Omixon HoloTYPE and NanoTYPE kits were used on the Illumina MiSeq and Oxford Nanopore MinION Sequencing platforms, respectively. The BAG Diagnostics HISTO TYPE Rainbow kit was used on a Bio-Rad CFX Opus Real-Time PCR instrument. Methods were compared based on quality and accuracy of results, as well as parameters such as DNA concentration and rapid turnaround time to result. All methods provide accurate HLA typing for the purpose of histocompatibility analysis between donors and recipients for BM and solid organ transplantation. Whilst the Illumina NGS platforms are known for their high throughput, the Oxford Nanopore Sequencing is better at assembly and portability, has an easy workflow, absence of capital investment for the instrument, and offers less hands-on work. Oxford Nanopore also offers the option of less samples per run, with as little as 3.5 hours for HR typing of a single sample. On the other hand, the RT-PCR method, is fast and easy with results in less than 80 minutes and automated interpretation of data. This is applicable for one sample analyzed per run, making it the ideal option when on-call HLA typing is required. The Illumina and Oxford Nanopore Sequencing platforms are ideal for routine HLA typing.

P193 | Nanopore kit for the detection of new alleles

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With its ability to deliver high-resolution HLA genotyping, NGS technology has revolutionized HLA compatibility for transplantation. But the innovative high-speed Nanopore technology now makes it possible to apply this revolution to organ transplantation, suggesting that donor selection could eventually be based on molecular compatibility. We present here the Marseille laboratory's experience with the NGS-Turbo® (GenDX®) technique. Six samples, 2 internal quality controls and 4 patient/donor samples for which a new allele was found using the Illumina NGS technique (NGmix, EFS) were tested. Of these 4 samples, only one could be tested using the NanoType technique (Omixon®), due to interference with the DNA extraction method (Chemagic) for the other 3. The NGS-Turbo protocol comprises three reaction mixes and requires 30 ng of DNA per mix, that is, 90 ng for typing the 11 HLA loci. The

bead library is prepared in three steps (end-repair, ligation and elution) in 30 min. Finally, sequencing on flowcell R.10 and Mk1C or Mk1B provides genotyping in 30 min to 1 h, using NGSengine-Turbo software (IMGT v3.52). Genotyping of the 11 HLA loci from internal quality controls and samples was confirmed, and the 4 new alleles were found independently of the extraction technique. On the one hand, the results produced by NGS-Turbo® are in accordance with those obtained by NGmix and NanoType techniques, and on the other, the advantages are easy implementation and rapid results.

P194 | Nanotype and nanopore sequencing—A rapid and reliable technology for routine HLA typing

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Nanopore sequencing is an innovative technology that relies on a nanoscale protein pores, or 'nanopores', that serve as a biosensor and allow direct passage of DNA or RNA molecules. This study presents our first experience using the nanopore technology for HLA typing in order to evaluate the feasibility of implementing this method in our routine. We have selected a total of 96 (76 fresh/20 frozen) EDTA samples from patients, related/unrelated stem cell donors. A number of 12 uncommon alleles in our population, 2 null alleles and 3 potential new alleles were included. The target loci were HLA-A, -B, -C, -DRB1, -DQB1, -DQA1 and -DPB1. All samples were previously tested by NGS LR-PCR (Holotype HLA 96/7, Omixon Inc) on an Illumina MiniSeq platform. For the new study, the NanoTYPE 24/11 kit (Omixon) and MinION sequencer have been chosen. The method consists of amplification, quantitation, normalization and library preparation including ExoSAP purification, fragmentation, barcoding, pooling, purification and rapid adapter attachment followed by sequencing, that was divided into 11 runs with different number of samples, 6/8/12/24/1 per run. For HLA assignment we used NanoTYPER software, IPD-IMGT/HLA Database release 3.52. Comparison with reference results: 5.2% (5/96) ambiguities on HLA-A respectively the 3 potential new and 2 null alleles, all other HLA-A, -B, -C, -DQA1, -DQB1 showing unambiguous 6 digits results. Regarding HLA-DRB1/DPB1, the results could be defined in G/P groups and were concordant 98% for DRB1 and 100% for DPB1. We closely monitored each sample sequencing quality parameters. The key exon imbalance was under the ideal range in 5.2% of DRB1 7% of DQB1 and 13.52% of DQA1 and the coverage was slightly lower in 3.12% of HLA-A and 1% of HLA-B and DRB1 loci. In conclusion, the NanoTYPE HLA typing assay is optimally designed and supported by a clear protocol, so that the workflow could be easily integrated into any H&I laboratory in order to be used in daily routine, in combination with any other available technique.

