ORAL PRESENTATIONS

Best Abstracts

O1 | Forward or reversed binding of peptides within the HLA-DP peptidome is mainly determined by the HLA-DPB1 allele but with a key role for the HLA-DPA1 chain

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A central dogma over the last 50+ years has been that peptide-binding to HLA-molecules is mediated by the docking of side chains of particular amino acids in the peptide into pockets in the HLA-molecules in a conserved N- to C-terminal orientation. We have shown in our large-scale identification of HLA-DP peptides that 9 out of 14 HLA-DP frequent allotypes can present peptides in two orientations that are both functional and can be recognized by CD4 T-Cells. The occurrence of the reverse peptide binding motif, in an N- to C-terminal orientation, is mainly dependent on the DPB1 chains in the allotypes with the DPB1-84DEAV87 sequence at P1. We investigated the role for DPA1 and DPB1 chains by making several cis and trans HLA-DP heterodimers and introducing specific mutations. When we analyzed the presentation of peptides by peptide elution we found that not only the sequence of the DPB1 chain is crucial in the presentation of peptides in the reversed orientation, but we also showed a key role for the DPA1 chain in modifying the peptidome. This leads to the conclusion that specific heterozygous HLA-DP peptidomes will be shaped depending on the potential cis and trans heterodimer-combinations. In the context of HLA-DP-mismatched allogeneic stem cell transplantation, mismatched HLA-DP alleles can provoke profound allo-HLA-DP-specific immune responses from the donor T-cell repertoire dependent of the composition of the mismatched peptidome. Our results illustrate unique features of HLA-DP molecules that substantially broaden the HLA-class II bound peptide repertoire to combat pathogens, eliminate cancer cells and can have significant influence on the outcome of allogeneic stem cell transplantation.

O2 | Single cell transcriptomics to identify leukemia-intrinsic and -extrinsic bone marrow correlates of immune escape and post-transplantation relapse

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Allogeneic Hematopoietic Cell Transplantation (allo-HCT) represents the most successful therapeutic option for many patients suffering from Acute Myeloid Leukemia (AML). Nevertheless, tumor cells frequently enact strategies to evade immune recognition and re-emerge, by either impairing their recognition mediated by T cells through reduced antigen presentation and enforcement of inhibitory checkpoints, or by taking advantage of their own tumor microenvironment (TME). Here, we used single-cell RNA-sequencing to detail the changes that occur in the bone marrow of patients who experience
AML relapse after allo-HCT. We profiled 114,273 cells from 18 AML patients with different mechanisms of leukemia relapse, including down-regulation of HLA class II (n = 5), up-regulation of inhibitory ligands (n = 4), and HLA loss (n = 9), 10,080 cells from 3 healthy donors and 16,832 cells from 3 post-transplant AML patients in complete remission. First, we used different gene signatures representing successive stages of normal hematopoietic development to calculate a gene module score for each relapse modality. HLA loss relapses showed a more immature expression profile, suggesting that the hematopoietic cell of origin impacts on the mechanism of relapse. Focusing on the TME, we analyzed monocytes, and observed the presence of an interesting cluster of donor origin characterized by an anti-inflammatory gene signature, which might play a facilitating role in leukemia immune evasion. Lastly, we leveraged on TCR sequencing to describe the complexity of the T cell repertoire in different relapse settings, and found out that AML relapses characterized by HLA class II downregulation displayed a more clonal TCR repertoire. Findings from this study will improve our understanding of how leukemic cells exploit their TME to escape immune surveillance in the different patients, and identify new vulnerabilities to be exploited for personalized therapeutic approaches.

O3 | Highly specific latent membrane protein 2A-targeting T-cell receptor-engineered T cells with inducible Interleukin-18 expression as promising tool to treat Epstein-Barr Virus-associated malignancies

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Epstein-Barr virus (EBV) infects more than 90% of the population and remains in B-cell compartments life-long, passing through several latency stages. While in healthy individuals, strong immune responses control EBV reactivation, in immunocompromised patients, infections and reactivations can lead to severe EBV-associated malignant complications, such as post-transplant lymphoproliferative disease (PTLD). These patients may benefit from EBV-specific T-cell therapy. In latency stages II/III, latent membrane protein 2A (LMP2A) is expressed and therefore associated with PTLD as well as various lymphomas and carcinomas. Recently a clinically protective TCR recognizing an LMP2A-derived peptide in context of HLA-A*02 was identified. Based on this, we developed TCR-engineered T cells and further equipped these with an inducible cassette for locally restricted IL-18 release (LMP2A_iIL18_TCR-Ts), which was shown to convert T cells into pro-inflammatory effector cells, preventing loss of function and exhaustion, and redirecting the immunosuppressive tumor microenvironment. As LMP2A_iIL18_TCR-T functionality is hypothesized to be superior compared with endogenous T cells, we analyzed their phenotype, replicative capacity, activation and exhaustion state as well as cytotoxicity towards HLA-A*02+EBV+ target cells. In this context, LMP2A_iIL18_TCR-Ts showed HLA-A*02:LMP2A specificity without signs of HLA cross-reactivity or recognition of an irrelevant HLA-A*02-restricted peptide, which was further confirmed by Ca2+ flux analysis. Combination with inducible IL-18 expression increased cytotoxicity measured by 7-AAD staining, live cell imaging and detachment of target cells in real-time impedance measurements. In conclusion, ex vivo isolated protective TCRs could be redirected into T cells from third-party donors with the potential to attract innate immune cells and alter the tumor environment, thereby widening the applicability of T-cell therapy to refractory viral infections.

O4 | Proteome analysis of drug susceptible HLA-B*57:01+ cells reveals the pivotal mechanisms of HLA-mediated carbamazepine hypersensitivity

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Measures of drug-mediated immune reactions that are dependent on the patient’s genotype determine individual medication protocols. Despite extensive clinical trials prior to the license of a drug, certain immune reactions cannot be predicted. The need for acknowledgement of the proteomic state for selected individuals under drug administration becomes obvious. The association between certain HLA molecules and drugs or their
metabolites has been analyzed in recent years. Dependent on the patient's genotype, Carbamazepine (CBZ) hypersensitivities cause diverse disease pictures as MPE, DRESS or more severe diseases SJS or TEN. Not only the association between HLA-B*15:02 or HLA-A*31:01 but also between HLA-B*57:01 and CBZ administration could be demonstrated. This study illuminates the mechanism of HLA-B*57:01-mediated CBZ hypersensitivity by full proteome analysis. Genetically engineered human B-LCLs expressing sHLA-B*57:01 molecules were treated with CBZ or its metabolite carbamazepine-10,11-epoxide (EPX). The incapability of these B-LCLs to metabolize CBZ makes them an ideal system for analyzing the influence of CBZ and EPX orthogonally. HLA/drug complexes were purified and the availability of CBZ or EPX was monitored by mass spectrometry. The data reveal significant stronger engagement of B*57:01 to EPX than to CBZ. Subsequent full proteome analysis of engineered B*57:01+ cells following CBZ or EPX treatment uncovered an unknown mechanism; EPX introduced drastic proteomic alterations as the induction of inflammatory processes through the upstream kinase ERBB2 and the upregulation of NFkB and JAK/STAT pathway implying a pro-apoptotic, pro-necrotic shift in the cellular response. Simultaneously, anti-inflammatory pathways and associated effector proteins were downregulated. Fundamental knowledge of drug-susceptible HLA molecules, their drug antagonist and the disequilibrium of the proteomic content will certainly facilitate personalized and safe medication.

O5 | The evolution of MHC class I loss in a newly emerged transmissible cancer in Tasmanian devils

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Downregulation of Major Histocompatibility Complex (MHC) molecules is often key to evasion of the immune system by tumors and viruses. Transmissible cancers transmit between individuals, in a manner akin to a metastatic event, providing a unique opportunity to study the evolution of MHC loss in the face of selective pressure from the immune system. Tasmanian devils are infected with two genetically distinct transmissible cancers which transmit via biting behaviors. Devil Facial Tumor Disease (DFT1) emerged over 25 years ago, while DFT2 was identified in 2014. In contrast to DFT1, DFT2 tumors express MHC class I molecules; but recent evidence shows some are MHC class I negative, suggestive of evolving immune escape by this cancer. We hypothesize that as DFT2 spreads through the population, encountering hosts with disparate MHC class I genotypes, selection for MHC class I loss is occurring. Here we have used immunohistochemistry to assess MHC class I expression and CD3+ cell infiltration in DFT2 primary tumor biopsies (n = 26). Classical MHC class I expression was highly variable between tumors, with 9 tumors negative by immunohistochemistry. Tumors were also variable for expression of 2 non-classical MHC class I genes, which could play a role in immunosuppression. To investigate drivers of MHC loss we have genotyped (n = 17) host animals at three classical MHC class I loci. Host-tumor comparisons at MHC class I reveal potential immunogenic alleles driving immune escape in DFT2. These results demonstrate that DFT2 is evolving immune evasion mechanisms as it transmits between individuals in the population, with the potential for more rapid dissemination if MHC-negative subclones gain dominance. However, this data can be used to predict DFT2 transmission dynamics and inform more effective management of the population. Further, this study provides a platform to investigate the mechanisms behind MHC loss in a cancer under sustained pressure from the immune system.

O6 | Bw4 ligand and direct T-cell receptor binding induced selection on HLA-A and -B alleles

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The HLA region is the hallmark of balancing selection, argued to be driven by the pressure to present a wide variety of viral epitopes. As such the peptide-binding positions have been the center of interest for the detection of selection. However, the human MHC molecule also directly binds to the T-cell Receptor and KIR. We here use the HLA allele frequencies in over six-million donors with a novel machine learning based method to estimate selection to show that: (a) the allele frequency can be predicted from its sequence; (b) The strongest selection is actually in KIR binding regions, followed by the peptide-binding cleft; (c) the selection from the direct interaction with the KIR or TCR is centered on positively charged residues (mainly arginine); and (d) some positions in the peptide-binding cleft are not associated with
the allele frequency, especially the ones with tyrosine residues. These results suggest that the balancing selection for peptide presentation may be combined with a positive selection for KIR and TCR binding.

The 18th International HLA and Immunogenetics Workshop (IHIWS) HLA immunogenic epitope project

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In solid organ transplantation, the formation of de novo donor-specific antibodies (dnDSA) is associated with inferior graft function and survival. dnDSA are induced by mismatched configurations of polymorphic amino acids, often referred to as eplets. While eplet mismatches have been associated with dnDSA formation, not every eplet mismatch appears to be immunogenic. To implement HLA eplet matching in allocation in order to prevent dnDSA formation, it is essential to define non- and immunogenic eplet mismatches. Therefore, the immunogenic epitope project of the 18th IHIWS was aimed to define the most immunogenic HLA eplet mismatches. Data from first-time, non-sensitized solid organ transplant recipients that either developed dnDSA or not after transplantation were collected. High resolution HLA typing of recipient and donor, and Luminex single antigen bead results pre- and post-transplantation, at first detection of dnDSA, or follow up of ≥5 years without DSA were collected. For each mismatched donor, HLA allele eplet mismatches were determined using HLA-EMMAv2.0beta, which contains all eplets from the HLA Eplet Registry. Subsequently, we determined how often a specific eplet was mismatched and how often it resulted in dnDSA (DSA count). A total number of 809 patient donor couples was submitted to the IHIWS component. In these couples, certain eplets were never mismatched and considerable overlap in the number of eplet mismatches between the no DSA and dnDSA group was observed. Many eplet mismatches never resulted in antibody formation. Per locus the immunogenicity score for each eplet per locus was defined by dividing DSA count by the eplet mismatch count from which the most immunogenic eplets could be defined. While a first inventory of the most immunogenic eplet mismatches was made, extending 18th IHIW cohort with recipients from other HLA backgrounds during the 19th IHIWS is crucial to move towards implementing eplet matching in clinical transplantation.

Spatial composition of decidual immune cells in oocyte donation pregnancies in relation to fetal-maternal HLA incompatibility

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Oocyte donation (OD) pregnancies are related to a higher degree of fetal-maternal HLA mismatching and a higher risk of complications compared with naturally conceived pregnancies. Nevertheless, many OD pregnancies maintain healthy until term. We hypothesize that in OD pregnancies with high HLA dissimilarity, the immune response at the fetal-maternal interface (the decidua) is divergent to maintain a healthy state. Here we focus on myeloid cells as we previously found these cells to be highly frequent in the decidua and they are essential in maintaining a healthy pregnancy. We performed imaging mass cytometry (IMC) using a 42-antibody panel on
decidua tissues of 8 uncomplicated singleton OD pregnancies. Single cell masks were created using cell segmentation. Child and mother were typed for HLA-A, -B, -C, -DRB1, and -DQB1, and fetal-maternal HLA mismatches were calculated. Based on the number of HLA mismatches, samples were separated into a semi-allogeneic group (≤5 HLA mismatches, n = 4) and a fully allogeneic group (>5 mismatches, n = 4). Myeloid cells represented the most abundant (~60%) immune cell population in the decidua. Thirteen phenotypically distinct subclusters were identified within the myeloid cell lineage. The IMC gave a possibility to study the microenvironment of each cell. The fully allogeneic group showed a higher frequency of maternal myeloid cells in the maternal T cells microenvironment than the semi-allogeneic OD group (p<0.050). Most notably, a higher extent of interaction between CD163+CD206+HLA-DR+ myeloid cells and CD4+ T cells was observed in the fully allogeneic group (p<0.050). Our results show the phenotypic diversity of decidual myeloid cells and their prominent frequency in uncomplicated OD pregnancies. By interacting with T cells, decidual myeloid cells might perform immune regulatory functions to compensate for the higher fetal-maternal HLA mismatch load in OD pregnancies.

MHC Evolution, Population Genetics

O9 | From global population genetic profiles to detailed individual molecular variation in humans and chimpanzees: a new turn in our understanding of MHC diversity and evolution

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The wide application of high-throughput DNA sequencing methods in the typing of human and non-human MHC genes since the last decade has boosted the generation of big data in immunogenetics, where the identification of thousands of alleles defined at very high resolution can now be used to investigate detailed molecular variation at the individual level rather than global genetic profiles in populations. This poses new challenges in data analysis, as new biostatistical and computer tools need to be developed to visualize this substantial amount of molecular diversity and decipher it in terms of genetic evolution. Following the work carried out by the EFI Population Genetics Working Group and the Population Genetics, Anthropology and Evolution (PGAE) Component during the last (18th) IHI Workshop, we here present the results of extensive NGS data analyses at 11 HLA loci in a large set of more than 300,000 individuals from Europe, North Africa, Asia and America, as well as at 5 MHC loci in 63 chimpanzees and 43 bonobos, that we compared withgether by implementing and redesigning the hla-net.eu bioinformatics pipeline now available as a
fully user-interactive interface. While the data of the 13th to 18th Workshops compared at the 2nd-field do improve the global map of HLA population diversity, mostly correlated with geography, the NGS data analyzed at the 3rd- and 4th-field significantly refine the characterization of HLA variation at the inter-individual level, emphasizing remarkable differences between individuals within some isolated populations. This high-resolution dataset also allowed estimating HLA peptide-binding affinity thereby highlighting potential functional differences among individuals of different populations. Finally, we performed direct comparisons of HLA and homologous Patr and Papa genes among individuals of the different species, providing new insights into the evolutionary history of MHC in humans and their closest relatives.

O10 | Creating fully representative MHC reference haplotypes

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The 5Mbp MHC genomic region is characterized by immense sequence and structural variation and is the most significant region of the genome affecting immune mediated disease. Despite this medical importance, the MHC remains inadequately represented in the current version of the human reference genome. We recently completed the sequences from the original MHC consortium cell lines, to include eight full-length haplotypes, including representatives from the four major HLA class II structures -DR1, -3, -4 and -5. Although this represents a substantial improvement from that previously available, these samples are of European descent and do not capture the full complexity of the global population. To expand the MHC reference set further, we first made technical improvements to our approach. To increase the sequencing efficiency, we developed new targeted nanopore long-read methods, resulting in greater depth of coverage through the MHC. We also modified our bioinformatics pipeline to utilize both long and short sequencing read data. Our combined methods provide the scaffolding and high depth of coverage to perform a fully comprehensive de novo assembly that can identify and integrate structural variations that were not previously characterized. We have now expanded the project to complete 100 reference haplotypes representing diverse ancestries with a goal of expanding to include another 500 MHC homozygous samples we have collected through multiple collaborations. These references will be integrated into the human reference genome. They will also be used to construct a population reference graph (PRG) that can in turn be used to study associations with disease through the entirety of ~180 genes and intergenic segments that comprise the MHC genomic region.

O11 | MICA Copy Number Variations are the Result of Numerous Independent Non-Allelic Homologous Recombination Events

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MICA and MICB are stress-induced ligands of the NKG2D receptor that regulate the activity of NK- and T cells. The MICA and MICB genes are located within the MHC complex and are in strong linkage disequilibrium with HLA-B. Since 2019, we have genotyped over two million samples for MICA and MICB and thereby identified 22,883 samples with a MICA gene duplication (1.0%) and 9,262 samples with a MICA gene deletion (0.4%). In certain populations, these frequencies can even exceed 5%. Since copy number variations might alter the level of MICA expression and consequently the immune responses against cancer or infectious diseases, we aimed for a detailed characterization of the underlying genomic organization. By using whole genome sequencing and targeted PCR on 53 samples with MICA duplications and 17 samples with MICA deletions, we mapped the recombination events to CT-repeat regions approximately
Telomeres protect the ends of normal chromosomes from degradation or abnormal fusions, with the consequent maintenance of genome stability. They consist of tandem repeats of nitrogenous bases and their number determines the maximum lifespan of a cell. The aging process is very complex and is determined by genetics, environmental, and lifestyle factors. Previous studies within the IHIWS component “Immunogenetics of Ageing” showed that longevity is associated with positive selection of HLA-DRBI*11 and DRBI*16 associated haplotypes, shown to be protective against diseases. Within the 18th IHIWS, we aimed to investigate the relevance of telomere length for successful ageing and its association with classical HLAs. In total 857 individuals: 316 unrelated elderly (>76 years) and 541 young controls (age 18–35 years), from Bulgaria, Turkey, Romania, and Poland were investigated. Telomere length was assessed using the Absolute Human Telomere Length Quantification qPCR Assay Kit. The comparison of telomere length in the analyzed groups showed some significant differences between the studied populations (p<0.0001). Elderly from Romania had the shortest telomeres, while those from Turkey had the longest ones. Among young controls, Poles had the shortest while Romanians had the longest telomeres. Furthermore, an association between telomere length and the presence of HLA-DRB1 alleles was found. DRBI*11 was associated with longer telomere length in Bulgarians (p = 0.004). This relationship was observed in both elderly (p = 0.053) and young (p = 0.019) Bulgarians. Furthermore, we found that Bulgarians carrying HLA-DRBI*11 and/or DRBI*16 had significantly longer telomeres than those with other HLA alleles (whole study group, p = 0.001; elderly, p = 0.007; young controls, p = 0.001). These results highlight the inter-population differences in telomere length and suggest some associations with HLA. In addition, these data could help to identify immunogenetic profiles associated with longevity.
HLA proteins play a vital role in our adaptive immune responses, and thus is crucial in understanding the human evolutionary process. Several non-competing natural selective regimes exist to explain how the MHC locus evolved, including balancing, and pathogen-driven positive and negative selection. However, owing to the complicated genomic structure of the MHC locus, it remains challenging to nominate specific genomic variations and haplotypes driving the observed selection signatures. In this work, we studied 1,832 African American (AA) individuals with majority African (74.4%) and European (25.6%) ancestry, and 594 Latino American (LAT) individuals with majority African (4.4%), European (49.7%) and Native-American (45.9%) genetic ancestry. By using whole-genome sequences in two admixed populations within the United States, we inferred 726 unique classical HLA alleles at two-field resolution including three classical HLA class I (HLA-A, -B, -C) and five class II (HLA-DPA1, -DPB1, -DQA1, -DQB1, -DRB1) genes. In LAT, we observed 4.4% African ancestry genome-wide versus 7.7% in the extended MHC region, representing a 1.75-fold increase. In contrast, in AA, we did not observe a genome-wide significant deviation in local ancestry. Furthermore, we nominated specific classical HLA alleles (e.g., B*14:02, C*08:02 and DQB1*05:01) and long-range haplotypes (e.g., A*33:01~B*14:02~C*08:02 and DQA1*01:01~DQB1*05:01~DRB1*01:02) that are under recent selection and responsible for deviations in local ancestry in LAT. Our work provides clarification of the dynamics that shaped the MHC locus, and thus improves our understanding of how selection-driven alleles contributed to immune-mediated disease susceptibility today.