P195 | Deceased donor HLA typing by next generation sequencing—Ready for prime time!

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In the U.S. the most common method for deceased donor (DD) HLA typing is RT-PCR. DD typing must be reported as serological equivalents as RT-PCR does not provide 2 field high resolution. DD typing reporting at 2-field would allow the HLA lab to accurately identify allele level antibodies in sensitized patients. The constraint for using next generation sequencing (NGS) for DD has been the length of time it takes to perform the assay. With the advent of newer technologies turnaround time (TAT) is no longer an issue. The average TAT from DNA extraction to reporting results using GenDx Turbo NGS is routinely under 5 h although the time has begun to shorten as proficiency is increasing. Here we report the implementation of NGS-Turbo for routine DD HLA typing. From Nov. 1st 2023 through to Feb. 14th 2024 a total of 83 local DD typings were performed by both Turbo NGS and RT-PCR. The 2-field hi-res data provided by NGS has allowed the HLA and clinical team to better assess offers for highly sensitized patients who have allele level antibodies and thus selecting appropriate donors for these patients. In addition, NGS has resolved ambiguities seen in RT-PCR which may be impactful. For example, NGS typing called a DPB1*1037:01 outright whereas the RT-PCR gave multiple combinations of

rare alleles making assignment difficult. Although there was very good correlation between RT-PCR and NGS there were some clinically relevant discrepancies. The most significant one was a sample where RT-PCR called the common typing as C*03:03 (Cw9) and NGS typed the donor as C*03:421N. NGS Turbo has also identified at least one new allele in the 83 typings. Validation was quick as the lab was already routinely performing NGS and training staff was not an issue. The assay is robust with only 1 sample needing repeat. This technology is easy to integrate into the DD workflow and although it takes a little longer than standard RT-PCR the increased level of resolution is worth pursuing.

P196 | Comparing MFI and MCI in single antigen HLA antibody detection on alternative platforms (Luminex vs HISTO SPOT microarrays)

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The aim of this study was to establish if there is a correlation between the measurement values generated with three alternative methods for single antigen HLA antibody identification. Though the MFI (Mean Fluorescence Intensity) measured on the established Luminex platforms is no direct measure of antibody titer or risk for organ rejection, it is often used as an indicator for the clinical relevance of an HLA antibody in the transplantation context. Therefore, it is important to know if the MFI values generated with the two different vendors on the Luminex platform are comparable and if the MCI (Mean Color Intensity) generated on the MR.SPOT platform can be used as an equivalent to the MFI. In this study the donor specific antibodies of 88 patients who showed clinical symptoms of rejection after kidney transplantation were tested with the LIFECODES LifeScreen LSA (Immucor), the LABScreen Single Antigen (ThermoFisher/One Lambda) and the HISTO SPOT HLA AB ID Kits (BAG Diagnostics). There was a strong correlation between all tests, with the correlation between the two Luminex tests being slightly higher than between the Luminex and the HISTO SPOT tests. None of the differences in the qualitative results between the Luminex (fluorometric) and HISTO SPOT (colorimetric) platforms were statistically significant. In conclusion, the measured values provide equivalent information, yet it is crucial to consider the inherent limitations of using these values as indicators of clinical relevance.

P197 | Utilization of PreSorb beads in removing nonspecific reactivities in HLA antibody test by Luminex solid phase single antigen beads assay: A single center experience

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Accurately determining HLA antibody specificities is critical for successful virtual crossmatch and donor-specific antibody interpretation in transplantation patients. Although the Luminex solid phase single antigen beads (SAB) assay has become the golden standard in assessing HLA antibodies, it is still challenging when non-specific reactivities are present. In our previous study, we have shown that PreSorb beads, a new serum cleanup reagent, from One Lambda, can effectively remove C1/12/15 and Pan-DR in sera without significant changes in mean fluorescence intensity (MFI) values of HLA antibodies. Since December 2022, our laboratory treated sera with non-specific reactivities using PreSorb beads. In this study, we retrospectively investigated the effectiveness of PreSorb beads in the removal of non-specific antibodies in transplant patients. From December 2022 to January 2024, 206 sera from 156 transplant patients with non-specific reactivities tested by HLA SAB assays were treated with PreSorb beads according to the manufacturer's instruction, and further HLA class I and / or class II SAB assays were repeated. HLA antibodies using LabScreen SAB assay (One Lambda) and read on FLEXMAP 3D system (Luminex). Our results showed that PreSorb beads caused neither false positive nor false negative HLA antibodies in 206 sera. ProSorb beads effectively removed Pan-DR non-specific reactivities in 6 patients. Among these 6 patients, 5 had pre-existing C1/12/15 specificities. However, other non-specific reactivity patterns, such as nonspecific self-antibodies, Pan-C, DP1/DP5/DR53, other nonspecific -DQ, and -DP, and high background were not effectively removed in other sera. These results confirmed our previous study and further

suggest that PreSorb may be valuable in reducing non-specific HLA antibody reactivities in the sera of certain transplant patients, however, it had a limited effect on other non-specific reactivity patterns.

P198 | Rapid and accurate monolocus HLA typing using Oxford Nanopore Technology: New routine for the "HLA and disease" activity at Bordeaux University Hospital

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The Oxford Nanopore Technology (ONT), coupled with its MinION sequencer, is revolutionizing the field of HLA by enabling rapid high-resolution typing from long reads in just a few hours. In June 2023, ONT (NanoTYPE Mono, Omixon) replaced PCR-Sequence-Specific Oligonucleotides (SSO) (LABType SSO XR, One Lambda) for HLA monolocus typing at the Bordeaux University Hospital Immunogenetics Laboratory. HLA monolocus typing is ideally suited for our "HLA and diseases or drug hypersensitivity reactions susceptibility" activity. We have expanded its application to rapidly confirm organ or hematopoietic stem cell donors/recipients typing. The implementation, evaluation, and optimization of this technology in the laboratory, based on sequencing metrics and samples analyzes, allowed us to establish validation criteria for typing interpretation. A total of 415 alleles (including patient and external proficiency scheme samples) were compared to PCR-SSO and short-read sequencing (Illumina or Ion torrent) typings. Despite residual ambiguities, most interpretation difficulties, such as cis-trans ambiguities, were resolved. For instance, 96 out of 96 2nd field typed alleles (100%) were found in the PCR-SSO's ambiguity list. Additionally, 313 out of 319 3rd field typed alleles (98.1%) by short-read sequencing matched ONT results. Discrepancies included 3 dropouts and 3 misinterpreted new alleles. Optimizations showed that 4 h are enough to sequence a set of 48 loci and consumes on average 270 pores. 2500 reads/FASTQ lead to typings of sufficient quality. Even though some essential improvements are required, the use of NanoTYPE Mono kits with ONT offers new perspectives for HLA typing and laboratory organization.

P199 | Genotyping of six HLA-G SNPs by oligonucleotide ligation assay: Optimization of the technique

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HLA-G belongs to the non-classical major histocompatibility complex class Ib (MHC Ib), including membrane and soluble isoforms. HLA-G regulates the function of several immune cells. It is one of the important immunological mechanisms of maternal-fetal tolerance and tumor immune escape. The HLA-G locus is highly polymorphic in the 3′ UTR (untranslated region) and 5′ URR (upstream regulatory region). Genetic variations in the HLA-G gene affect its expression at both transcriptional and post-transcriptional levels. The aim of our study was to develop a new method for genotyping six HLA-G 3′ UTR SNPs (rs1707, rs1710, rs17179108, rs1063320, rs9380142, rs1610696) detected by Luminex using the Oligo Ligation Assay (OLA). Two groups were included: Group 1; individuals homozygous for the HLA region from consanguineous families and their parents. Group 2; unrelated healthy controls. SNP genotyping was performed in 3 steps. Genomic target sequence amplification which amplifies the region of the 3′ UTR of the HLA-G gene containing the six SNPs. Multiplex oligoligation assay reaction, where for each SNP two primers were used, the first labeled at 5′ with an oligonucleotide probe and ending at 3′ with an allelic variant, and the second is a biotinylated reporter primer. Hybridization on MagPlex-TAG microspheres: two beads were used for each SNP. After adding streptavidin-R-phycoerythrin (SAPE), the beads were analyzed on Luminex 3D. As a result, we confirmed the homozygosity of the six SNPs in the offspring of consanguineous families and the segregation of the alleles within these families. In the control group we will

provide the frequencies of the different alleles and the corresponding haplotypes. In conclusion, this preliminary study provides promising results for the use of OLA for HLA-G SNP genotyping in Luminex-owning laboratories. Validation of this method in a larger population of controls is necessary.