O14 | HLA evolutionary divergence (HED) calculator

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HLA molecules are highly polymorphic proteins and essential for transplantation. With the exception of red blood cells, almost all of our cells include HLA-A, -B, and -C (class I), whereas immune cells primarily contain HLA-DR, HLA-DQ, and HLA-DP (class II). It is believed that people heterozygous at HLA loci will exhibit a wider variety of pathogen-derived peptides than homozygotes, increasing the likelihood of inducing a particular immune response. This heterozygote advantage hypothesis, when sequence level is considered lead to the idea of a divergent allele advantage. Furthermore, HLA genes’ high polymorphism may also be explained by this mechanism. The Grantham distance between the two alleles of each HLA locus can be calculated using the HLA evolutionary divergence (HED) metric. Likewise, HED is a straightforward replacement for the size of the repertoire of peptides presented by the HLA molecules. A new application to calculate HED values for class I and class II loci is available from here: https://txor.shinyapps.io/ched/. Protein sequences corresponding to the peptide-binding domain (exons 2 and 3 for class I and exon 2 for class II) of each HLA molecule were obtained from the IPD-IMGT/HLA Database. This application was built in shiny, an R package for the development of interactive web apps. Genotyping at two-field resolution for HLA-A, -B, -C, -DRB1 and -DQB1 can be used as input for this HED calculator. The application allows calculations for individual loci where each pair of alleles are single picked and also using files with several HLA genotypes. From a clinical perspective, HED has been associated liver transplantation rejection, response to checkpoint medications in some malignancies, improved HIV management, and hematopoietic stem cell transplantation outcome. HED calculator is easy-to-use and do not require any programing skills from their users.
highly variable genomic sequences. Traditional assembly approaches relying on reference genome alignment can leave short read data sets partially unaligned, or improperly aligned, and variation is missed. Long read sequencing is an option for overcoming such limitations in the future, though it is still cost-prohibitive and comes with a high error rate. Genome graphs have great potential but are still in developmental stages and are limited by the extent of sequenced MHC regions available as graph backbones. As an alternative to such challenges, we implement a hybrid approach: a reference genome-guided, de novo assembly method, previously established to scaffold genomes without a reference sequence to the genome of a related species. Aligned and unaligned reads are de novo assembled separately, after which, unique contigs are integrated into a single scaffold. We apply this to a human multiple sclerosis case-control cohort, comprised of 3,000 individuals with full MHCs, sequenced at high coverage using Illumina NGS. In this way, we rapidly and sensitively characterize both coding and non-coding sequence variation associated with multiple sclerosis. Achieving high-resolution MHC sequences for previously inaccessible disease cohorts could transform our understanding of the MHC in human disease.

New Technologies and New Approaches in Immunogenetics

O17 | The beneficial impact of HLA-E mismatching for patients receiving a less than 10/10 HLA matched hematopoietic cell transplant

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The role of HLA-E in hematopoietic cell transplantation (HCT) has been investigated by a few studies but mostly for patients receiving well HLA matched transplants. Here we describe our analysis of HLA-E matching in 1,513 HCT UK patients and their unrelated donors. Genotypes were generated by full-length PacBio Single Molecule Real-Time DNA sequencing. We investigated HLA-E matching at both the protein and full allelic levels. A total of 943 (62.3%) patients were matched for HLA-E (HLA-Em) at the second field, whilst 821 (54.3%) were HLA-Em at full allelic resolution. In all 584 patients received a <10/10 HLA matched transplant, 290 (49.7%) of which were also mismatched for HLA-E (HLA-Emm). After adjusting for clinical factors that affected outcome prognoses, multivariate analysis showed patients receiving a <10/10 HLA matched donor, who were also HLA-Emm had a significantly reduced risk of relapse (HR 0.55; 95% CI 0.36 to 0.84, p = 0.005) compared with their HLA-Em counterparts. Correspondingly, HLA-Emm was associated with a significantly reduced probability of Event-Free Survival (EFS) (HR 0.72; 95% CI 0.54 to 0.97, p = 0.03). A beneficial but not significant effect on Overall Survival (OS) and Transplant-Related Mortality (TRM) was also observed for <10/10 HLA-Emm patients compared with HLA-Em cases (OS: HR 0.82,
We hypothesize that HLA-Emm in an already HLA mismatched setting is providing enough genetic disparity to improve the Graft-versus-Leukemia effect. In a complimentary study at our center, 59.5% of patients with a choice of only <10/10 donors at verification typing were also HLA-Emm. Of those cases, 76.1% had a choice of more than one HLA-Emm donor. Together this suggests that in patients receiving a less well HLA matched HCT, who would otherwise have poorer post-transplant outcomes, HLA-Emm is beneficial for patient survival and reducing risk of relapse and is feasible to include in donor selection.

O18 | Clinical relevance of cell-free DNA quantification and qualification during the first month after lung transplantation

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Many studies have reported the relevance of donor-derived cfDNA (dd-cfDNA) after lung transplantation (LT) to diagnose and monitor acute (AR) or chronic rejection or infection (INF), but analysis of cfDNA fragment size is not studied. The aim of this study was to determine the clinical relevance of dd-cfDNA and cfDNA size profiles in events (AR and INF) during the first month after LT. This is a prospective, single-center study including 62 LT patients at the Marseille Nord Hospital (LARA project). Total cfDNA quantification was performed by fluorimetry and digital PCR, dd-cfDNA by NGS (AlloSeq cfDNA-CareDX®) and the size profile by BIABooster (Adelis®). After selection of small (<160 bp) cfDNAs by BIABooster technology, chimerism analysis by ddPCR (HLA KMR marker, GenDX) was performed. A biopsy at D30 allowed us to establish the following groups: stable, nonstable (AR, INF and AR+INF). Quantification of total cfDNA, for all methods, was not correlated with the patient's status at D30, the percentage of dd-cfDNA was significantly higher for non-stable patients at D30 (p = 0.0004). A threshold of 1.72% of dd-cfDNA determined the non-stable patients (NPV: 91.4%). The analysis of small sizes (80-120 bp) identified the INF with a threshold of 3.7% (PPV: 100%). An algorithm combining the two analyses allows to significantly differentiate the type of lesions due to allografts. Our algorithm aims at guiding the performing of biopsies, which are invasive and risky for the patient. This combined non-invasive biomarker of allograft injury requires to be confirmed. For this purpose, a control cohort is being included and a multicenter study is planned.

O19 | Full-gene sequence characterization of HLA-DMA, -DMB, -DOA, and -DOB in a panel of International HLA and Immunogenetics Workshop cell lines

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The non-classical HLA class II molecules, HLA-DM and -DO, regulate peptide loading to other HLA class II molecules. They have been less well studied than classical HLA genes and are thought to be less polymorphic. Given their position in the major histocompatibility complex, these genes may be good candidates as haplotype markers. We have used PacBio Single Molecule Real-Time (SMRT) DNA sequencing to perform full gene sequencing of HLA-DMA, -DMB, -DOA, and -DOB from 49 IHW cell lines. PCR amplicons encompassing the entire gene were pooled into barcoded adaptor libraries and SMRT DNA sequencing was performed on a PacBio Sequel. An in-house bioinformatics pipeline was used to analyze this data. A total of 17, 26, 17 and 18 unique sequences were generated for HLA-DMA, -DMB, -DOA, and -DOB, respectively. We observed 13 novel alleles, all with SNPs in intronic regions. Six reported typing results, previously observed to be homozygous, were shown to have two different intronic variants of the same coding allele. None of these results occurred in cell lines reported to be homozygous or consanguineous. Concordance with previously available typing for these genes was high, 96 out of 99. The differences between the previously reported typings and that derived here were all located with coding regions. A discrepant HLA-DOA typing was shown to differ from the previously reported sequence by a single SNP. The observation of multiple novel alleles within a small sample size suggests there is greater polymorphism within these genes than previously
thought. However, it should be noted that IHIW cell lines cover a range of ethnicities and cannot be considered as a random sample of a population. The identification of zygosity changes within intronic regions when completing full gene sequencing highlights the importance of sequencing the entirety of these genes. These results therefore establish the foundations for larger-scale, more comprehensive future studies of HLA-DM and -DO.

O20 | A randomized trial to assess the clinical utility of renal allograft monitoring by urine CXCL10 chemokine

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Urine CXCL10 is a promising non-invasive biomarker for detection of renal allograft rejection. The aim of this study was to investigate the clinical utility of renal allograft monitoring by urine CXCL10 in a randomized trial. We stratified 241 patients, 120 into an intervention and 121 into a control arm. In both arms, urine CXCL10 levels were monitored at three specific time points (1, 3, and 6 months post-transplant). In the intervention arm, elevated values triggered performance of an allograft biopsy with therapeutic adaptations according to the result. In the control arm, urine CXCL10 was measured, but the results concealed. The primary outcome was a combined endpoint at one-year post-transplant (death-censored graft loss, or clinical rejection between month 1 and one-year, or acute rejection in one-year surveillance biopsy, or chronic active T cell-mediated rejection in one-year surveillance biopsy, or development of de novo donor-specific HLA-antibodies, or estimated GFR <25 ml/min). The incidence of the primary outcome was not different between the intervention and the control arm (51% vs. 49%; RR 1.04 [95% CI 0.81–1.34]; p = 0.80). When including 175/241 (73%) patients in a per-protocol analysis, the incidence of the primary outcome was also not different (55% vs. 49%; RR 1.11 [95% CI 0.84–1.47]; p = 0.54). The incidence of the individual endpoints was not different as well. The CXCL10 burden, calculated as the mean of measurements at the three monitoring time points, predicted rejection in one-year surveillance biopsies defined by the Banff 2019 classification (p = 0.01). CXCL10 values often reflected inflammation processes in the allograft (rejection, polyomavirus-BK infection). In conclusion, this study could not demonstrate a beneficial effect of urine CXCL10 monitoring on one-year outcomes. However, CXCL10 can provide important information on the inflammatory status of the renal allograft (ClinicalTrials.gov_NCT03140514).

O21 | Characterization of chimpanzee KIR haplotype organizations using Cas9 enrichment and Oxford Nanopore sequencing

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Chimpanzees are humans’ closest living relatives, and they share a common ancestor that lived about 5–7 million years ago. The human KIR gene family consists of 17 members, which may show polymorphisms. Each KIR haplotype contains a variable number of genes, including four framework genes; KIR3DL3, KIR3DL2, KIR2DL4, and KIR3DP1. Chimpanzees possess a comparable KIR gene system, with a total repertoire of 13 genes, which includes the above mentioned framework genes. Only three genes, KIR2DL4, KIR2DL5, and KIR2DS4, are considered orthologous based on their high nucleotide sequence similarity in humans and chimpanzees. The KIR repertoire in chimpanzees is further characterized by an expansion of the lineage III KIRs (both D1-D2 and D0-D1-D2 structures). In the past, three chimpanzee KIR haplotypes have been characterized by sequencing different BAC libraries. Twenty additional KIR haplotypes have been deduced by KIR genotyping of a panel of chimpanzees. Recently, we have characterized the KIR transcriptome of a West-African chimpanzee cohort, and deduced KIR haplotypes based on segregation analyses. However, some of the deduced haplotypes contain different combinations of KIR genes that are not covered with the BAC-library sequenced haplotypes, and therefore gene combinations and locations could only be inferred.
In the present study, our aim was to resolve the chimpanzee KIR gene organizations by characterizing a variety of Patr-KIR haplotypes using Cas-9 enrichment and Oxford Nanopore Technologies sequencing approach. The data provides a detailed insight on the location of KIR genes and shows possible duplication of genes on a haplotype. Moreover, we discovered that some haplotypes can contain recombinant KIR genes, which may impact the binding and signal potential of the encoded receptors.

**O22 | TXMatching – A novel software for kidney paired donations**

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Kidney pair donation (KPD) is one of a few efficient strategies to improve the chance of receiving a compatible kidney for patients with incompatible living kidney donor. Czechia is involved in an international KPD together with Austria (since 2016) and Israel (since 2019), where the Prague transplant center is the coordinating center for these exchanges. In 2020 we developed a software called TXMatching to simplify the process of finding optimal matching between donor recipient pairs, especially for sensitized patients. There are two key features of the tool. First, it supports calculation of HLA matching and virtual crossmatching for both high- and low-resolution typing. This includes a novel algorithm that determines whether the reactivity of an antibody is against the alpha or beta chains of HLA-DQ and DP. Second, it allows broad customization to accommodate the needs of all the countries and pairs. This includes recipient’s MFI cut-off or acceptance criteria for donor (blood group, weight, age, etc.). Moreover, when calculating the optimal solution, maximum sequence length, debt between centers, HLA scorers and other parameters are configurable. The software runs as a web application and is fully accessible from any PC or mobile device. The design ensures that the app is easy to use and at the same time gives each transplant center full control over their patients and donors. As proof that the software is useful, we present a case study of an immunologically complicated patient actively waiting for 5 years for his second kidney transplantation in both KPD and deceased donor programs that found compatible kidney on the first attempt with the new algorithm. This donor would have been rejected based on two antibodies that would have been considered donor-specific if the virtual cross match was not performed on a high resolution and no discrimination was made between DQA and DQB reactivity. The patient is now 18 months since transplant without any rejection incidents.

**O23 | HLA loss detection by NGS using STR markers within the MHC region on chromosome 6**

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Genomic loss of mismatched HLA alleles is a possible driver of relapses after hematopoietic stem cell transplantation, especially in a haploidentical transplant. Early detection of HLA loss is needed for comprehensive clinical decision-making. In case HLA loss did not occur and mismatched HLA alleles are still present, donor lymphocyte infusion or modulating immunosuppression is a treatment option. In the case HLA loss occurs, a second allo-transplant from a different donor is a treatment option. HLA loss can be measured by quantifying the levels of markers within the region where HLA genes are located. This can be measured by quantitative PCR, which is highly accurate. However, the number of markers currently available in commercial kits using qPCR are insufficient to cover the complete population and cannot detect all types of markers. We are investigating if this method can be developed using Next-Generation Sequencing (NGS) for increased informativity and combination with NGStrack chimerism measurement. HLA genes are clustered in the MHC region on the short arm of chromosome 6. Multiple short tandem repeat (STRs) can be found in between the HLA genes, with the number of repeat units being highly variable among individuals. These can be used as informative markers for detecting HLA loss using NGS. For this purpose, we have developed PCR assays for over 15 STRs interspersed between HLA-A (on one side of the MHC region) and HLA-DPB1 (on the other side of the MHC region). The amplicons are sequenced on an Illumina MiSeq and automatically analyzed in a new analysis tool. This tool 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 this workflow, showing 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.

O24 | Assessment of a universal blood donor genotyping platform

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This study, part of a collaboration undertaken by a consortium of global blood services, industry and academia, assessed the performance of a universal blood donor genotyping platform capable of typing clinically relevant red cell (HEA), leukocyte (HLA) and platelet (HPA) antigens in a single test. DNA samples from consented blood donors were submitted by 7 blood services from Europe, Africa, North America and Australia with a genetic ancestry of 76% European, 14.3% African, 4.7% Hispanic, 3% South Asian, 1.4% other, 0.6% East Asian. Samples were collected centrally, plated and distributed for testing in laboratories in 3 different blood services. Genotyping of samples was performed in our laboratory using the GENETITAN-PROPEL workflow in batches of 94 on the Applied Biosystems™ UK Biobank v2.2 Axiom™ Array. HEA and HPA antigen inference was performed using the BloodTyper algorithm. HLA class I and II were imputed using Applied Biosystems™ HLA Analysis v2.12.0RC1 algorithm. Existing data on HEA, HLA and HPA types of the donors were used for concordance analysis. In phase one of the study, 4,510 samples were tested in our laboratory. Results showed that for HEA genotyping, a total of 58,856 comparisons were performed with concordant results in 99.88%. HLA and HPA typing records were available for a subset of donors. Initial analysis of 380 samples showed a 99.2% to 99.9% concordance rate for HLA- A, -B, -C, -DRB1 and -DQB1 but 98.6% for HLA-DPB1 at second field when compared typing previously performed by Next Generation Sequencing and PCR-SSOP. HPA typing results were available for HPA1,2,4,5,6,15. The probes for HPA-3 did not perform satisfactorily. For all other HPA antigens concordance was 100% except HPA-6 where a small number of discordances require further analysis. The study shows the genotyping array delivers highly concordant and reproducible results with a high level of agreement for samples tested at all three blood service laboratories.