P200 | Assessment of automated cell-free DNA extraction utilizing Promega Maxwell

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Donor-derived cell-free DNA has emerged as a valuable biomarker in transplant medicine, providing non-invasive insights into graft health. Efficient extraction of cell-free DNA is a critical step for accurate downstream monitoring assays and the potential early detection of allograft rejection. However, most manual cell-free DNA extraction methods are labor-intensive and require significant hands-on time. Moreover, the manual approach poses technical challenges that can result in potential contamination or poor recovery rates. In this study, we evaluated an automated system for the extraction and purification of cfDNA. We assessed total cfDNA yield and purity using the Qubit Fluorometer and the Tapestation 4150 System, respectively. Additionally, we measured the total time to completion and the hands-on time required. Blood samples from healthy donors were collected into collection tubes (Paxgene Blood ccfDNA Tubes or Streck Cell-Free DNA BCT RUO Tubes). Up to 8 mL of plasma was then centrifuged and processed according to the manufacturer's instructions. Samples were prepared following the instructions of the Maxwell ccfDNA Plasma Kit and loaded onto the HSM 2.0 Instrument. After completion, samples were transferred to prepared Maxwell cartridges, and the LV ccfDNA method was performed on the Maxwell CSC instrument in research mode. In conclusion, the utilization of an automated system for the extraction and purification of cell-free DNA presents a promising advancement in transplant medicine. Our study demonstrates the efficiency of this automated approach in achieving high yields of cell-free DNA with minimal hands-on time compared to manual methods. Additionally, the automated system offers improved consistency and reduces the risk of contamination, addressing key challenges associated with manual extraction techniques.

P201 | Analytical and clinical validation of the One Lambda™ Devyser Accept cfDNA kit

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Our team has published that %dd-cfDNA can identify patients free of early lung transplantation (LTx) events (acute rejection (AR), infection), 30 days after LTx. Our biopsy decision algorithm for suspected AR is based on a pre-analytical and analytical process defined by our laboratory. However, it is known that different pre-analytical (extraction) or analytical processes, have a major influence on the dd-cfDNA interpretation. The aim of this study is to check the performance of the One Lambda™ Devyser Accept cfDNA kit, and to compare the results with our gold-standard (AlloSeq cfDNA-CareDX®). Six dd-cfDNA control, 5 artificial dd-cfDNA (0.5%–10%) and 3 LTx patients (at day 0, 15, 30, 90 and 180 of LTx) were tested. Use of the One Lambda™ Devyser Accept cfDNA kit requires manual steps lasting 2:10, such as preparation for two PCRs and purification on beads (equipment/incubation time of 3:40). Two libraries are prepared, one for screening and the other for monitoring, then loaded onto the sequencer (MiSeq, micro 300 v2, sequencing time $\sim$ 16 h). The artificial panel highlighted the good analytical performance of the Accept cfDNA kit. All patient points were comparable to our reference and consistent with our clinical algorithm (stable, AR or infection). The One Lambda™ Advyser Solid Organs software is user-friendly, with the option of selecting markers for %dd-cfDNA calculations. Many steps were performed manually, but few functions automate the process of importing fastq into the correct timepoint, for example. Concerning the need for pre-LTx DNA, whereas the laboratory has not systematically donor pre-LTx DNA, his availability reduced background noise. Our experience with the One Lambda™ Devyser Accept cfDNA kit validated its analytical performance and comparability with our

reference (AlloSeq cfDNA-CareDX®). Patients are classified identically in our algorithm. This method is promising, and we're going to test it on the entire cohort of LTx patients.