O25 | Chromosomal rearrangements in the KIR gene cluster as evolutionary strategy to protect against evading pathogens

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The killer cell immunoglobulin-like receptor (KIR) cluster displays extensive chromosomal rearrangements that reshuffle gene content, reflected by a variable number of genes per haplotype. These recombination events not only diversify haplotype configurations, but might also generate novel hybrid entities, which consist of segments from two different genes. The common human KIR gene repertoire comprises 17 members, but is expanded by multiple reports of hybrid genes. For example, the IPD-KIR Database documents 7 allelic variants for a hybrid KIR3DL1-KIR3DL2 gene, which are referred to as alleles of KIR3DL1. Most conventional typing techniques, however, might lack detection of these hybrid genes, suggesting that the pool of novel KIR entities may be larger. Other hybrid entities are discovered using haplotype sequencing and are often accompanied by gene deletions or introductions. Long read transcriptome and ultra-long read haplotype sequencing, utilizing PacBio and ONT platforms respectively, allowed definition and detection of hybrid KIR genes in macaques. We documented over 20 novel gene entities, which are represented by more than 60 allelic variants, expanding the previously defined gene repertoire of 22 macaque KIR genes. Chromosomal rearrangements mostly involved lineage II genes (KIR3D), but novel entities were also defined comprising segments of KIR3DL20 (lineage V), KIR1D (lineage III), KIR2DL04 (lineage I), and pseudogene KIR2DP. Each hybrid KIR gene is in-frame and comprises all coding exons. The sophisticated mechanism that drives gene diversification is not completely understood yet. However, the extensive reshuffling of binding and signaling domains appears to generate receptors with differential biological functions.
might indicate an evolutionary strategy to protect against pathogen evasion and subversion.

**O26 | Variegated expression of KIR regulated by conserved and diverged promoter regions in humans and macaques**

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The members of the killer cell immunoglobulin-like receptor (KIR) family display a variegated expression pattern on NK cells. This stochastic activation of KIR genes is orchestrated by multiple promoter regions that contain different sets of transcription factor binding sites. The proximal promoter, located directly upstream of each first exon, functions as a bidirectional switch that generates sense or antisense transcripts associated with gene activation or silencing, respectively. A distal promoter seems to modulate the activity of this switch via splicing of transcripts, whereas the intermediate promoter might be involved in tissue-specific expression. In humans, sequence variation in these promoter elements correspond with differential expression of KIR. For example, distinct promoter regions are defined for KIR2DL4, which displays non-stochastic expression, whereas for KIR3DL3, expression is lacking on circulating NK cells. Moreover, decreased expression of specific KIR allotypes might associate with SNPs in the distal promoter. In this study, we performed long read sequencing using an Oxford Nanopore Technologies platform to assemble complete KIR haplotypes in rhesus macaques, a species that is relevant for preclinical studies. These high-resolution haplotypes provide insights into the conservation and diversification of the KIR promoter regions. The bidirectional switch and the distal promoter seem to be largely conserved, indicating a strong selective pressure for these regulatory elements. In contrast, the intermediate promoter displays more sequence variation, especially for KIR3DL3 when compared with its counterpart in macaques, KIR3DL20. This latter gene is abundantly transcribed by circulating NK cells in macaques, indicating a diverged biological function. These insights enhance the currently limited understanding of KIR expression in macaques, and might help to translate the biological relevance of the differential KIR expression patterns.

**O27 | Polymorphism of HLA and KIR affects severity of COVID-19 by shaping innate and adaptive immunity to SARS-CoV-2**

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Extreme polymorphism of HLA and Killer-cell Immunoglobulin-like Receptors (KIR) differentiates immune responses across individuals. Additional to T-cell receptor interactions, subsets of HLA class I act as ligands for inhibitory and activating KIR, allowing natural killer (NK) cells to detect and kill infected cells. We investigated the impact of HLA and KIR polymorphism on the severity of COVID-19. High resolution HLA class I and II and KIR genotypes were determined from 403 non-hospitalized and 1,575 hospitalized SARS-CoV-2 infected patients from Italy collected in 2020. We observed that the activating KIR2DS4*001 allotype is
associated with severe disease (OR = 3.74, 95% CI 1.75-9.29, pc = 0.003). KIR2DS4*001 in presence of its specific HLA ligands and inhibitory KIR3DL2*002 in absence its HLA ligand are also enriched in severe COVID-19 patients (OR = 1.64, 95% CI 1.09-2.50, p = 0.019), suggesting this combination acts in tandem to increase risk of developing severe COVID-19. We also observed the HLA class II allotype, HLA-DPB1*13:01 protects SARS-CoV-2 infecting patients from hospitalization (OR = 0.49, 95% CI 0.33-0.74, pc = 0.019). These association analyses were replicated using logistic regression with sex and age as covariates. Autoantibodies against IFN-α associated with COVID-19 severity were detected in 26% of hospitalized patients. HLA-C*08:02 was more frequent in patients with IFN-α autoantibodies than those without, and KIR3DL1*01502 was only present in patients lacking IFN-α antibodies. We intend to expand this analysis in Greek and Spanish COVID-19 cohorts. These findings show that KIR and HLA polymorphism may play important roles in determining the clinical outcome following SARS-CoV-2 infection, by influencing the course both of innate and adaptive immunity.

O28 | The role of natural killer cells in recurrent pregnancy loss: Evaluation of natural killer cell education

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NK cells can target cells lacking HLA class I molecules. This is regulated by inhibitory and activating signals from KIR and NKG2 receptors which can interact with highly polymorphic HLA molecules. HLA and KIR are inherited independently. To ensure self-tolerance, NK cells undergo a process termed education. Education makes the NK cell more receptive to activating stimuli. Uneducated NK cells are largely hyporesponsive. Human cytomegalovirus (HCMV) has been shown to impact the NK cell receptor repertoire and could therefore affect NK cell education. In reproduction, the role of education is still unknown. In early pregnancy, most of the leukocytes are NK cells and they regulate placentation and trophoblast invasion. We hypothesized that NK cell education is altered in women with recurrent pregnancy loss (RPL) compared with women with successful pregnancies (controls). Additionally, the effect of CMV infection on NK cell education was investigated. Protein expression of KIRs and NKG2A was assessed by flow cytometry. HLA typing was done using PCR-SSO (Luminex). HLA alleles were divided into KIR recognition epitopes. KIR expression was similar between RPL (n = 41) and controls (n = 16) with KIR2DL2/3 having the highest percentage positive cells followed by KIR2DL1 and KIR3DL1. No differences were found in the percentage of NKG2A educated NK cells between RPL (49.98%) and controls (55.50%) (p = 0.33) or in the percentage of single KIR educated NK cells (RPL: 10.07%, C: 13.27, p = 0.74). Only very few NK cells were double or triple KIR educated in both the RPL and the control group. Furthermore, CMV infection did not influence the percentage of educated NK cells in women with RPL. In conclusion, the percentage of educated NK cells is not associated with RPL. Our results also suggest that CMV infection does not impact the percentage educated NK cells in these women.

O29 | KIR2DL2/C1: A potential predictive immunogenetic marker to COVID-19 severity in Spanish patients

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COVID-19 has aspects on its pathogenesis that still need elucidating and an analysis of clinical and immunogenetic factors in each cohort of patients is paramount to understanding how genetic variability can explain the multiple clinical spectra seen in patients infected with SARS-CoV-2. The aim of this study was to correlate the KIR polymorphism/HLA class I ligand interactions from patients and healthy subjects with either the susceptibility or severity to COVID-19. Genotyping of HLA-A, -B, -C and KIR genes were carried out from 459 symptomatic as well as 667 non-infected Spanish Caucasians individuals using Lifecodes HLA-SSO and KIR-SSO kits (Immucor™, USA) and analyzed in the Luminex® in this uni-centre case-control study performed at the University Hospital of Salamanca, Spain. Comparative KIR gene analysis showed that KIR2DS4 was significantly more representative in healthy versus infected individuals. When comparing subgroups of infected patients, KIR2DS3 had a higher frequency in those who progressed to a more severity disease and yet with higher mortality rate. Three functional combinations were significant on univariate analysis: KIR2DL2/C1, KIR2DS2/C1, and KIR2DS3/C1. However, in the multivariate analysis, only the KIR2DL2/C1 interaction remained significant (OR = 15.2 (95% CI 1.5-147), p = 0.0189). Compared with the solo-clinical characteristics predictive model, that included well-known comorbidity variables such as hypertension, age, sex, diabetes, C-reactive protein, dyslipidemia, smoking, ferritin, and fibrinogen, the clinical-and-KIR-based model showed a better ability to discriminate between severe and non-severe patients with higher sensitivity and specificity. Our results support a fundamental role of KIR/ligand interaction in the clinical course of COVID-19. Since the KIR2DL2 gene has a high frequency in Spain (60%), the analysis of the KIR2DL2/C1 in symptomatic patients who require hospitalization could be helpful to better determine their prognosis.

O30 | Nanopores may replace SMRT reads for dual redundant reference sequencing (DR2S): Characterization of more than 600 novel KIR alleles

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Killer-cell immunoglobulin-like receptor (KIR) genes regulate NK cell activity and thereby influence the immune response to infectious diseases and malignant cells. However, the underlying molecular interactions resemble a giant and largely unsolved puzzle. Variability in KIR gene repertoire is derived from KIR region haplotype diversity with varying KIR gene composition and high allelic diversity of each of the 17 KIR genes. Since we started allele-level KIR genotyping in 2018, we identified hundreds of novel KIR alleles and successfully characterized and submitted 478 novel alleles for KIR2DL1, -2DL4, -2DL5, -2DS1, -2DS2, -2DS3, -2DS5, -3DL3 and -5DP1 to the international database IPD-KIR in 2020. For these samples, we used our well-established dual redundant reference sequencing (DR2S) protocol of short-read Illumina and long-read PacBio sequencing to obtain fully phased sequences in reference grade quality. However, recent advances in nanopore sequencing technology offered the opportunity to use an alternative platform for KIR gene characterization. As proof of concept, we first confirmed more than 30 PacBio/Illumina-characterized KIR2DL1 alleles using the novel ONT/Illumina-based approach with perfect concordance. Subsequently, we applied the ONT/Illumina process for the characterization of novel KIR2DL1, -2DS1, -3DS1 and -2DP1 alleles. By now, we finished reference grade sequence characterization of over 600 novel alleles. Among them are 52 novel KIR3DS1 alleles, which multiply the amount of full-length KIR3DS1 alleles in the newest IPD-KIR release (2.12, December 2022). These novel KIR sequences provide additional insight into KIR gene diversity and will support KIR genotyping in the future. Furthermore, since ONT requires far less capital investment compared with PacBio, these results lower the entry threshold for applying reference grade sequencing for the characterization of novel KIR alleles.

O31 | Natural killer cell receptor variation is associated with more aggressive subtypes of breast cancer

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Breast cancer (BC) is the most common malignancy among women, with tumors varying in aggressiveness and histological and immunohistochemical subtypes. Natural killer cells (NK) are lymphocytes critical for recognizing and killing neoplastic cells and express the killer-
cell immunoglobulin-like receptor (KIR) molecules on the cell surface, which modulate NK cell responses by recognizing HLA molecules. Despite the well-established role of KIR-HLA in diseases, the variation of these genes in high resolution has never been analyzed in BC. Here, we analyzed the full extent of the KIR polymorphism in the context of their HLA ligands. We applied a state-of-the-art next-generation sequencing method and bioinformatics pipeline to evaluate a cohort of 550 patients with BC and 747 controls. The significance threshold was set at 0.05 after Bonferroni corrections. The presence of three copies of KIR2DL5 was associated with protection (OR = 0.21, pcorr = 0.027), while the presence of three copies of KIR3DL1S1 was associated with increased risk to BC (OR = 2.44, pcorr = 0.009). We observed that the duplication of KIR3DP1~KIR2DL4~KIR3DS1 significantly increases the risk of sporadic BC (OR = 2.42, 95% CI 1.30-4.61 p = 0.007). Moreover, the pair KIR2DL3*001+HLA-C1 ligand was significantly reduced in patients with the aggressive non-luminal HER2+ compared with all other subtypes (OR = 0.23, pcorr = 0.016), in addition to a strong association of KIR2DS1*002+HLA-C2 in patients with aggressive triple-negative tumors compared with other subtypes (OR = 3.34, pcorr < 1.4 × 10^-5). Finally, we observed a protective effect of the pair KIR2DL1*001+HLA-C2, significantly increased in patients with less severe BC grade I compared with the most severe grades II+III (OR = 0.25, pcorr = 0.038). In summary, we have provided solid evidence that the variation of KIR genes is implicated in BC and associated with its aggressive subtypes, pointing them as candidates for immunotherapies.

**O32 | HLA class I epitope and KIR diversities in multiple myeloma**

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Multiple myeloma (MM) is a hematological malignancy caused by the clonal expansion of malignant plasma cells in the bone marrow. Myeloma cells are susceptible to killing by natural killer (NK) cells, but NK cells fail to control disease progression, suggesting immunosuppression in the MM setting. The strength of NK cell effector function is regulated by interaction between KIRs and self HLA class I molecules, during a process called ‘education’. The HLA and KIR repertoires are extremely diverse, thus this study aimed to characterize potential variances in genotypic composition of HLA class I epitopes and KIRs between patients with MM and healthy controls. For this, 16 KIR genes and HLA group C1, C2, and Bw4 epitopes were analyzed in 172 MM patients and 196 healthy controls using SSO and LumineX-analysis. Compared with healthy controls, we did not observe specific KIR genes or genotypes, or HLA epitopes with higher prevalence among MM patients. The presence of all three HLA epitopes was not related to the occurrence of MM disease (30.4% in MM vs. 32.5% in controls; chi-square, p = 0.672). However, MM patients were more likely to be C1-/C2+/Bw4+ (chi-square, p = 0.05, OR 2.0). In line with this, the occurrence of both HLA-Bw4 and KIR3DL1 was higher in MM patients (chi-square, p = 0.023, OR 1.6). Furthermore, MM patients were less likely to only have a single HLA-KIR pair (chi-square, p = 0.02, OR 0.6). In contrast, MM patients were more likely to have two HLA-KIR pairs (chi-square, p = 0.003, OR 2.0), specifically HLA-C2/KIR2DL1 and HLA-Bw4/ KIR3DL1 (chi-square, p = 0.008, OR 2.6). In conclusion, our results reveal an HLA epitope combination that is associated with the occurrence of MM. There were no specific KIR genotypes associated with MM. Based on HLA-epitope triple-positivity, haplo-SCT combined with KIR-ligand mismatching is feasible for MM patients. The role of having different HLA-epitopes, HLA-KIR pairs, or different amounts of HLA-KIR pairs should be further investigated.

**Immunogenetics in Organ Transplantation**

**O33 | Immunogenomic exploration in a large kidney transplantation genetic cohort reveals a kidney graft failure association with HLA-B*40:01 and KIRD2L2/HLA-C2 combination**

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Kidney transplantation remains the best therapeutic option against end-stage kidney disease. However, current
immunosuppression poorly influences chronic rejection and the graft half-life is still around 10 years. Identification of biomarkers to anticipate risk of allograft injury may conduct to the design of innovative therapeutics adapted to the patient's own risks (i.e., personalized medicine) to prevent graft failure and favor patients care. The KIT-GENIE genetic cohort is composed of 1,504 complete donor/recipient kidney transplant pairs from European ancestry from Nantes since 2000. We imputed the HLA alleles from SNPs genotyping using HIBAG to obtain high resolution typing, we used Easy-HLA (hla.univ-nantes.fr) to determine KIR ligand groups from HLA alleles. In parallel, we typed 247 recipients for KIR gene content. We performed an HLA genetic association study for time-to-kidney graft failure using Cox regression models. We added several covariates in the model such as recipient/donor age, recipient/donor sex, ancestry components from genomic analysis, epitopic mismatch score. We defined a Bonferroni multiple testing threshold of significance at \( p \leq 8 \times 10^{-4} \). We identified \( HLA-B^*40:01 \) from the donor as significantly associated with time to graft failure \( (p = 5.9 \times 10^{-4}, \text{HR} 1.8, \text{frequency} = 4.3\%, \text{dominant genetic model}) \). Moreover, we identified the KIR2DL2 recipient + HLA-C2 donor combination as significantly associated with time to graft failure \( (p = 2.2 \times 10^{-4}, \text{HR} 3.0, \text{frequency} = 19\%, \text{dominant genetic model}) \). Overall, these results report important new findings on kidney transplantation graft failure: the first reported significant association with an HLA allele from the donor as well as a new KIR/HLA association. These very promising results need further investigation with a larger number of patients.

**O34 | In the era of precision medicine: lncRNAs as probable biomarkers to predict allograft rejection**

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To evaluate the possibility of predicting acute renal allograft rejection the present study evaluated lncRNA expression at various time points pre- and post-transplant. Study enrolment included ESRD patients who underwent renal transplantation and were grouped as Group 1-subjects with well-functioning grafts and Group 2- subjects that experienced acute allograft rejection. RNA seq was performed on a selected cohort using samples collected pre-transplant, at the time of rejection, and post anti-rejection therapy time points. Differentially expressed (DE) RNAs and related biological pathways were identified from the RNA Seq data. Database for Annotation, Visualization and Integrated Discovery (DAVID) and Gene Ontology (GO) helped analyze the major function(s) of DE genes. Using the 2-side Fisher exact test, we determined the statistical significance of enrichment. In the present study, we compared lncRNAs expression among two data sets obtained from RNA obtained from (i) set 1: Well-functioning grafts versus those experiencing rejection and (ii) set II: those experiencing rejection versus the same cases post anti-rejection therapy. Over 2000 RNAs were upregulated and >850 were downregulated in the set 1 analysis whereas in set 2, a lower number of differentially expressed RNA were identified (~360 upregulated and ~ 150 downregulated). These GO terms were associated with leukocyte activation, degranulation and regulation of cytokine secretion. Further, the involvement of pathways like cytokine-cytokine receptor interaction and TNF signaling was suggested on using Kyoto Encyclopedia of Genes and Genomes pathway (KEGG) analysis. qPCR was performed on samples obtained from an independent cohort of subjects experiencing acute rejection and this helped in validation of lncRNA identified from discovery cohort. The linkage of identified and validated lncRNAs with immune pathways indicates few of these might play key role in leading to allograft rejection.

**O35 | Gene expression profiles in 3-month biopsies associate with progression to kidney transplant rejection before detection of histological changes**

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Allograft rejection remains a major cause of kidney function loss after transplantation. The histological analysis of graft biopsy is the gold standard to diagnose rejection and guide immunosuppressive treatment. However, histology can yield inconclusive results, leading to a delay in optimal treatment. Moreover, once detected in histological analysis, injury is often irreversible. Therefore, we compared gene expression profiles of biopsies from patients without versus with early and late rejection to identify molecular changes associated with progression to rejection before histological changes occur. RNA was isolated from frozen biopsies, all taken at 3 months after transplantation, including samples from patients without rejection in the first year (n = 6), with biopsy proven rejection at 3 months (n = 4, early rejection group) and with no rejection at 3 months, but biopsy proven rejection at 12 months, (n = 4, late rejection group). Gene expression analysis was performed with Nanostring’s Human Organ Transplant panel. Distinct gene expression profiles were observed between control and early rejection groups (138 genes differentially expressed). Importantly, gene expression, 103 genes were differently expressed at 3 months in the late rejection group as compared with control group. Interestingly, 83 of these genes were also differentially expressed between early rejection group and control and 3 month expression pattern of the late rejection group closely resembled that of the early rejection group. This pattern includes genes involved in oxidative stress, inflammatory and NLR signaling such as NOX4, FAS, JAK2, NFkB1, BCL2 and BMP7 (p-Adj < 1.641e^{-02}). Our results demonstrate that a distinct gene expression profile precedes histological evidence of injury. This can potentially be used to predict rejection and adjust treatment to prevent it.

Liver transplantation is the main available treatment for acute and chronic hepatic failure. Since the transplant outcome is still limited and influenced by the ischemia-reperfusion injury grade of the graft, hepatic function is carefully monitored through the analysis of multiple hematological parameters. However, a single, specific and early marker of post-transplant liver damage has not been identified yet. Donor-derived cell-free DNA (dd-cfDNA), which is a potential biomarker of rejection and graft tissue damage in heart, lung and kidney transplanted patients, could represent this missing marker. The aim of this study was to recruit a cohort of adult liver transplant recipients to validate dd-cfDNA quantification as a powerful and non-invasive method for early identification of graft failure. We included 40 patients (male sex 80.0%; mean age 58.01±9.97 years). Blood samples were collected before transplantation, daily during the first 7 days post-transplant, then at day 28 and at months 3 and 6. Dd-cfDNA quantification was obtained from 418 samples using droplet digital PCR, targeting a list of 8 alleles of HLA-DRB1 gene. Results were correlated to hematological measurements, clinical exams and liver biopsies collected in case of suspicion of rejection. Dd-cfDNA levels showed a common trend in the entire cohort with a peak at the time of reperfusion of the organ and a progressive decrease of its levels over time, reaching percentages similar to those physiologically secreted by the liver after 7 days. The percentage of dd-cfDNA reflected the grade of ischemia-reperfusion injury and significantly correlated with hepatocytolysis indices used in clinical practice (p<0.0001). In the long term, it increased in patients with hepatic complications, such as acute rejection and increase of hepatocytolysis. Our results demonstrated the value of dd-cfDNA as a reliable biomarker of hepatic injury reflecting the ischemia-reperfusion damage in liver transplantation.

O36 | Precision medicine in liver transplant recipients: donor cell-free DNA as an early marker of post-transplant hepatic injury

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O37 | Acute and chronic rejection monitoring of pediatric heart transplant recipients through a ddPCR assay based on HLA-DRB1 polymorphism

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Rejection represents one of the main causes of graft failure and loss after heart transplantation. It is generally monitored by histopathological evaluations of endomyocardial biopsies (EMB), that are limited by interobserver variability, poor sensitivity, and invasiveness, particularly in pediatric patients. In addition, biopsies are often performed for clinical reasons and therefore when graft injury is possibly already present. Donor derived cell-free DNA (dd-cfDNA) is a potential biomarker of rejection and graft injury in solid organ transplantations. The study aims to assess the role of dd-cfDNA in rejection monitoring in a pediatric cohort of heart transplant recipients. We enrolled a pilot cohort of 21 pediatric heart recipients with an average age at transplant of 4.7 ±4.3 years and a transplant survival of 6.9±5.2 years. cfDNA was obtained from 38 plasma samples (1.8 samples/patient) and dd-cfDNA quantification was performed exploiting a ddPCR assay based on HLA-DRB1 polymorphisms that discriminate between donor and recipient DNA. Results were correlated with clinical and biotic data collected contextually with blood samples. dd-cfDNA quantification was significantly higher in relation to graft injury events (acute or chronic) compared with stable patients (p = 0.0080). We obtained a significant correlation with the EMB rejection grade (p = 0.0146) and the pro-BNP value (p = 0.0135). To assess the performances of the test, we performed a ROC analysis, obtaining an AUC of 0.74. With a positive cut-off of 0.13%, the test had 80.95% sensitivity and 64.71% specificity. Our preliminary results demonstrated that dd-cfDNA is a valid and reliable biomarker of both acute and chronic rejection in pediatric heart transplant recipients, and its quantification can help in the post-transplant management of patients with the aim to avoid unnecessary and invasive EMB when there is no suspicion of rejection.

**O38 | A genome-wide survival study identifies a novel association between donor genotype and antibody-mediated kidney graft rejection**

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Donor-recipient mismatches in HLA genes have been associated with a poorer kidney graft survival. However, HLA mismatches alone do not explain long-term graft function decline. We ran a genome-wide survival study on a large monocentric cohort of kidney transplant donors in order to characterize non-HLA genetic factors associated with HLA and non-HLA antibody mediated rejection (ABMR), the main transplant complication causing graft loss. The Kit-GENIE French genetic cohort comprises 1647 donors of European ancestry for kidney transplants performed in Nantes since 2000. After genotyping and polymorphism (SNP) imputation with the TopMed reference panel, we performed a genetic association study on >8.2M SNPs for biopsy proven ABMR. Cox proportional hazards models notably adjusted for HLA mismatches were fit to assess the association between donors’ genotypes and biopsy proven ABMR. Statistical tests were computed using a saddle point approximation with the SPACox R package in order to limit the number of false positives. p-values below the genome-wide multiple testing Bonferroni correction threshold (5 × 10⁻⁸) were considered significant. We identified a statistically significant association in an intergenic region of chromosome 6 (p = 3.5 × 10⁻⁶, HR = 2.9) outside of the HLA complex, near a gene implied in vasculogenesis. Our genome wide analysis in a large homogeneous monocentric cohort revealed a non-HLA variant in donors associated with ABMR. Further validation in external cohorts and non-European individuals will be performed.

**O39 | The number of donor HLA-derived T-cell epitopes available for indirect antigen presentation determines the risk for vascular rejection after kidney transplantation**

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Kidney transplantation is the preferred treatment option for many patients with end-stage kidney disease. The outcome of such a treatment is most optimal when recipient and donor are HLA matched. Through the activation of alloimmune T and B cells, HLA mismatches may lead to T-cell- or antibody-mediated rejection. During antibody-mediated rejection, the indirect pathway of allorecognition plays an important role. However, the role of the
indirect pathway of allorecognition in acute T cell-mediated rejection (aTCMR) is not well defined. In this study, we aimed to evaluate the role of the indirect pathway of allorecognition in aTCMR using the Predicted Indirectly ReCognizable HLA Epitopes algorithm (PIRCE-II), which is an algorithm that quantifies the amount of theoretical T-cell epitopes available for indirect allorecognition. As a measure for the potential indirect CD4+ T-cell alloreactivity, the PIRCE-II score was calculated for 688 donor kidney-recipient combinations. A diagnosis of aTCMR was made in 182 cases; 121 cases of tubulo-interstitial rejection cases and 61 cases of vascular aTCMR. A strong association between the PIRCE-II score and the incidence of first-time aTCMR was observed, in particular vascular rejection. This association was found mainly for the peptides derived from donor HLA-DR/DQ (PIRCE-II DR/DQ): at one year after transplantation, the cumulative percentage of recipients with a vascular rejection was 12.7%, 8.6% and 2.1% within respectively the high, medium and low tercile of the PIRCE-II DR/DQ score (p<0.001). In a multivariate regression analysis this association remained significant (p<0.001 for PIRCE-II DR/DQ terciles). In conclusion, indirect antigen presentation of donor HLA-peptides may significantly contribute to the risk for acute vascular rejection. This finding increases our current understanding of the pathogenesis of aTCMR and may contribute to risk stratification following kidney transplantation.

O40 | Impact of HLA diversity on humoral response to SARS-Cov-2 and HBV vaccines in liver transplant recipients

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Organ transplant recipients show weaker immune responses to vaccines than immunocompetent individuals, which may be related to the repertoire of HLA-bound vaccine antigens presented to T lymphocytes. The HLA evolutionary divergence (HED) metric, which quantifies pairwise allele divergence at each HLA locus, provides a primary measure of the breadth of the immunopeptidome. We recently showed that high class I HED of the donor is a strong and independent driver of allograft rejection in a large cohort of liver transplant recipients. Here, in the same cohort, we explored the relation between HED, the size of the predicted immunopeptidome derived from vaccine antigens, and the quality of vaccine responses. We analyzed humoral response to the SARS-CoV-2 BNT162b2 vaccine (n = 310 patients; undetectable anti-spike IgG titers considered as no response, ≤250 BAU/mL as moderate and >250 BAU/mL as strong response) and Hepatitis B virus (HBV) vaccine (n = 424 patients; anti-HBs IgG <10 mIU/mL considered as no response, 10-100 mIU/mL as moderate and ≥100 mIU/mL as strong response). HED at HLA-A, -B, -C, -DRB1, -DQA1 and -DQB1 loci were measured using the Grantham distance. NetMHCIIpan-4.0 was used to predict the binding to HLA-DQ molecules of all possible 15mer peptides derived from the Spike and HBS sequences. For each vaccine, HED at the DQB1 locus, but not at the other loci, was significantly higher in responders than in non-responders (p = 0.0003), independent of response-associated covariates (age, time since transplant, immunosuppression). Moreover, for both vaccines, there was a strong relationship between DQB1 HED, the diversity of the immunopeptidome and the quality of the vaccine response. In conclusion, DQB1 HED is a critical determinant of humoral response to vaccines in liver transplant recipients. This metric could guide the design of future vaccines as it predicts the magnitude of the repertoire of vaccine-derived peptides presented to CD4 helper T cells.

Bioinformatics, Data Analysis in Immunogenetics

O41 | Unexposed individuals are fully equipped at the genetic level in terms of peptide coverage and T-cell repertoire against SARS-CoV-2: analysis in a cohort of healthy donors and alloHSCT recipients

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T-cell recognition of antigenic peptides presented by HLA molecules at the cell surface is critical for mounting an efficient adaptive immune response during acute viral infection such as COVID-19 caused by SARS-CoV-2. Recent data suggest that the depth of peptide coverage and the breadth of T cells that are able to respond are
both important parameters associated with disease outcome. Strong T-cell responses against SARS-CoV-2 have also been reported in unexposed individuals, pointing to a possible role of heterologous immunity. In this study, we performed immunosequencing of the TCR CDR3β region in a large cohort of 116 alloHSCT recipients and their corresponding healthy donors collected prior to the emergence of SARS-CoV-2. We used bioinformatics analyses and a large database of about 150,000 SARS-CoV-2 specific T-cell sequences in order to investigate the composition of the TCR repertoire regarding the presence of SARS-CoV-2 specific clonotypes in unexposed subjects among the more than 3.5 million CDR3β sequences that we retrieved by immunosequencing. We also performed peptide binding predictions based on the reference proteome of the virus and by using the HLA class I high resolution typing data of the 116 patients. We could show that every individual is equipped with a large and diverse repertoire of clonotypes sharing their CDR3β sequence with a SARS-CoV-2 specific T cell. Furthermore, the composition of the anti-SARS-CoV-2 repertoire was very similar among individuals, in healthy donors but also in the context of immune reconstitution in recipients, despite significant differences previously reported when accounting for the whole repertoire or for CMV-specific clonotypes only. In addition, each individual had the potential to cover a diverse repertoire of SARS-CoV-2 derived peptides (i.e., a few thousands strong and weak binders), but, interestingly, some inter-individual differences were observed when only accounting for a strong affinity level of binding.

O42 | A new hla-mapper algorithm for alignment optimization of HLA sequences from RNA-seq

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Massively Parallel Sequencing or Next-Generation Sequencing (NGS) allowed an in-depth evaluation of thousands of whole genomes and transcriptomes. While the NGS data analysis for most genes is now trivial, with many well-documented protocols, some genes require special attention to achieve reliable genotyping and expression-level estimations. This is the case for most HLA genes, where sequence similarity and high levels of polymorphism jeopardize genotyping and expression analysis when using conventional tools. Here we present a new algorithm implemented in the hla-mapper package that supports RNA sequencing (RNA-seq) data. This algorithm minimizes read alignment errors, allowing more accurate downstream analysis such as genotyping, haplotyping, detection of alternative transcripts, and expression level estimations. To assess the hla-mapper RNA algorithm performance, we used simulated transcriptomes to compare the results obtained with the aligner STAR and after the hla-mapper optimization. We also demonstrate that, when using conventional tools for sequence alignment, real transcriptomes present the same pattern observed in the simulated ones: underestimation of the expression levels for most HLA genes, overestimation of others, and many genotype errors, particularly for HLA-B, HLA-C, and HLA-DRB1. Alignment errors are particularly intense in alleles that present many nucleotide differences from the reference genomes. These alignment errors are minimized or prevented with hla-mapper, allowing accurate genotyping and expression-level estimations for HLA genes, including allele-specific expression levels. After optimization, the expression levels in real transcriptomes are very different from the original reported by STAR. Therefore, post-processing transcriptome with hla-mapper is essential for reliable HLA genotypes and expression estimates in RNA-seq data.

O43 | HLA-3Diff: Redefining donor-recipient HLA matching based on three-dimensional structure prediction

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The HLA genes are a major component in tissue compatibility for solid organs and hematopoietic stem-cell transplantation. The differences between donor and recipient HLA alleles, called HLA mismatches, is a major factor for de novo Donor-Specific Antibodies (dnDSA) development. Several methods have been proposed to evaluate
the immunogenicity of the graft and thus the risk of developing dnDSA. Among these, amino-acid matching, and eplet matching showed promising results. However, these methods do not consider the allele immunogenicity in its entirety. In this project, we hypothesized structural differences relate to immunogenicity. We developed a score based on predicted 3D structure superposition for all HLA class I (HLA-A, HLA-B and HLA-C) alleles. In practice, we retrieved all HLA amino acid sequences and performed homology modeling on all class I HLA alleles in multi-templates mode using available solved structures from the Protein Data Bank (PDB). We then superimposed modelled structures two by two resulting in a square symmetrical matrix of Root Mean Square Distance (RMSD). For a given pair of donor recipient, the calculated score corresponds to the sum of RMSD of each unknown alleles (by the recipient in solid organ and by the donor in HSCT) for each HLA locus. We compared this score to the epitopic mismatch score and found a partial correlation ($R^2 = 0.55$). Finally, we validated our score on a kidney transplant cohort by performing cox regression on longitudinal data. Our HLA-3Diff score was strongly associated with class I dnDSA occurrence (FDR = 5.3e-5, HR = 1.89) as well as rejection (FDR = 0.01, HR = 1.58). In perspective, we plan to perform the same work on HLA class II alleles and refine our score on the basis of available scientific knowledge. Altogether, this new metric could help matching donors and recipients and minimize the risks of rejection and dnDSA occurrence especially for non-familial transplantation in a context of graft shortage.

**O44 | HLA variation is associated with Cytomegalovirus seropositivity**

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Cytomegalovirus (CMV) is a β-herpes virus that is ubiquitous in all human populations, with worldwide prevalence of seropositivity ranging from 60 to >90%, reaching 80% in some parts of the USA. While both acute and chronic infection are benign for the majority of infected individuals, CMV can cause severe effects in immunocompromised individuals, including transplant recipients. Additionally, CMV has the ability to evade immune response causing a persistent asymptomatic infection. Downregulation of HLA class I expression is one of the mechanisms that CMV uses for evading the immune system. HLA mismatch is also associated with CMV reactivation, with high risk of opportunistic infection after transplant. Here, we evaluated the role of HLA variation with CMV seropositivity in 397,672 individuals registered with Be The Match. We examined 5 HLA loci (HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQB1) for association with CMV serostatus, while adjusting for sex, age, ancestry and socioeconomic status. Eleven risk alleles were identified, with C*03:04 and DRB1*04:01 demonstrating strongest association in European ancestry individuals (OR = 1.07; CI97.5% = 1.04 - 1.10; p = 1.23E-08, and OR = 1.06; CI97.5% = 1.04 - 1.09; p = 3.40E-08 respectively). Ten protective alleles identified included HLA-DRB1*01:03 in both Hispanic and European ancestry (OR = 0.52; CI97.5% = 0.44 - 0.61; p = 2.14E-14, and OR = 0.71; CI97.5% = 0.671 - 0.75; p = 1.35E-32 respectively). Interestingly, HLA-DRB1*01:01 was identified as a risk allele (OR = 1.05; CI97.5% = 1.032 - 1.08; p = 2.36E-06) in individuals with European ancestry. HLA-DRB1*01:01 and -DRB1*01:03 have 98.48% homology, differing in only three amino acids residues at positions 67, 70 and 71 in the peptide binding groove. Despite their overall similarity, these differences appear to mediate opposing effects in CMV seropositivity. Our results provide population-scale evidence of the role of HLA in mediating control of this common and important virus.

**O45 | A large, improved and ancestry-diverse reference panel to impute HLA classical and non-classical class I alleles**

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The HLA class I genes encode key molecules for antigen presentation, recognition of self and non-self-antigens, and the modulation of NK cell activity. HLA polymorphisms influence transplant outcomes and the susceptibility to many autoimmune and infectious diseases. The SNP-HLA Reference Consortium (SHLARC) aims to accelerate immunogenetics studies by improving HLA imputation from SNP data. Imputation accuracy depends on a suitable reference panel. However, HLA genes are highly polymorphic, and the available reference panels are mostly European, resulting in poor accuracy when imputing diverse populations. Here, we tested imputation performance for HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, HLA-G, HLA-H, MICA, and MICB genes. We used a bioinformatics pipeline developed to minimize genotype errors for HLA genes, obtaining the genotypes (SNPs and indels) and the HLA alleles for 4,592 samples from 80 different populations, including 1,170 admixed Brazilians. The generated data were used to build reference panels to predict HLA alleles in 192 samples (test set) extracted from the data but not used for the reference. The F1 score was used to evaluate these models. Our models predicted alleles with an F1 score ranging from 0.80 (HLA-B) to 0.86 (HLA-A) for the classical HLA genes, while for other HLA loci and MIC genes the F1 score ranged from 0.80 (HLA-B) to 0.86 (HLA-F) to 1 (HLA-G). We detected a poor performance for HLA-E (F1 = 0.70) due to a misclassification of HLA-E*01:11 to the common HLA-E*01:03 allele in only one sample. Overall, imputation provided a fast and simple method for inferring HLA alleles from SNPs, with accurate results for all genes, including those not commonly evaluated in imputation (e.g., MICA). Imputation errors are mostly related to rare and population-specific alleles, such as Native-American alleles, which emphasizes the importance of larger and more diverse reference panels for improving HLA imputation accuracy.