P202 | Validation of NGS and intermediate resolution methods for HLA typing at the Laboratorio Nacional de Inmunogenética—INCUCAI of Argentina

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The importance of HLA compatibility at high resolution level in hematopoietic progenitor cells (HSC) transplantation is well known and their use is expanding to solid organ transplants for enhanced accuracy in virtual crossmatch predictions and for post-transplant follow-up. NGS typing is the gold standard method for HLA and SSO can be used as an alternative method with intermediate resolution. The HLA typing of deceased donors requires a faster typing method like Real Time PCR (RT-PCR). In our laboratory we validate NGS, SSO and RT-PCR typing in order to cover the need for enrollment unrelated HSC donors to our National Registry, solid organ programs and typing of deceased donors. Twenty-four reference samples were extracted from EZ2 Qiagen DNA Blood kit and sequenced for 11 HLA loci using AllType and AllType FASTplex on an Illumina MiniSeq platform. Fifteen of the total samples were additionally typed for 8 HLA loci using SSO Lifecode and 11 loci using Real Time LinkSeq. Results were analyzed with TSV, matchIT DNA and SureTyper software respectively. In the panel of 24 reference samples, we found a concordance rate of 100% for HLA-A, -B, -C, -DRB1/3/4/5, -DQA1, -DPA1, -DPB1 and 96% for HLA-DQB1 using the AllType FASTplex kit, all discordant results were due to dropout of one allele. While the concordance rate using the AllType kit was 100% for all 11 loci. On the 15 samples typed by SSO and Real Time, there were no discrepant results at any loci. The total time to generate a typing on a deceased donor by real time PCR was from 2.5 h. In this analysis we found a good correlation rate for high-resolution typing by two NGS typing kits, but AllType FASTplex uses a simplified workflow that minimize pipetting, consumables and it is easier to implement than AllType. We are going to extend this validation with at least 50 samples. The RT-PCR typing meets our local requirements for deceased donors and SSO will be used as a complementary method.

P203 | NanoTYPE—From single locus testing to high throughput multiplexing in 96 format

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In recent years, next-generation sequencing (NGS) has emerged as the preferred method for achieving high-resolution HLA typing, surpassing Sanger sequencing due to its notable advantages, including increased throughput, comprehensive gene characterization, reduced turnaround time, and minimized ambiguity. Despite these benefits, conventional NGS platforms face challenges in sequencing lengthy fragments, leading to cis-trans ambiguities. Additionally, the prolonged turnaround time (2–3 days) and the necessity to batch samples for cost-effectiveness pose limitations, particularly in urgent scenarios or laboratories with lower throughput. Omixon has expanded its product portfolio on the nanopore sequencing platform by introducing tailored solutions for diverse testing scenarios. This expansion includes NanoTYPE mono, designed for single locus testing, NanoTYPE COMBI for dual plex testing, and a presentation showcasing data generated using the advanced NanoTYPE 96/11 version. This specific version is crafted for high-throughput testing on a novel nanopore sequencing platform. The presentation will delve into the analysis of data generated from 96 samples, highlighting the efficacy and versatility of the NanoTYPE solution.

P204 | Evaluation of LabScreen PreSorb to proficiently remove pan-reactive DR antibody reactivity

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False positive results from LabScreen single antigen assays (LS-SAB) may result from denatured HLA

molecules and may be suspected from own-type reactivity. False negative results may result from prozone. High background may result in repeated failure to obtain valid results. We use a second SAB assay to overcome these issues. To evaluate the use of LabScreen PreSorb to remove pan-reactive DR antibodies. Sera for a control cohort ($n = 13$) with varied HLA CII antibody positivity and pan-reactive DR cohort ($n = 18$) were tested neat, EDTA, PreSorb Neat and PreSorb EDTA. PreSorb was used as per manufacturers' instruction. Lifecodes SA CII was also performed on pan-reactive DR cohort. In our control cohort, Prozone was seen when PreSorb was used in the absence of EDTA, that is, EDTA treatment is required. Antibody assignment was comparable with EDTA SA with the exception of 12/1274 beads that became negative and 30/1274 beads that became positive. PreSorb EDTA MFI tended to be higher than for EDTA SA, and use would require review of reference cut-off MFI value. In the pan-reactive DR cohort, 2/18 PreSorb EDTA were invalid as negative control MFI > 1500. In 15/16 valid results, PreSorb EDTA treatment decreased DR antibody MFI. One case remained DR pan-reactive, and no valid Lifecodes result was obtainable. Otherwise, any DR positivity detected by PreSorb EDTA was concurrently detectable by Lifecodes SA. A positive to negative MFI change was noted for 8/16 and 6/16 samples for several DQAB and DPAB beads respectively. PreSorb treated sera requires subsequent EDTA treatment to mitigate prozone. PreSorb EDTA removed pan-reactive DR in 15/16 cases. However, MFIs were altered, and some DQAB and DPAB beads were also affected.