A strong link between COVID-19 severity and HLA-C*04:01 allele has been replicated in several Caucasian populations including Armenians. The results have led to an idea that HLA-C*04:01 may affect the immune response via three biological mechanisms: (i) disruption of the HLA-C mediated protection harnessing natural killer cells (NK); (ii) causing NK hypo-responsiveness through KIR2DL1; or (iii) over-activation and exhaustion of CTL and NK cells by stimulating functional KIR2DS4. To test those hypotheses, we re-analyzed HLA-genotypes and RNA-sequencing data of Overmyer et al. [Cell Systems 2021; 12:23-40]. An ordinal regression of patients' status (i.e., non-COVID vs. COVID-non-ICU vs. COVID-ICU) against HLA-C has corroborated the increase in the disease severity with increasing HLA-C*04:01 dosage (p < 0.003). DESeq2 analyses of the transcriptome (16444 loci) within COVID subset mapped 3586 down-regulated and 4031 up-regulated loci to the disease severity at FDR p<0.05. The results of enrichment analyses of those 7617 genes indicated aberrations in processes, such as T cell activation, inflammatory response, positive regulation of both NK-mediated cytotoxicity and interferon-gamma production. However, only 563 down- and 341 up-regulated loci had nominally associated with the HLA-C*04:01 carriage, reflecting its genetic association with severe symptoms. Using GTEx data and rs5010528 as proxy for HLA-C*04:01 (R² = 0.97, 1kG EUR cohort), we found that HLA-C*04:01 was associated with multiple tissue (e.g., lung, heart and blood) RNA expression and splicing changes in >10 protein-coding loci situated close to HLA-C. The ontology analysis of the loci implicated HLA-C*04:01 in altering antigen processing and presentation of endogenous peptide antigen via HLA class I via ER pathway (FDR p < 0.0001), protection from NK-mediated cytotoxicity (p < 0.004), and innate immune response to other organisms (p < 0.009). The work was supported by the Science Committee of RA (grant E17).

O46 | Possible Biological Mechanisms Underlying the Association between COVID-19 Severity and HLA-C*04:01

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O47 | The expanded role of microRNAs in controlling the HLA class I phenotype: Relationship between the 3' UTR and post-transcriptional Gene Regulation

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HLA genes are strongly associated with transplantation outcome, disease onset and severity, while their comprehensive study utilizing state-of-the-art methods represents one of the major goals of immunology. The introduction of Next Generation Sequencing (NGS) can provide the opportunity to investigate non-coding regions of the HLA genes and to associate them with disease prevalence. MicroRNAs (miRs) represent single-stranded non-coding RNAs that can efficiently modulate the HLA phenotype, through sequence-specific interactions with their corresponding mRNAs. This study aimed to investigate the potential association between the 3’ UTR of HLA class I (HLA-A, -B and -C) genes with specific miRs, that can affect the HLA phenotype. Initially, we used the genetic sequence (indexed in the IPD-IMGT/HLA Database) of the most frequent HLA class I alleles in Greece, as has been previously by our laboratory, to find potential associations with specific miRs. For this purpose, miRbase and miRDB, were used to search potential miRs related to the 3’ UTR genetic sequence of HLA class I alleles. Moreover, the function of the detected miRs was identified, using the available databases such as Target Scan, miR path v3, miRWalk2.0, miRanda and miRNAmap. The results of this study showed the presence of about 149, 175, and 152 associated miRs with HLA-A, -B and -C alleles, respectively. For each locus allele, approximately over 30 miRs were differentially detected. Specifically, for the A locus, the hsa-mir-7111-3p, hsa-mir-7111-5p, hsa-miR-4436b-3p, hsa-miR-7114-3p, for B locus, the hsa-mir-125a-3p, has-miR-1207-5p and for C locus, the has-miR-103a-5p and has-miR-4646-3p, were found to play a major role in post-transcriptional HLA gene regulation. Based on the above results, the identification of specific miRs may reveal significant data regarding the HLA class I phenotype modulation, thus playing important role in decision-making for transplantation or disease administration.

O48 | **Analysis of "Big Data" reveals a new MHC Class I sequence, HLA-OLI, and the location of HLA-Y**

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The Pangenome (PGP) and Thousand Genome Project (1KGP) sequence data provide new opportunities for understanding MHC diversity. Dot matrix analysis of 47 fully phased MHC sequences from the PGP revealed a 60kb DNA indel between HLA-W and HLA-J on 27 of the 47 PGP sequences. A homology search of the 60kb indel sequence with sequences maintained by the IPD-IMGT/HLA Database identified a sequence in the indel with 100% homology to HLA-Y*01:01 and a second sequence with 88% identity with HLA-P*01:01:01:01. Subsequent phylogenetic analysis placed the second sequence in a broader cluster with alleles of HLA-P and HLA-W. Assuming this to be a novel HLA class I sequence we named it HLA-OLI. Subsequent analysis of 3202 whole genome sequences from 1KGP with Type Stream Visual optimized for genotyping whole genome sequence data revealed that HLA-Y and HLA-OLI (Y/OLI) were present together in 886 of the 3202 samples. Y/OLI was associated with HLA-A*29:01:01, 30:01:01, 33:01:01, 33:03:01 and 34:01:01, confirming HLA-A~HLA-Y associations of previous studies. We could not confirm an association with HLA-A*02:03 and 02:05 but did identify a possible new association with HLA-A*01:01:01:11. Further analysis suggests a segmental replication structure of the alpha block of the MHC with at least 4 homologous segments that include HLA-V and HLA-P, HLA-H and HLA-T, HLA-A and HLA-W and HLA-Y and HLA-OLI. The HLA-H/HLA-T segment is absent on haplotypes with HLA-A*23 and HLA-A*24 and the HLA-Y/HLA-OLI segment is present only on some haplotypes with the HLA-V/HLA-H segment and HLA-A/HLA-W segment being present on all (or most haplotypes). This study of the MHC using “big data” has provided new insights into the structure and evolution of the MHC alpha block. We have identified a new HLA class I sequence, the location of HLA-Y and provide evidence that the MHC alpha block is made up of varying numbers of replicated segments containing HLA class I genes and pseudogenes.

**Immunotherapy, Gene Therapy, Cellular Therapy**

O49 | **Polymorphic KIR3DL3 expression modulates tissue-resident and innate-like T cells**

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Most human killer cell immunoglobulin-like receptors (KIR) are expressed by Natural Killer (NK) cells and recognize HLA class I molecules as ligands. Uniquely, KIR3DL3 is a conserved but polymorphic inhibitory KIR recognizing a B7 family ligand, HHLA2, and is implicated for immune checkpoint targeting. KIR3DL3 is also the most genetically diverse KIR, with a subset of polymorphic residues having genetic patterns consistent with the action of diversifying natural selection. Because the expression profile and biological function of KIR3DL3 remained elusive, we searched extensively for KIR3DL3 transcripts, revealing expression is highly enriched in γδ and CD8+ T cells rather than NK cells. These KIR3DL3 expressing cells are rare in the blood and thymus, but more common in the lungs and digestive tract. High resolution flow cytometry and single cell transcriptomics showed that peripheral blood KIR3DL3+ T cells have an activated transitional memory phenotype and are hypofunctional. The TCR usage is biased towards genes from early rearranged TCR-α variable segments or Vδ1 chains. Using calcium flux and luciferase reporter assays, we show TCR-mediated stimulation of γδ T cells can be inhibited through KIR3DL3 ligation. Surface plasmon resonance shows KIR3DL3 binds HHLA2 with similar affinity as KIR3DL1 binds Bw4+HLA class I. Whereas we detected no impact of KIR3DL3 polymorphism on ligand binding, variants in the proximal promoter and at residue 86 can reduce expression. In summary, we demonstrate that KIR3DL3 is upregulated in response to unconventional T cell stimulation and that individuals may vary in their ability to express KIR3DL3. These results have implications for the personalized targeting of KIR3DL3/ HHLA2 checkpoint inhibition.

O50  |  Memory CD4+ T cells efficiently recognize divergent HLA-DP immunopeptidomes relevant in allogeneic hematopoietic cell transplantation

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Naïve T-cells (TN) are considered the main mediators of alloreactivity after hematopoietic cell transplantation (HCT), prompting clinical trials with TN-depleted allografts to prevent graft-versus-host disease (GvHD). Both minor histocompatibility antigens (mHAg) and HLA-DP mismatches can be alloreactive CD4+ T-cell targets. We have demonstrated that immunopeptidome divergence between mismatched HLA-DP, regulated by the peptide editor HLA-DM, determines the strength and T-cell receptor (TCR) diversity of alloresponses. Here, we hypothesized that immunopeptidome divergence could also influence memory T cell (TM) alloreactivity against HLA-DP. TN (CD45RA+CD45RO-) and TM (CD45RA-CD45RO+) cells from healthy individuals were stimulated with HLa cells expressing single matched (DPB1*04:01) or permissively (DPB1*04:02) or non-permissively (DPB1*09:01) mismatched HLA-DP allotypes, with/without HLA-DM. In the presence of HLA-DM, TN alloresponses (quantified by CD137 upregulation) against matched (14.4±13.9% vs. 5.9±7.7%, p = 0.0041) and permissively mismatched (16.7±14.3% vs. 3.0±3.3%, p = 0.005) HLA-DP were significantly stronger than TM responses. In contrast, TM responses against non-permissive mismatches were strong and not significantly different from TN (40.0±18.1% vs. 33.4±22.3%, p = 0.08). Using immunopeptidomics and comparative whole-exome sequencing, we show that the absence of HLA-DM significantly increases the number of candidate mHAg presented by HLA-DP (7.9±5.1 to 18.9±7.2, p = 0.016), concomitantly increasing the strength and TCR α+β diversity of alloresponses to comparable levels in TN and TM (33.9±14.6% vs. 36.3±15.7%, p = ns). In conclusion, TM cells contribute substantially to alloresponses against divergent immunopeptidomes from non-permissive HLA-DP mismatches and deregulated mHAg. These findings bear implications for TN depletion strategies to prevent GvHD, and suggest potential new avenues to harness mHAg alloreactivity in cellular therapy.
O51 | Discovery of the human cytomegalovirus-specific peptide repertoire naturally processed and presented by infected human antigen presenting cells

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Complications due to infection with or reactivation of human cytomegalovirus (HCMV) remain a clinically challenging problem in immunocompromised patients. Knowledge of viral targets is critical to improve monitoring of high-risk patients and to optimize antiviral T-cell therapy. We aimed to identify naturally presented HLA-A*11:01-restricted HCMV-derived T-cell epitopes from HCMV infected professional antigen presenting cells (APCs) to expand the spectrum of immunogenic targets. Monocyte-derived and genetically-engineered dendritic cells (DCs) were generated to provide a stable platform for soluble (s)HLA-A*11:01 production. After infection of sHLA-A*11:01-secretting cells with wild type HCMV or a mutant lacking known immune evasion molecules (US2-6 + 11), sHLA-A11-bound peptides were isolated by w6/32 immunoaffinity chromatography followed by mass spectrometric analysis. More than 50 peptides were identified and screened for their viral protein origin and binding strength to HLA-A*11:01 using established databases. The 25 highest scoring candidates were selected for in vitro evaluation of immunogenicity, cytotoxicity, clinical suitability and relevance, and specific T cells for 5 can-
tides were detected in healthy CMV donors by IFN-γ-EliSpot. Their complex stability was demonstrated by in vitro peptide binding assays. Highly proliferative and cytotoxic memory T cells were detected after stimulation with the UL36-derived A11SAL and UL122-derived A11SVS peptides in healthy CMV donors and HCMV-infected patients. Surprisingly, A11SAL-specific memory T cells exhibited functional properties at levels comparable to those T cells against the known immunodominant pp65-derived A02NLV peptide. The A11SAL peptide is a new major target of the anti-HCMV immune response. The newly identified HCMV peptides expand the repertoire of immunodominant targets and will improve strategies for identifying high-risk patients, and enhancing therapeutic options with HCMV-specific T cells.

O52 | Exploring the cryptic HLA-DP immunopeptidome for new targets of T cell immunotherapy in acute myeloid leukemia

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HLA molecules present a large repertoire of peptides (immunopeptidome) including epitopes relevant for T cell immunity. A fraction of these peptides originate from alternative open reading frames (ORF) embedded in canonical genes or in long non-coding RNA (lncRNA), or interspersed in intergenic regions (cryptic immunopeptidome). This represents an unexplored reservoir of epitopes potentially useful in cellular immunotherapy of cancer. Here we characterized the cryptic immunopeptidome of class II HLA-DP allotypes in the monocytic leukemia cell line THP-1. Single HLA-DP expressing THP-1 cell lines were generated by CRISPR/Cas9 knockout of endogenous HLA class II genes followed by transduction with lentiviral vectors encoding 3 frequent prototype HLA-DP antigens: DP10, DP401 and DP402. HLA-DP peptides were eluted after immunoaffinity chromatography and analyzed by tandem Mass Spectrometry. Peptide identification was performed using a THP-1 specific protein database built from the 3-frame in silico translation of transcripts in total RNA sequencing data. A total of 2104, 1963 and 2206 peptides were identified in DP10, DP401 and DP402, with 10.0%, 5.4% and 8.1% derived from cryptic ORFs, respectively. Among all 6273 peptides identified, 117 (1.9%) carried amino acids corresponding to public or THP-1 specific genetic variations, that is, potential minor histocompatibility antigens or neoepitopes. In addition, 569 (9%) cryptic peptides were identified overall, the majority arising from alternative ORFs in canonical genes (83.8%) or in lncRNA (10.7%). One peptide was derived from the lncRNA CCDC26 whose expression levels have been associated with poor prognosis of AML, and 16 peptides were found from alternative ORFs of 15 tumor associated antigens including TP53,
GFI1 and ETV6. In conclusion, we show here that a relevant fraction of the HLA-DP immunopeptidome in THP-1 is of cryptic origin and includes potential new targets for T-cell based immunotherapy.

O53 | Generation and characterization of third party donor derived AdV, CMV and EBV multivirus specific T cells for therapeutic intervention in patients undergoing hematopoietic stem cell transplantation

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HSCT donor-derived virus-specific T cell (VST) therapy is an alternate prophylactic treatment for viral infections after allogeneic HSCT. However, HSCT-donor derived T cells have the major limitation of requiring a prolonged time for the generation. Therefore, it is necessary to have off-the-shelf, ready-to-use, third party donor derived VSTs. We have generated and characterized in vitro adenovirus (AdV), cytomegalovirus (CMV), and Epstein Barr Virus (EBV) - specific T cells from third-party donors. These donors (n = 145) were selected based on common HLA alleles namely HLA-A*02, A*24, A*11, B*40, B*15, DRB1*07, DRB1*15 in an Indian population and the seropositive status (n = 50) against respective target viruses. The VSTs were generated by co-culturing donor-derived PBMCs with donor monocyte-derived dendritic cells pulsed with virus-specific overlapping peptides namely BLZF1/LMP2/EBNA-1 for EBV VST, with pp65 for CMV-VST, Hexon and Penton for AdV-VSTs. During the standardization of protocols we have compared VSTs generated using both in house and commercial available peptides. The results based on characterization were comparable. Immunophenotyping of the VSTs showed prominent CD3+ CD8+ and CD3+CD4+ subset having effector memory CD45 RA- CCR7 - (TEM), terminal effector memory (TEMRA) CD45 RA+ CCR7 - subsets in all the VSTs generated. Also, CD3+ CD8+ and CD3+CD4+ subsets had high percentage of TC1 and Th1 cells. We then functionally characterized the VSTs using IFN gamma secretion assay post antigen stimulation, which was found to be in the range of 38 to 45% for EBV, AdV in the total live cell population and 90% positive for CMV VSTs. Our data suggests that with current approach of generation of VST bank, a broad range of HSCT patient's population can be targeted for VST adoptive immunotherapy. Further toxicity of VSTs will be analyzed in immunocompromised mice models.

O54 | Optimal population coverage for cellular therapies

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HLA compatibility is not commonly considered in the development of other allogeneic IECTs, such as CAR-modified cells. However, HLA matching, or knocking out problematic HLA, could improve the persistence and duration of efficacy of such “HLA-independent” therapies. However, even in situations where finding HLA compatible starting material is usually easy, there is no need to manufacture “HLA redundant” products. Optimally sizing the bank to maximize population coverage while minimizing manufacturing costs is an open problem. To address this aspect of IECT development, we developed an optimal coverage problem, combined with graph algorithms for donor selection, under different, clinically plausible scenarios where the cost of additional donor recruitment and/or the cost of preventing class I or class II HLA expression through gene editing were of interest. We compared the efficiency of different optimization algorithms – a greedy solution, a linear programming (LP), and integer linear programming (ILP) versus random donor selection over millions of candidate donors. The additional population coverage per donor decreases with the number of donors. All proposed algorithms consistently achieve the optimal coverage with far fewer donors than the random choice. The number of randomly selected donors required to achieve a desired coverage increases with increasing population. However, when optimal donors are selected, the number of donors required may counterintuitively decrease with increasing population size. Gene editing was generally more expensive than recruiting additional donors. When choosing the donors and patients from different populations, the number of random donors required drastically increases, while the number of optimal donors does not change. Random donors fail to cover populations different from
their original populations, while a small number of optimal donors from one population can cover a different population.

O55 | The stimulation of memory B cells for the identification of unacceptable antigens in solid organ transplantation

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Identification of HLA antibody utilizes bead-based technology, which offers a snapshot of the antibody profile at the time of collection. However, antibody profiles can fluctuate with time, specifically a current serum profile may not detect historical sensitization. Memory B cells in circulation specific to HLA-alloantigen can be present without detection by SAB assay, though may be differentiated into plasma cells and rapidly produce high affinity HLA specific antibodies. Karahan et al. published a protocol for stimulation of memory B cells and characterization for the presence of HLA specific IgG. The aim of this project is to reproduce the findings of Karahan et al. in the Western Australian cohort. The study subject was selected based on the history of sensitization. Peripheral blood and serum samples were collected from healthy donors consisting of multiparous women, and individuals who had never been exposed to alloantigens. Specifically, B cells from PBMCs were incubated with agonists and growth media for 10 days at 37°C. The stimulated cells were characterized by Immunophenotyping assay using flow cytometry. The supernatant was purified for IgG using a protein G affinity purification method. The purified products were then evaluated for HLA IgG specificity. We observed an expansion of CD19+ B cells in 10 days of stimulation and those cells carried a characteristic antibody-secreting-cell phenotype. In immunized individuals, 3/5 were found to have HLA-specific IgG antibody in concentrated supernatants. The HLA specificities detected in the supernatants were absent in the current serum, and accounted by a single mismatch eplet from the sensitizing partner. In all unsensitized individuals, the supernatant was negative for HLA specificity by SAB assay as expected. Therefore, the stimulation assay was shown to be specific to B cell memory, however, further investigation is required to examine the assay sensitivity and producibility.

O56 | New regulatory dimensions for transplantation, genetics and stem cell research in the French bioethics law. Consequences for European collaborations

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The bio-ethical regulatory framework applicable to immunogenetics is different in various countries, although common principles and supra national texts do exist; it impacts on medical and scientific collaboration between countries. In France, a bioethics law has existed since 1994 covering the use of body elements for research and therapy, stem cells, genetic testing, human genomics, transplantation and other domains without impact on immunogenetics. It has been periodically revised (2004, 2011, 2021). After a public consultation in 2018 and reports from bodies such as the National Bioethics Committee, the State Council and a parliamentary commission the revised law was discussed, voted for in parliament and published on August 2, 2021. Some decrees are still pending for the application of the law. However, a good picture of the framework for immunogenetics can be drawn and allows to better organize lawful collaborations, be it for clinical care or for research. Of special interest is the analysis of the measures proposed by citizens and what was kept of them in the final text, as an example of societal dialogue with possible impact on regulations. We will present the relevant modifications for the practice of immunogenetics and their possible consequences on collaborative practices in Europe, for transplantation, genetic testing, genomic medicine and stem cell research. The new measures deal with living cross organ donation, intra-family donation from minors or vulnerable persons, for organs and haematopoietic stem cells, the transmission of genetic information concerning a deceased person, for the medical benefit of a family member, the conditions for revealing incidental findings occurring in the course of testing and not related to the aim of the test prescribed, algorithms and massive data management in the context of medicine, new conditions for research on embryos and stem
Permissive T-cell epitope (TCE) HLA-DP mismatches (mM) are an established criterion for unrelated donor (UD) selection in hematopoietic cell transplantation (HCT). We have recently demonstrated that frequent permissive mM can be stratified according to their immunopeptidome divergence into core versus non-core subsets. Here, we sought to refine the definition of these permissive subsets and explore how their directionality affects their associations with HCT outcome. HLA-DP matching status was scored in 8420 adult patients who received a 1st HCT from 10/10-matched UD for AML, ALL, or MDS from 2005 to 2020. TCE group 3 (TCE3) permissive pairs (N = 3178) were stratified into core and non-core mM, and compared with allele-matched (N = 2390) and non-permissively mM (N = 2598) pairs. An extended core allele definition including alleles with 0-5 exon-2 amino acid differences respect to the high-frequency core alleles (DPB1*02:01, 04:01, 04:02) was applied. Multivariable models were tested in parallel to the standard TCE model, including graft-versus-host (GvH) and host-versus-graft (HvG) vectors. In the standard TCE model, non-relapse mortality (NRM) risks were significantly increased for non-permissive (HR 1.3 [99% CI: 1.0–1.5]; p = 0.0011) but not for permissive (HR 1.1 [0.9–1.3]; p = 0.08) mM compared with allele matches. Both permissive and non-permissive mM were associated with increased acute GvH disease (aGvHD) and lower relapse. Among permissive pairs, only GvH non-core mM showed significantly increased risks of aGvHD (HR 1.5 [1.3–1.8]; p<0.0001) and reduced risks of relapse (HR 0.77 [0.63–0.93]; p= 0.0005) compared with allele-matched pairs. Importantly, none of the permissive subsets associated with increased NRM, resulting in better relapse-free survival for non-core GvH mM (HR 0.86 [0.74–1.0]; p = 0.0121). Our results confirm the clinical relevance of the refined definition of core alleles, and identify GvH non-core permissive mM as a subset associated with improved disease control.

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We recently isolated a multiple myeloma-reactive T-cell receptor (TCR) that appeared to be restricted for the HLA null allele C*04:09N. HLA-C*04:09N differs from HLA-C*04:01 in a frameshift within its cytoplasmic domain resulting in 32 additional amino acids that prevent cell surface expression. Based on our observations in multiple myeloma, we hypothesized that HLA-C*04:09N can...
present antigen sufficient for T cell activation in an appropriate microenvironment. We asked experimentally whether HLA-C*04:09N i) is detectable on the cell surface, and ii) can present peptides to T cells. We generated an HLA class I-deficient osteosarcoma cell line (U2OSHLA-KO) and established single cell-derived cell lines that stably expressed HLA-C*04:01 or HLA-C*04:09N. In line with previous observations, HLA-C*04:09N expression was detectable intracellularly but not at the cell surface by flow cytometry and conventional fluorescence microscopy. Yet, we asked whether antigen presentation could still be sufficient for T cell activation. We expressed an HLA-C*04:01-restricted TCR specific for the QYDPVAALF (QYD) epitope in human peripheral blood T cells (TQYD) to probe for antigen recognition in different HLA contexts. TQYD were incubated with (i) U2OSHLA-KO or (ii) U2OS expressing only HLA-C*04:09N, both previously transfected with a QYD-minigene or a different epitope control minigene. Only U2OS cells expressing HLA-C*04:09N and the QYD-minigene activated TQYD as indicated by interferon-γ and granzyme B secretion. T cell activation was blocked by an HLA class I-specific antibody, which suggests that HLA-C*04:09N-T-cell interaction occurred at the cell surface. In summary, HLA-C*04:09N expression is sufficient for antigen presentation and T-cell activation in vitro. We assume that certain in vivo situations will enable efficient antigen presentation on HLA-C*04:09N and suggest consideration of this allele in clinical context such as allogeneic stem cell transplantation.

O59 Longitudinal tracking of T-cell receptor repertoire reconstitution after allogeneic hematopoietic stem cell transplantation
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Reconstitution of T-cell immunity after allogeneic stem cell transplantation (aHSCT) is essential towards achieving immune protection and is linked to several transplant related complications. We thought to investigate the spatiotemporal dynamics of T-cell receptor (TCR) repertoire in a cohort of 32 aHSCT recipients and their respective donors. We performed high-throughput TCR complementarity-determining region 3 (CDR3) β sequencing as a measure of T-cell immunity on serial peripheral DNA samples at 4 time points during the first year post-aHSCT. Recipient’s TCR diversity was significantly reduced after aHSCT (p < 0.001) reflected by an increased clonality and reduced TCR richness. This reduced diversity sustained during the entire follow-up period and remained stable.Recipient-donor overlap was persistently low post-aHSCT (median morisita index of 0.04), with increasing similarity of recipient’s repertoire during immune recovery. R+/D+, R+/D- CMV configurations were subject to significant higher clonalities than in R-/D+, R-/D- recipients (p < 0.001) and the clonality within the different groups didn’t change throughout the follow-up. Cumulative frequency and the number of anti-CMV clonotypes didn’t differ pre and post-aHSCT and didn’t significantly correlate with clonality in R+/D+, R+/D- CMV configurations. In conclusion, clonal diversity was stably maintained throughout the time course suggesting changes in the repertoire immediately after transplantation. Repertoire reconstitution mainly consists of de novo clonotype generation and reaches statis by 9 to 12 months post-HSCT. CMV specific clonotypes follow homeostatic stability and expansion of clones in CMV positive recipients seems to be rather driven by a bystander effect than antigenic selection.

O60 Associations between HLA Evolutionary Divergence and clinical outcome of matched related or unrelated stem cell transplantation: A study from the EBMT Cellular Therapy and Immunobiology Working Party
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HLA Evolutionary Divergence (HED), a numerical metric calculated from the amino acid variability in the peptide binding pocket of HLA allotypes representing predicted immunopeptidome diversity, has been associated with outcome of hematopoietic cell transplantation (HCT). Here we explored the impact of HED scores as continuous variable (rather than cohort-specific cut-off values) in HLA-matched HCT. 17,525 adult patients with acute leukemia or myelodysplastic/myeloproliferative disease, transplanted from 10/10 HLA-matched related (N = 3682) or unrelated (N = 13,843) donors between 2010 and 2019, were included. Cause-specific proportional hazard models including common predictors were used to investigate the association of HLA-A, -B, -C, -DRB1, and -DQB1 locus-specific HED scores as continuous variables with a linear effect, with overall survival (OS), relapse-free survival (RFS), non-relapse mortality (NRM), relapse, acute and chronic graft-versus-host disease (GvHD). The distribution of HED scores was similar in related and unrelated transplants (0-15 for HLA-A, -B, -C; 0-20 for HLA-DRB1, -DQB1). No significant associations were found between HED scores at any locus and any outcome endpoint after related donor HCT. In contrast, in the unrelated donor setting, increasing HED scores at HLA-B (HED-B) were associated with improved OS (hazard ratio [HR] 0.99 per unit difference, p = 0.004) and RFS (HR 0.99, p < 0.001), as well as lower NRM (HR 0.98, p = 0.004) and aGvHD (grade 2-4 (HR = 0.99, p = 0.014). For RFS, we observed a significant interaction between HED-B and B-Leader status (strongest protective HED-B effect for B-Leader MM). In conclusion, we show a weak but consistent protective association between increasing HED-B scores and survival after HLA-matched unrelated but not related HCT for malignant disease, possibly mirroring effects on T-cell reconstitution and/or B-Leader related mechanisms. These findings will be of aid in patient risk stratification after allogeneic HCT.

O61 | The role of Recipient Specific Antibodies (RSA) in transplant outcome. Analysis of a group of family donors selected for patients undergoing haploidentical transplantation

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Hematopoietic stem cell transplant (HSCT) is an effective therapeutic procedure for the treatment of patients (R) with onco-hematological disorders. Improved
transplant protocols have brought to increasingly satisfactory results in the field of transplant from haploidentical donors (D). Recently, a hypothesis has emerged that, in addition to DSA (donor specific antibodies), RSA may play an important role in transplant outcome, especially in relation to GvHD. To evaluate the role of RSAs, 51 D/R pairs (41 adults and 10 pediatrics) undergoing haplo-HSCT (2016–2022) were analyzed. The donors were tested for Ab-HLA with Luminex technology, using an MFI = 1000 as positivity cut-off. 23 (46%) were Ab-HLA+. 8/23 (35%) had RSA and 15/23 (65%) were RSA-. aGvHD occurred in 37% of Rs (18/48) while cGvHD occurred in 25% (10/40). 100% of RSA+ recipients (8/8) experienced acute or chronic GvHD versus 40% of RSA- recipients (p<0.01).

75% of RSA+ recipients developed aGvHD versus 30% of RSA- recipients (p = 0.04, OR = 6.69). 67% of RSA+ recipients developed cGvHD versus 18% of RSA- recipients (p = 0.025, OR = 8.63). Multivariate analyses confirmed the relationship between RSA and chronic and acute GvHD (p = 0.008, p = 0.01), as already described in few papers. Interestingly, the only low MFI (959) was found in a son vs. father transplant with aGvHD-I while 3 other papers. Interestingly, the only low MFI (959) was found in a son vs. father transplant with aGvHD-I while 3 other cases, with MFI: 17772, 24542 and 16227, had aGvHD-III and cGvHD, aGvHD-II, aGvHD-III and cGvHD, respectively. These data seem to indicate that the level of MFI could correlate with incidence and severity of GvHD, although the result needs to be confirmed in larger cohorts. In addition, all Rs were DSA-, suggesting that RSAs are the key players in an immunological milieu in the D/R pair that could lead to the development of GvHD. It is therefore possible to hypothesize that donor immunization may be involved in B-mediated alloreactivity toward the recipient, leading mainly to the development of cGvHD, but also to aGvHD.

Hematopoietic stem cell transplantation from an HLA-haploidentical donor (haplo-HSCT) is now commonly used to treat hematological malignancies in the absence of a fully-matched donor. HLA typing is not considered the first criterion for donor selection (absence of donor specific antibody, age, gender and CMV). HED, a measure of HLA allele diversity, has been proposed to predict response to cancer immunotherapy, solid organ transplant rejection and outcome of HLA-matched HSCT. We retrospectively analyzed the impact of HED on survival and relapse in 107 haplo-HSCT recipients, transplanted between 2014 and 2022 in Saint-Louis hospital, Paris. HED was calculated as genetic distance between pairwise HLA-A, -B, -C, -DRB1, -DQB1 and -DPB1 alleles in recipients (HED-R), donors (HED-D) and mismatched haplotypes (HED-MM). Both 1st quartile and median HED values at each locus were used to stratify recipients, donors and MM as “high” or “low” HED. Survival analysis, using Kaplan-Meier curves, was performed for progression-free (PFS) and overall survival (OS). Cumulative incidence of relapse (CIR) and non-relapse mortality (NRM) were calculated using the method of Fine and Gray with their competing risks (death and disease recurrence, respectively). We found impaired OS in recipients with low (<1st quartile) class I HED (log-rank p = 0.014). This effect of low class I HED-R was also significant when analyzing death from relapse (fine-gray test p = 0.032) and PFS (p = 0.036). No impact of HED-D was found on outcomes. Interestingly, OS and PFS were decreased with a high HED-MM (p = 0.051 and p = 0.022 respectively), while the number of mismatches was not associated with outcomes. Thus, outcome of haplo-HSCT is not only impacted by class I HED-R, as previously shown for matched-unrelated donor HSCT, but also by the level of class I HLA divergence between the unshared haplotypes. These results suggest that HED-MM could be considered as a selection criterion for haploidentical donors.

O62 | HLA evolutionary divergence (HED) influences the outcome of haploidentical hematopoietic stem cell transplantation in adult patients with hematological malignancies

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O63 | Combined imputation of HLA genotype and race leads to better donor-recipient matching

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Hematopoietic Stem Cell Transplants (HSCT) are a curative treatment for many hematologic diseases. Such transplants require high resolution HLA-matched donors. Current HLA matching algorithms depend on imputation which in turn relies on a self-identification of race and ethnicity (SIRE), which presents a challenge for multi-racial/ethnic individuals or those with unknown race/ethnicity. We present a novel algorithm MR GRIMM “Multi-Race Graph IMputation and Matching” that simultaneously imputes the ethnic group and high-resolution HLA genotypes using the SIRE as a Bayesian prior, and propose a novel method to impute typing inconsistent with current haplotypes. The prediction performance of MR GRIMM was measured using a validation dataset of over 170,000 donor-recipient potentially matched pairs from US registry searches. A higher AUC was observed compared with single-race method on all populations. Recall of the race/ethnic group imputation from the HLA was measured by comparing to ethnic self-definition- using the SIRE. Accuracies of 0.74 and 0.55 were obtained for the prediction of 5 rollup and 21 detailed US population groups using the HLA typing respectively. Improved race/ethnic group designation can improve the accuracy of match predictions as well as increase the accuracy of genetic association studies. We anticipate that the operational implementation of this algorithm in the registry search would help reduce disparity in access to HLA-matched HSCT.

O64 | Genetic variation in HLA genes: impact on transplant compatibility in a Brazilian admixed population

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Brazilian populations carry large components of European, African, and Native American ancestry. This has implications for finding a compatible donor for bone marrow transplants. Here, we investigate HLA polymorphism in a Brazilian population to understand how admixture impacts the chances of finding a compatible donor for transplant. Using whole genome sequencing data of 2629 individuals from the Recipient Epidemiology and Donor Evaluation Study-III Brazil Sickle Cell Disease Cohort (REDS), we applied the ADMIXTURE and Gnomix approaches to infer each individual’s global genetic ancestry, as well as their local ancestry in the MHC region, and the HLA allelic diversity. Because of the sequence similarity between HLA genes and their high variability, we used a specific approach to make the HLA genotyping and allele calls (HLA-mapper software). The global ancestry proportion of the cohort is 47.9% African, 43.6% European, and 6.7% Native American. There are 87 HLA-A predicted proteins, 129 HLA-B, 69 HLA-C, 158 HLA-DRB1, 89 HLA-DQB1, and 63 HLA-DPB1, some not fully described in the IPD-IMGT/HLA Database. To understand the impact of admixture on the chances of finding a donor in REDOME, the Brazilian Marrow Donor Registry, we treated REDS as patients looking for a donor in REDOME. The match proportion decreases markedly when we attempt to match over more loci, going from 80% (6/6) to 1.3% (12/12). Considering IBGE self-reported categories, individuals who self-identify as “white” have a higher average proportion of matches to potential donors than those who identify as “mixed” or “black” (e.g., 16% and 10% for 10/10 match). Also, individuals with higher African ancestry have lower chances of finding a compatible donor when compared with individuals with less African ancestry (e.g., 16% and 7% for a 10/10 match). These results, generated using new and accurate HLA allele calls, confirm the finding that the proportion of African ancestry influences the chances of finding a compatible donor in REDOME.
Autoimmunity, Infection, Reproduction and Cancer

O65  |  A Protective HLA Extended Haplotype Outweighs the Major COVID-19 Risk Factor Inherited from Neanderthals in the Sardinian Population

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During the first and second waves of coronavirus-19 disease, Sardinia had one of the lowest hospitalization and related mortality rates in Europe. However, in contrast with this evidence, the Sardinia population showed a very high frequency of the Neanderthal risk locus variant rs35044562, considered to be a major risk factor for a severe SARS-CoV-2 disease course. We evaluated 358 patients who had tested positive for SARS-CoV-2 and 314 healthy Sardinian controls (Italy). Patients were divided according to WHO classification: 120 patients asymptomatic, 90 pauci-symptomatic, 108 with a moderate disease course and 40 severely ill. The allele frequencies of Neanderthal-derived genetic variants reported as being protective (rs1156361) or causative (rs35044562) for severe illness were calculated in patients and controls. The Thalassemia variant (rs11549407), the HLA haplotypes, the KIR genes, as well as KIRs and their HLA class I ligand combinations were also investigated. The rs35044562 and rs1156361 Neanderthal variants revealed a distribution in Hardy–Weinberg equilibrium (HWE) both in SARS-CoV-2 patients and the control population (X2HWE = 0.82, p = 0.37 and X2HWE = 0.13, p = 0.72, respectively). Our findings reported an increased risk for severe disease in Sardinian patients carrying the rs35044562 high-risk variant [OR 5.32 (95% CI 2.53-12.01), p<0.0001]. Conversely, the protective effect of the HLA-A*02:01~B*18:01~DRB1*03:01 three-loci extended haplotype in the Sardinian population was shown to efficiently contrast the high risk of a severe and devastating outcome of the infection predicted for carriers of the Neanderthal locus [OR 15.47 (95% CI 5.8 – 41.0), p<0.0001]. This result suggests that the balance between risk and protective immunogenetic factors plays an important role in the evolution of COVID-19. A better understanding of these mechanisms may well turn out to be the biggest advantage in the race for the development of more efficient drugs and vaccines.

O66  |  HLA-A*03:01 significantly predicts strong humoral response at six months after mRNA vaccination: Results from the observational prospective cohort study RENAISSANCE

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While several studies have been conducted on HLA and Covid-19 infection, few investigated the role of HLA polymorphism on vaccination response. We previously analyzed the HLA allele and haplotype frequencies in a hundred weak responders (antibody levels <5th percentile) after mRNA anti-Covid vaccine and found some suggestions about specific alleles and one haplotype. We moved on typing individuals enrolled in the same cohort study (“RENAISSANCE”) with antibody titers above the 95th percentile at six months after vaccination, in order to search for any alleles predictive of strong humoral response. Individuals with clinical history of COVID-19 or positive anti-nucleocapsid antibodies were excluded. Allelic frequencies were compared with those of weak responders and of the general population, taken from the national bone marrow donor registry, IBMDR. N = 123 evaluable individuals presented with >95th percentile antibody titers at six months after BNT162b2 vaccine. One-third of them had >2080 BAU/mL, lowest value was 1261 BAU/mL. Comparison of allelic frequencies with weak responders showed a significant different proportion of individuals carrying A*03:01, A*24:02 and DRB1*16:01 (21.5% vs. 3.6%, 7.7% vs. 14.9% and 3.2% vs. 8.1% respectively). Moreover, when looking at alleles of the ancestral haplotype A3-B35-C4-DR1, we observed frequencies of 4.47%, 0.84% and 0% in the present cohort, IBMDR and weak responders, respectively. After adjusting for age, gender and BMI, the presence of A*03:01
confirmed to be statistically significant (p<0.0001) and was predictive of high antibody titers at six months with an odds ratio of 12.5 with respect to weak antibody levels. Age was the only other significant variable, with an odds ratio of 0.96. Clinical collection data is underway to correlate Covid-19 infection rates in both cohorts, in an attempt to find a definite correlation between HLA and vaccine protection.