P205 | Detection and characterization of six novel HLA alleles by next-generation sequencing in a Spanish population during the last year

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Next-generation sequencing (NGS) has benefited immunology labs since it obtains phased sequences, increases coverage, reduces ambiguities and permits the identification of novel HLA alleles. HLA typing of volunteer bone marrow donors was performed with NGSgo-MX11-3 (GenDx) and MiniSeq (Illumina). Data were analyzed according to NGSengine. A novel null allele, DPA1*02:66:02N: showed a single difference relative to DPA1*02:01:01:03 in exon 2 at genomic position (gp) 3825 (C > T), codon 50 (CGA > TGA) producing the replacement of Arginine by a premature stop codon. A novel allele with an exon 1 change, DRB1*04:354, presented a single change relative to DRB1*04:06:02 at gp 38 (C > T), codon -17 (GCA > GTA), causing the replacement in the leader peptide of Alanine -17 to Valine. A novel allele with an exon 4 change, DQA1*05:71, showed a single transition relative to DQA1*05:01:01:02 at gp 5238 (G > A); codon 208 (GGC > GAC), causing the replacement in the transmembrane domain of Glycine 208 to Aspartic Acid. A novel allele with intronic changes, DQB1*03:02:01:14, showed one change in intron 1 and three in intron 2 located at gp 577 (A > G), 2155 (A > G), 3466 (A > G) and 3933 (T > C) relative to DQB1*03:02:01:11. The sequence was aligned with all DQB1 alleles and we noticed that reported mismatches were shared with DQB1*03:02:01:01. However, DQB1*03:02:01:14 showed 10 changes from intron 3 to intron 5 which were shared with DQB1*03:03:02:01. DQB1*03:02:01:14 would have arisen by a recombination event between DQB1*03:02:01:01 and DQB1*03:03:02:01. DQB1*05:02:01:13: showed a single change in intron 5 (gp 6036:T > C) relative to DQB1*05:02:01:01. DQB1*05:02:01:14: presented a single change in intron 2 (gp 2453:T > C) relative to DQB1*05:02:01:01. NGS has allowed the exhaustive description of HLA polymorphism, including its origin and distribution along HLA genes. These data could be useful in clinical practice to improve transplantation outcome and to understand the association between HLA and some diseases.

P206 | Deciphering alloreactivity: An educational website tailored to teach and learn alloreactivity

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Although many textbooks have exhaustively described the immune mechanisms that occur after transplantation, most of them require a strong biological background

to understand. This educational website aims to be an attractive resource for the immunology novice, while accurately describing the processes of alloreactivity in solid organ transplantation. The website is organized around three panoramas representing the main periods of transplantation: pre-transplantation, per-transplantation and post-transplantation. Each of these three panoramas can be viewed from two different perspectives thanks to a toggle button, highlighting either the actors or the mechanisms involved. They can all be developed with a single click for an interactive and enjoyable experience. In total, almost 20 actors of innate and adaptive immunology are represented—such as the complement system, antigen-presenting cells, dendritic cells, NK cells, T lymphocytes, HLA molecules, KIR receptors, lymph nodes, as well as more than 15 key mechanisms—such as direct and indirect alloreactivity, cross-presentation, lymphocyte activation, NK activation by the "missing self," acute and chronic rejection. The attractiveness lies in the conciseness of the explanations, which are entirely focused on transplantation. The content of each page can be downloaded in a readable format. This website aims to provide an insight into the players and mechanisms involved in alloreactivity after transplantation. It has been designed to be understandable without a strong background in immunology and is available on desktops and smartphones to reach a wide audience, from undergraduate biology students to medical and paramedical staff and patients wishing to better understand the transplant journey. The website is available from the home page www.comprendrelalloreactivite.fr in French as well as English versions and was designed with the support of Sanofi.

DOI: 10.1111/tan.15481

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ABSTRACT

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