**O67 | NEGR1 genetic variants and risk for virological failure in the HIV-positive Botswanan population**

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The Tshepo study is a clinical trial exploring non-nucleoside reverse transcriptase inhibitor (NNRTI)-based antiretroviral therapy (ART) in HIV-positive patients from Botswana. Here, we propose to explore for the first time the impact of host genetic factors on the development of virological failure (VF) among ART-treated adults in Botswana. Participants were genotyped and after quality control and SNP imputation, 798,232 SNPs were tested for association with VF in 46 cases vs. 521 controls. Logistic mixed models were performed and adjusted for gender, age, CD4 levels, virus load, BMI, and ancestry. Ten SNPs located on the first chromosome reached the genome-wide significance threshold (p-value < 6.26e-8). These SNPs overlapped the NEGR1 promoter in a 200kb high linkage disequilibrium region, which suggests they all derived from the same haplotype. Among the significant SNPs, rs145668083 (p-value = 2.31e-8, odd ratio or OR = 44.66) is a 5bp deletion directly located in a regulatory region of NEGR1, suggesting a potential effect on its expression. NEGR1 was previously associated with psychological disorders and BMI, but BMI was similar among patients with and without VF (p>0.05). In addition, four SNPs located near the UBE2H promoter exhibited a suggestive negative association with VF (p-value = 2.09e-6, OR = 0.005). UBE2H encodes an E2 ubiquitin ligase involved in MHC-I presentation and UBE2H knockdown was previously shown to disrupt early steps of HIV in vitro replication. This study provides compelling evidence that NEGR1 variations are strongly associated with VF development among ART-treated adults in Botswana, independently from BMI. This is the first report to identify a non-HLA gene associated with VF. Non-adherence to ART is often a multidimensional issue involving HIV mutational resistance but also psychosocial factors (including mental illness) that could explain the association between NEGR1 variants with VF in our cohort.

**O68 | Copy number variation of the C4L gene isoform is associated with risk for multiple sclerosis**

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Multiple sclerosis (MS) is a multifactorial disorder characterized by the destruction of the myelin sheath and progressive neurodegeneration. The HLA allele HLA-DRB1*15:01 has been consistently demonstrated to be the factor exerting the largest genetic contribution to MS susceptibility, along with other classical HLA alleles. However, the complement system has been suspected to play a role in neuroinflammation due to the presence of complement activation products in MS lesions. The Complement 4 (C4) gene is located within the HLA region and has been associated with experimental autoimmune encephalomyelitis in C4-deficient animal models. However, analysis is difficult due to structural and genetic variation within the HLA region. C4 gene products consist of two isoforms (C4A and C4B) and two isoforms, long (C4L) and short (C4S), owing to a H-ERV insertion. We used our novel bioinformatics tool, C4Investigator, to interrogate the role of C4 copy number variation in MS. Analysis of C4 copy number, controlling for sex and HLA-DRB1*15:01, in affected individuals of European (428) and African (199) ancestry against ancestry-matched healthy controls (438 and 124 respectively) found higher C4L copy number (3+) in European
ancestry MS patients compared with healthy controls \((p = 6.26 \times 10^{-3}, \text{OR} = 1.51, 95\% \text{ CI 1.12-2.03})\). In comparison, affected individuals of African ancestry had lower C4L copy number (0-1) compared with healthy controls \((p = 1.18 \times 10^{-2}, \text{OR} = 0.48, 95\% \text{ CI 0.27-0.84})\). Overall, the median C4L copy number among healthy European individuals was 3, while among African individuals the median copy number was 2, suggesting that copy number differentials in one group may have opposing outcomes with respect to disease depending on ancestry. These findings suggest that the proportion of C4S/C4L may influence disease risk in multiple sclerosis, and that this variation modulates risk differently across ancestral groups.

O69 | HLA expression in HPV infected Cervical Carcinoma

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HLA-E is a unique, non-classical HLA class I molecule that modulates both innate and adaptive immunity via interaction with natural killer (NK) cells and T cell receptors. Tumor cells are reported to overexpress HLA-E. HLA-E interacts with CD94/NKG2A receptors on NK cells and prevents NK cell mediated lysis and contributes to the immune escape of tumor cells. The aim of the present study was to analyze the HLA-E protein expression in HPV infected cervical carcinoma. The tumor tissue was obtained from cervical squamous cell carcinoma patients. Clinically normal tissue was obtained from patients undergoing hysterectomy. Extracted DNA was subjected to Human Papillomavirus (HPV) genotyping. Western blotting was performed on the tissue lysates. The protein intensity data was acquired using the image software and normalized with internal control GAPDH. In addition, peripheral blood samples were collected as well and subjected to ELISA to determine the serum HLA-E levels. There were 32 cases of squamous cell carcinoma of cervix and 43 cases of clinically normal cervix for which tissue samples were available. The high-risk HPV was observed in all cases of carcinoma cervix cases enrolled for the study and the subtypes observed were HPV 16 alone in 31 (72\%) and rest 12 cases had multiple subtypes. HLA-E expression was significantly increased in cervical cancer tissue as compared with normal cervical tissue \((p = 0.000)\). The blood samples were available in 43 cases of carcinoma cervix and 55 cases of normal cervix. The soluble HLA-E levels were significantly increased in carcinoma cervix as compared with normal cervix group \((p = 0.038)\). The study provides support for exploring HLA-E based therapy which would plausibly function by inhibiting the immunoregulatory role of HLA-E.

O70 | HLA binding-groove motifs are associated with myocarditis induction after Pfizer-BioNTech BNT162b2 vaccination

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We found a higher incidence of myocarditis in young males who had received Pfizer-BioNTech BNT162b2 vaccinations as compared with historical controls and unvaccinated individuals. The analyses focused on risk following the first and second vaccine in adults and adolescents, as well as risk in adults following the third (booster) vaccine. Males, mainly aged 12-30 years, were found to be at higher risk. However, the question remains what causes lead one specific young male, but not another, to develop post-vaccination myocarditis. The HLA molecule is known to play an important role in infectious and auto-inflammatory diseases. We hypothesized that differences in HLA alleles could lead to either protection or susceptibility to vaccination-induced myocarditis. On this basis, HLA typing was performed using next-generation sequencing technology for the HLA-A, -B, -C, -DRB1, -DQB1 and -DPB1 loci, in 21 well-characterized patients who developed myocarditis after the second Pfizer BNT162b2 vaccination. The HLA genotypes were compared with high-resolution HLA data of 272 healthy controls from the Hadassah Bone Marrow registry samples, who are representative of HLA frequencies in the Israeli population. Our findings demonstrated that in HLA class II, DRB1*14:01 (19.04\% vs. 5.3\%,
Pcorr = 0.028, OR = 4.17), HLA-DQB1*05:03 (19.04% vs. 6.06%, Pcorr = 0.034, OR = 3.64) and DRB1*15:03 (7.14% vs. 0.0%, Pcorr = 0.003, OR = 41.76) were significantly associated with disease susceptibility. We further discovered susceptibility motifs in the HLA-DR peptide-binding grooves: His60 (Pcorr = 0.01, OR = 3.52) and Arg70 (Pcorr = 0.0047, OR = 3.43). Our findings suggest that immunogenetic fingerprints in HLA peptide-binding grooves may have changed the binding affinity of different peptides derived from the Pfizer-BioNTech BNT162b2 vaccination, and induced myocarditis.

O71 | HLA-Bw4 is Associated with Pediatric Acute-Onset Neuropsychiatric Syndrome (PANS)

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Pediatric Acute-Onset Neuropsychiatric Syndrome (PANS) is a relatively recently-described condition in which children exhibit sudden and inexplicable behavioral changes characteristic of obsessive-compulsive disorder (OCD) and is often accompanied by disordered eating or difficulty completing daily tasks. PANS has been associated with other autoimmune diseases and is observed most often in patients who have experienced a recent, prior infection or inflammation of the upper respiratory tract. The disease course of PANS has been defined as relapsing-remitting; most of the children impacted by PANS show improved symptoms over time and may relapse after an extended period. Given the apparent immunologic underpinnings in PANS pathology, we sought to elucidate the role of HLA in this little-understood disease. Using our custom next-generation sequencing methods, we genotyped 149 PANS cases with European ancestry for 6 HLA loci (HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1) and compared the results to 2026 previously genotyped ancestry-matched healthy controls. Analysis at the allele and amino acid-levels revealed a significant association of disease with the HLA-B locus, with the risk signal focused on positions 80-83. The amino acids at positions 80-83 comprise the Bw4 epitope, which mediates binding to the inhibitory receptor KIR3DL1 on the surface of natural killer (NK) cells. Of the four significant positions, HLA-B position 80-I (Isoleucine), which is known to increase binding affinity to KIR3DL1, displayed the strongest association (p = 2.63E-05, OR = 1.85, 95% CI = 1.36, 2.48). Our results provide strong evidence for a role of HLA-KIR interaction in PANS, suggesting the involvement of NK-mediated immunopathology in this enigmatic pediatric neurological disease.

O72 | Fight against COVID-19: functional and structural study of the T cell response

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T cells, and especially cytotoxic T cells are at the forefront of the fight against viral infection. The killer cells are able not only to distinguish between self and foreign peptides, but also to engage in the fight to clear the viral infection by eliminating the infected cells. Our lab is focused on understanding how T cells engage with viral peptide antigens, that are presented by highly polymorphic HLA molecules. T cells have receptors on their surface called T cell receptors (TCRs) that allow them to recognize the composite surface of the peptide-HLA complex. Using x-ray crystallography we can understand at the atomic level both peptide antigens presentation and TCR recognition, both important to determine the quality of the subsequent immune response. We can then link that structural information with our cellular assay that determines the strength and magnitude of the anti-viral response, providing the basis for peptide modification to reach stronger response or an understanding of viral mutation that led to viral escape. Our current work compared the T cell response, at the antigen level against 32 single epitope derived from spike, between COVID-19 recovered and vaccinated donors. We have shown that the booster shot (3rd dose) increases the antigen-specific T cell response, increases the level of T cell cross-reactivity against variant of SARS-CoV-2, but also alters the phenotype of the T cell. Those results are important to future guide vaccination advise and better understand the immune response to SARS-CoV-2 infection.
The immunological, structural and functional diversity of the TCR is skewed in cancer. TCRβ CDR3 regions represent primary sites of antigenic contact and contribute the most towards the T cell repertoire pool. The bcr-abl fusion peptides are a hallmark of CML and may affect the clonotypic abundance of TCRβ sequences in CML. High quality total RNA (RIN>8) was extracted from PBMCs of 35 CML patients, subjected to RNASeq NGS using SMARTer Human TCR αβ profiling kit 635014 from Takara Bio Inc. and Long read iR-profile kit H-Pro-10 from iRepertoire, Inc, USA. The libraries were sequenced with Illumina's MiSeq. Data was analyzed with MiXCR and iRweb. The top ten Vβ genes observed at frequencies greater than 5% in CML patients included the previously known CML associated Vβ2, Vβ9, Vβ7-2 genes and others such as Vβ10 and Vβ29. An immune convergence of shared Vβ clones 29-1 and 20-1 was also observed. The TRB J2-1 and J2-7 were observed at ~20% frequencies in patients. The top most TCR clonotypes were TCR Vβ10-2Dβ2-Jβ2-7, Vβ29-1Dβ2-Jβ2-1, Vβ29-1Dβ2-Jβ2-1 and Vβ19-1-Jβ1-2. N additions ranged from 2 to 19. This study has identified 23 common CDR3 sequences with an average length of 12 amino acids in CML. Interrogation of these sequences with TCRdb revealed that a few CDR3s including ASTRTSGSIYEQY, SVEDPGREEGEQF, SVEGLPSGRTY-NEQF may plausibly be pathogenic in CML and have not been reported among healthy volunteers. These sequences and clonotypes might be associated with CML and are a subject of future investigations including, but not limited to assessments of their HLA and antigen specificities. Together, the present study has identified multiple immunodominant T cell clones, along with their nucleotide and amino acid sequences in CML patients of Indian origin. The identified TCRs would be beneficial for understanding CML biology, diagnosis, and prognosis, and would be crucial for developing appropriate immunotherapies.
PBMC from healthy controls and MPN patients. Significantly strong T-cell responses were observed in controls with corresponding HLA class I alleles, while MPN patients showed weak or no response. In agreement with that, analysis of available datasets from Gene Expression Omnibus showed downregulation of HLA class I pathway key genes (HLA-A -B, TAPBP) in CD34+ cells from PV and ET. In conclusion our results provide further evidence on the role of HLA class I in immune editing of MPN driven by JAK2V617F and CALR exon 9 mutations. Furthermore, we identified immunogenic neo-epitopes that could be used for the development of cancer vaccines. Supported by grant KP-06-H41/2.

P3 | Humoral response against SARS-CoV-2 and other endemic corona viruses

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In a study of two Hospitals in Saxony (Chemnitz and Leipzig), we analyzed the antibody development towards SARS-CoV-2 and against a variety of endemic coronaviruses. Here we analyzed 760 sera from a Saxonian cohort for antibody reactivity against: common cold coronaviruses, HCoV-229E, HCoV-HKU 1, HCoV-NL63 and HCoV-OC43, MERS-CoV and SARS-CoV. For the SARS CoV-2 immune response we tested the following antigens: Spike, S1, S2, RBD and nucleocapsid. These 11 antigen determinants were tested in a commercial multiplex Luminex based assay. We tested sera from 544 individuals (347 females and 197 males; 498 SARS-CoV-2 PCR positive and 262 SARS-CoV-2 PCR negative) between May 2020 and March 2022. We observed up to 10% reactivity against the MERS virus in both the PCR positive and negative group. Against the common cold corona viruses 80%-90 % of the individuals in both groups show detectable antibodies. These 11 antigen determinants were tested in a commercial multiplex Luminex based assay. We tested sera from 544 individuals (347 females and 197 males; 498 SARS-CoV-2 PCR positive and 262 SARS-CoV-2 PCR negative) between May 2020 and March 2022. We observed up to 10% reactivity against the MERS virus in both the PCR positive and negative group. Against the common cold corona viruses 80%-90 % of the individuals in both groups show detectable antibodies. Regarding the antibody response against SARS-CoV a significant difference was observe. Only 19% of COVID-19 infected individuals show antibodies against the virus, while 81% of the PCR-positive individuals produced antibodies. The presence of antibodies against the SARS-CoV-2 is positively correlated with those against SARS-CoV (p = 0.001). No changes in endemic antibody responses were see in the two groups. The antibody status after first immunization event (infection/vaccination) shows differences in nucleocapsid-directed antibody production, found in the natural infection group (about 60%). In the vaccination group, more individuals (up to 95%) show an immune response against Spike, S1 and RBD compare with natural infection. In summary, the examined cohort shows a general immunization up to 90% against most endemic corona viruses. Correlation analyses show cross-reactivity between SARS-CoV-2 and SARS-CoV. Longitudinal antibody analyses are under way, as also correlations of humoral response with immunogenetic factors.

P4 | Diverse data in multiple sclerosis improves machine learning performance to predict the short-term evolution of disability: lessons from the EPIC cohort

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Multiple sclerosis (MS) is a typical multifaceted neurological disease requiring the integration of several modalities of data (clinical, imaging, biological, genetics) for the diagnosis and management. Machine learning could significantly support tackling the characteristic unpredictability of MS. We analyzed a prospective longitudinal cohort of 589 patients with MS from the EPIC study at the University of California San Francisco (UCSF), totaling 3,456 yearly clinical visits. We applied a supervised classification machine learning framework, considering each yearly clinical visit as a datapoint. Features were the clinical, imaging, genetic and quality of life (QoL) data at the index visit. The label was the short-term disability evolution at the next visit. The best predictive performance was achieved by the SVM classifier with all longitudinal features included in the model (AUROC: 0.782 ± 0.003). The addition of QoL and timed functional metrics to the usual minimal feature set of MS research significantly improved the predictive performances (AUROC increase of 0.04 and 0.05, respectively). This was not the case of the addition of brain MRI variables, which is the most important exam to monitor MS currently. Therefore, machine learning offers significant
short-term predictive performances in the follow-up of MS. It highlights the predictive value of QoL and timed functional data in comparison to the variables currently monitored in practice. The impact of variable selection on the predictive performance shows that the data selected as input is more important than the classifier.

P5 | **PRIMUS-Alpha: A clinical decision support system prototype for precision medicine in multiple sclerosis contextualizing patients’ evolutions in multi-source reference data**

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The clinical evolutions and therapeutic responses of patients with multiple sclerosis (MS) are heterogeneous. Digital tools may ease precision medicine approaches by helping physicians evaluate patients in the context of population subgroups. Here, we present PRIMUS-Alpha, a functional prototype of a clinical decision support system for MS management. It contextualizes patients in the individual data of pivotal randomized clinical trials and high quality prospective cohorts. We processed the individual data from 6 sources to build a harmonized reference database: 5 randomized clinical trials and the high quality cohort of the French MS registry OFSEP-HD. The resulting reference database contained 5064 patients and 41 627 patient-years of follow-up. Therapeutic scenarios involving 22 approved disease modifying treatments may be contextualized, including treatment switches and induction strategies. During the clinical visit, the neurologist may describe his patient with up to 9 predictive variables and compare several therapeutic scenarios in his context. A filter-based contextualization algorithm identifies the subgroup matching the patient’s characteristics and describes visually a projection of his evolution up to a 2-year horizon. The projection describes the rate of relapses, the rate of new T2 MRI lesions, the disability worsening and eventual changes of therapeutic strategies. Therefore, PRIMUS-Alpha describes a personalized subgroup by accessing multi-source and harmonized reference data in real-time during the clinical visit. Its modular architecture enables the addition of further sources in the future to diversify the documented profiles and therapeutic scenarios. The deployment of the database in a synthetic and distributed form will guarantee the confidentiality of patients and the usage control by each organization sharing data.

P6 | **HNA antibody association to HLA alleles and autoimmune neutropenia**

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Autoimmune neutropenia (AIN) in early childhood is caused by antibodies against epitopes on the immunoglobulin G (IgG) Fc receptor type 3b (FcγIIIb). Among Danish patients the most common epitope is Human Neutrophil Antigen 1a (HNA-1a). HLA class II molecules are known to be strongly associated with the risk of autoimmune disease. The association is often disease-specific and may represent the superiority of these HLA proteins in binding antigens with autoantigenic potential. Our aim was to establish the association between HLA class II and antibody specificity in Danish autoimmune neutropenia patients. We included Danish AIN cases and healthy and unrelated Danish blood donor as controls. HLA-DRB1 and HLA-DQB1 were determined by either next-generation sequencing or by real-time polymerase chain reaction using a commercially available HLA-DRB1 and -DQB1 tissue typing method. Presence of anti-neutrophil antibodies was determined by flow cytometric indirect granulocyte immunofluorescence test (Flow-GIFT). Our results showed that three HLA alleles were associated with AIN only for patients with anti-HNA-1a specific antibodies: DRB1*01 (OR = 2.63 (1.65-4.21)), DRB1*04 (OR = 0.29 (0.15-0.57)) and DQB1*03 (OR = 8.74 (3.29-23.23)) was specific for the remaining group of anti-FcγIIIb antibody positive patients. DRB1*13, DRB1*14, DRB1*16 and DQB1*05 were significantly associated with
P7  |  Association between T regulatory cell genes and autoimmune neutropenia

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Autoimmune neutropenia (AIN) in early childhood is caused by antibodies against epitopes on the immunoglobulin G (IgG) Fc receptor type 3b (FcγIIIb). Regulatory T cells (Tregs) are a specialized subpopulation of T cells that act to suppress immune response, thereby maintaining homeostasis and self-tolerance. Dysregulation in Treg cell frequency or functions, as a result of genetic polymorphisms, may lead to the development of autoimmune disease. The aim of this study was to establish the association between single nucleotide polymorphisms (SNPs) in Treg genes and AIN in Danish primary autoimmune neutropenia in early childhood patients. We included Danish AIN cases and healthy and unrelated Danish blood donors. 16 SNPs from 9 Treg genes: ADCY9 (rs1967309), CTLA4 (rs231775), IFIH1 (rs1990760), FOXP3 (rs3761547, rs2232365, rs3761548), IL-2 (rs2069762, rs2069763, rs2069772), IL-2RA (rs7093069), IL-10 (rs1800872, rs1800871, rs1800896, rs1800890), PTPN22 (rs2476601) and TGF-β1 (rs1800469) were genotyped by real-time polymerase chain reaction using TaqMan SNP Genotyping Assays (Applied Biosystems, US). Typing of 84 patients and 591 healthy female controls. Multivariate generalized linear models with age as a covariate were fitted for estimation of the association of HLA alleles and haplotypes. To access the global effect of HLA class I and class II genotypes on the presence of BRCA1 mutation we calculated Grantham distance as a measure of HLA allele's divergence. We did not find any difference between HED per locus as well as the mean HED in patient and control groups. One HLA class I allele: HLA-C*15:06 (OR = 6.221; p = 0.038) and HLA class I haplotype: HLA-A*24:02~B*51:01~C*15:06 (HS = 2.279; p = 0.022) showed positive association with the presence of BRCA1 mutation. DQB1*06:03 (p = 0.021) was found inversely correlated with the presence of BRCA1 mutation. Additionally, 4 HLA class II alleles were found significantly increased in patients; DQA1*01:01 (OR = 8.33; p = 0.013); DQB1*05:01 (OR = 2.73; p = 0.02) and HLA-DQB1*05:01-DRB1*01:02

P7  |  Association between T regulatory cell genes and autoimmune neutropenia

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Germline mutations in the BRCA1 gene are associated with breast (BC) and ovarian (OC) cancer. Although it has been shown that HLA could restrict development of some malignancies, its role in BRCA1 driven cancer remains to be established. Therefore, we aim to elucidate the putative role of HLA for the development of BC and OC driven by one of the most frequent frame shift mutations BRCA1 c.5263_5264insC. We performed NGS typing with Holotype HLA kit (Omixon) of 84 patients (34 BC and 50 OC) with BRCA1 c.5263_5264insC and 591 healthy female controls. Multivariate generalized linear models with age as a covariate were fitted for estimation of the association of HLA alleles and haplotypes. To access the global effect of HLA class I and class II genotypes on the presence of BRCA1 mutation we calculated Grantham distance as a measure of HLA allele's divergence. We did not find any difference between HED per locus as well as the mean HED in patient and control groups. One HLA class I allele: HLA-C*15:06 (OR = 6.221; p = 0.038) and HLA class I haplotype: HLA-A*24:02~B*51:01~C*15:06 (HS = 2.279; p = 0.022) showed positive association with the presence of BRCA1 mutation. DQB1*06:03 (p = 0.021) was found inversely correlated with the presence of BRCA1 mutation. Additionally, 4 HLA class II alleles were found significantly increased in patients; DQA1*01:01 (OR = 8.33; p = 0.013); DQB1*05:01 (OR = 2.73; p = 0.02) and HLA-DQB1*05:01-DRB1*01:02
P9 | HLA allele association studies with the kinetics of SARS-CoV-2 spike protein-specific IgG antibody responses to BNT162b2 mRNA vaccine

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BNT162b2, an mRNA-based SARS-CoV-2 vaccine (Pfizer-BioNTech), is one of the most effective COVID-19 vaccines and has been approved by more than 130 countries worldwide. However, several studies have reported that the COVID-19 vaccine shows high interpersonal variability in terms of humoral and cellular responses, such as those with respect to SARS-CoV-2 spike protein immunoglobulin (IgG), IgA, IgM, neutralizing antibodies, and CD4+ & CD8+ T cells. The objective of this study is to investigate the kinetic changes in anti-SARS-CoV-2 spike IgG (IgG-S) profiles and adverse reactions and their associations with HLA profiles among 100 hospital workers from the Center Hospital of the National Center for Global Health and Medicine (NCGM), Tokyo, Japan. DQA1*03:03:01 (p = 0.017; OR 2.80, 95% CI 1.05–7.25) was significantly associated with higher IgG-S production after two doses of BNT162b2 while DQB1*06:01:01 (p = 0.028, OR 0.27, 95% CI 0.05–0.94) was significantly associated with IgG-S declines after two doses of BNT162b2. No HLA alleles were significantly associated with either local symptoms or fever. However, C*12:02:02 (p = 0.058; OR 0.42, 95% CI 0.15–1.16), B*52:01:01 (p = 0.031; OR 0.38, 95% CI 0.14–1.03), DQA1*03:02:01 (p = 0.028; OR 0.39, 95% CI 0.15–1.00) and DPB1*02:01:02 (p = 0.024; OR 0.45, 95% CI 0.21–0.97) appeared significantly associated with protection against systemic symptoms after two doses of BNT162b2 vaccination. Further studies with larger sample sizes are clearly warranted to determine HLA allele associations with the production and long-term sustainability of IgG-S after COVID-19 vaccination.

P10 | Single Nucleotide Variation, associated HLA and Cytokines as possible biomarkers in the diagnosis of Psoriasis patients in Tshwane, South Africa

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Psoriasis is associated with molecular biomarkers such as HLA-C*06:02 and associated single nucleotide variants (SNVs). Furthermore, the circulatory cytokines, interleukins (IL)-17 and IL-23 are elevated in psoriasis patients. The current study has investigated the three biomarkers in a cohort of 40 psoriasis patients attending a dermatology clinic situated in a tertiary academic hospital with mainly African patients. The aim is to establish the incidence of HLA-C*06:02 and the single nucleotide variants (SNVs) associated with psoriasis, as well as the levels of IL-17 and IL-23 in this cohort of patients. The incidence of these markers in these individuals was compared with 40 healthy volunteers (controls) to elucidate the significance of the current data. HLA-C*06:02 was significantly elevated in our patient cohort with 53% (n = 21) of psoriasis patients found to express the HLA-C*06:02 allele.
compared with 15% (n = 6) of the healthy controls (p = 0.001). Circulating levels of both IL-17 and IL-23 were significantly increased in the psoriasis patients compared with the healthy controls (p = 0.0001 and p = 0.0005, respectively). The SNV, rs2248374, was shown to be associated with both IL-17 and HLA-C*06:02 in patients with psoriasis. Overall these novel findings are the first to be published for South African psoriasis patients and, indeed, African populations, in the public health sector. The finding of the current study corroborates international studies. Further validation through geographic and population expansion may assist in identifying individuals at increased risk of disease progression in psoriasis. These biomarkers may also be used as potential prognosticators, which will offer the opportunity for early medical intervention to reduce the burden of disease.

P11  |  Exploring genetic predisposition towards manifestation of Oral Submucous Fibrosis among tobacco consuming North Indians

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Oral Sub-Mucous Fibrosis (OSMF) is a pre-malignant condition that alters the fibro elasticity of oral submucosa and is predominantly confined to the South East Asian region. Variability in clinical course is intriguing, a fraction of betel nut users develop OSMF (0.1%–11%), while a majority show no signs and symptoms despite prolonged use. However, some severe forms are associated with very short history of tobacco use. A clear molecular mechanism involved in its pathogenesis still remains unexplored and this inconsistency in disease association can be indicative of genetic association, but specific role of any genes in defining its susceptibility remains largely unidentified. Investigating genetic predisposition to OSMF can potentially help characterize inter-individual variations in immune responses and identify susceptibility/protective HLA alleles for OSMF. The present study was undertaken to investigate HLA associations with OSMF. The demographic details, type, frequency, and history of tobacco habit was recorded. NGS based HLA class II genotyping was performed and data was analyzed using NGSengine (Gen Dx, The Netherlands). Mean age of the cohort was 42.2 yrs with almost equal number of OSMF patients in mild (36.7%), moderate (36.7%) and severe categories (26.7%). HLA-DRB1, -DQA1 and -DQB1 genotyping was performed on 96 patients (58 with OSMF and 28 without OSMF). Interim results show deviations in the frequency of HLA class II alleles in patients with OSMF when compared with those without OSMF. Compiled observation will be presented on completion of genotyping of remaining subjects. Identification of disease predisposing HLA alleles might be helpful in identifying subjects at initial disease stage. During treatment, close follow up of this cohort might help towards better clinical management.

P12  |  Analysis of HLA surface expression and the tumor microenvironment in Hodgkin lymphoma as a potential tumor immune escape mechanism in Egyptian patients

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While Hodgkin Lymphoma is one of the most treatable cancers, a significant proportion of patients revert after successful initial therapy or are predominantly resistant. This failure outcome could be related to the tumor escaping immunosurveillance. The aim of this study was to investigate the surface expression of HLA class I in primary Hodgkin lymphoma and if it can influence immune cell infiltration, especially cytotoxic T lymphocytes. Moreover, to study the cancerous tissue potential escape mechanism by analyzing the tumor microenvironment. Tissue biopsies were obtained from 27 Egyptian patients and were classified according to histological findings and different analysis techniques were used to test our hypothesis. HLA class I surface expression was examined using histochemical analysis at different tumor stages, where we were able to confirm different surface alterations in primary tumors and in the metastatic ones as well, these surface expression alterations would increase with the increase of tumor’s pathological state. HLA typing was performed to compare HLA class I loci in the affected tissue and blood of the patient, where we were able to see a case of loss of heterozygosity in the HLA-A genes. SNP analysis was further performed to test our
P13 | HLA alleles and SNPs association study with HBV-related liver cirrhosis and hepatocellular carcinoma in a Greek population

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HBV related hepatocarcinogenesis and decompensated liver cirrhosis worsen the survival of HBV infected individuals. Various immunogenetic factors have been associated with these complications. HLA polymorphism and other SNPs across the human genome have been associated with susceptibility to different HBV infection outcomes based on GWAS and their replication studies mainly at East Asians or Sub-Saharan endemic populations. The aim of our study was to extend our previous research on the association of HLA-A, -B, -C, -DRB1, -DQA1, -DQB1 and -DPB1 alleles with HBV-HCC/cirrhosis in North Greek HBV patients and additionally examine the possible association of two SNPs (rs9272105 and rs1110446). The HLA alleles of 161 HBV infected individuals (127 had a spontaneous clearance of HBsAg and 34 had complicated chronic HBV with cirrhosis/HCC) were genotyped by PCR SSP and their frequencies were compared with the HLA frequencies from the North Greece Bone Marrow Donor Registry (14506 samples – control group). SNPs were genotyped by TaqMan SNP Assays (Applied Biosystems – TM). Statistical analysis was performed by IBM SPSS Statistics for Windows v.25 and SNP association analysis by SNPStats. Our results showed an association of HLA-B*37 allele with HBV-cirrhosis/HCC when compared with the group of patients that spontaneously cleared HBSAg (6.3% vs. 1.0% p = 0.022). HLA-A*01 and DQA1*03 were found in a significantly higher frequency in the group of HBV-cirrhosis/HCC patients when compared with the control group (22.9% vs. 9.7% p = 0.002 and 14.3% vs. 3.8% p = 0.015 respectively). HLA DRB1*01, DQB1*03:02 (DQ8), DQA1*05 were significantly associated the HBV clearance when compared with the control group (11.7% vs. 6.7% p = 0.005, 8.9% vs. 3.8% p = 0.029 and 46.0% vs. 35.0% p = 0.031 respectively). HBV-related HCC/cirrhosis were associated with rs1110446 SNP located in the HLA region OR = 4, p = 0.029, 95% CI).

P14 | HLA variants associated with sarcoidosis and their tag single nucleotide polymorphisms in Czechs

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In sarcoidosis, an immune-mediated disease, genetic factors affecting immuno-inflammatory reactions have an essential role in disease onset and development. We aimed to detect HLA variants associated with sarcoidosis focusing on the link with relevant tag SNPs. 301 Czech, unrelated sarcoidosis patients diagnosed at the University Hospital Brno and 309 unrelated control subjects were genotyped for HLA using NGS. SNPs were determined by
the MassARRAY® method and compared with 203 unrelated controls. Associations were assessed by two-tailed Fisher's exact test with Bonferroni correction. Protective variants HLA-DRB1*01:01 (pcorr = 0.04) and rs3830135*A (pcorr = 4.63 × 10^-8) were identified. The presence of DRB1*01:01 and tag rs3830135*A coincided in 100% of cases. HLA-DRB1*15:01 was a risk factor (p = 0.01) as well as tag rs3135388*T (p = 0.05). The presence of HLA-DRB1*01:01 and tag rs3135388*T coincided in 99% of cases. HLA-DRB1*03:01 associated with better prognosis of sarcoidosis (CXR stage 1, remission, Löfgren syndrome (LS), no treatment) as well as with rs2040410*T (CXR stage 1 pcorr = 1.13 × 10^-6, remission pcorr = 1.93 × 10^-4, LS pcorr = 1.01 × 10^-11, no treatment pcorr = 4.80 × 10^-6). The presence of HLA-DRB1*03:01 and tag rs2040410*T coincided in 93%. HLA-DQA1*05:01 and -DQB1*02:01 associated with better prognosis as well as with tag rs2187668 (CXR stage 1 pcorr = 9.41 × 10^-4, remission pcorr = 0.01, LS pcorr = 4.19 × 10^-5, no treatment pcorr = 0.02). The presence of rs2187668*T coincided with HLA-DQA1*05:01 in 90%, and with DQB1*02:01 in 91%. In this study, we refined the rs3830135*A link with HLA-DRB1 to HLA-DRB1*01:01. Further, association of HLA-DQA1*05:01 and DQB1*02:01 with rs2187668*T, known to be linked with celiac disease, was observed. Previous reports associating HLA-DRB1*15:01 with rs3135388, and DRB1*03:01 with rs2040410 were confirmed. Defining links between HLA variants and corresponding tag SNPs may be applied in future sarcoidosis management. AZV: NV18-05-00134, CZ.02.1.01/0.0/0.0/16_019/0000868(ENOCH).

P15 | Immunogenetics and SARS-CoV-2 infection

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Since the beginning of the SARS-CoV-2 pandemic, in 2020, numerous data with respect to the influence of immunogenetics to the predisposition and infection severity have been reported worldwide. It is well accepted that immunogenetics plays a pivotal role in infection and vaccination, as well as vaccination failures and/or breakthrough. Factors of the major histocompatibility complex and the common ABO blood group system have been so far discussed. Here, we describe the association of HLA-A, -B, -C, -DRB1, -DRB345, -DQA1, -DQB1, -DP21, -DP61, and HLA-E, -F, -G, -H on the results of molecular detection of COVID-19 or in some cases on antibody detection upon first testing. Furthermore, we defined molecularly 22 blood group systems comprising 26 genes and 5 platelet antigen genes. We observed 37% COVID-19 PCR negative individuals and 63% positive. Within the negative subjects HLA-B*57:01, HLA-B*55:01, DRB1*13:01, DRB1*01:01, were enriched, and in the positive group the homoyzosity for DQA1/DQB1, DRB1*09:01 and DRB1*15:01. For HLA-DQA1 we observe an enrichment for DQA1*05:01, DQA1*02:01 and DQA1*01:03. For HLA-DQB1 we found HLA-DQB1*06:02 was enriched in the positive group while HLA-DQB1*05:01 and HLA-DQB1*06:03 in the negative group. We observed a significant enrichment of homozygosity for DQA1/DQB1 in the positive group. The homozygous platelet antigen HPA-1a was significantly enriched in the negative group, contrasting the result of HPA-1ab that was enriched in the COVID-19 infected group. Despite limitations of our study, the data presented here show clearly that COVID-19 infection and all the consequences of that are multifactorial and multigenetic. The virus is in a continuous mutation/selection process leading to escape possibilities. Therefore, associations are a momentum in science.

P16 | Role of HLA polymorphism in COVID-19 progression in the Bulgarian population

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The SARS-CoV-2 outbreak has impacted different socioeconomic aspects of our societies and represents a global...
The different rates of infection are heavily influenced by host genetic factors such as the variability in the HLA region. The aim of our study was to evaluate the role of specific HLA alleles in the Bulgarian population that contribute to COVID-19 progression. 76 Bulgarian patients (median age 59.4; range 25-84) with COVID-19, separated into 3 groups based on the severity of the infection, were included in the study. All patients were typed at allele level for HLA-A,-B,-C,-DRB1,-DQB1, and -DPB1. Data from a representative Bulgarian population control group of young individuals (n = 540; median age 28.5; range 18-38) with unknown infectious status was used for comparison. Among the patient group, HLA-A*23:01 (OR = 3.16; p = 0.016), and DQB1*05:03 (OR = 3.1; p = 0.0009) showed positive association with moderate course of COVID-19, whereas DRB1*07:01 (OR = 2.54; p = 0.018) was associated with more severe disease. We found that A*01:01 (OR = 1.82; p = 0.018), B*35:03 (OR = 2.22; p = 0.011), B*40:06 (OR = 23.59; p = 0.0001), and DRB1*14:01 (OR = 3.37; p = 0.015) strongly correlate with the disease progression and could be considered as high-risk alleles. Furthermore, A*02:01 (OR = 0.4; p = 0.02), and DQB1*03:01 (OR = 0.47; p = 0.025) were predominantly found in asymptomatic patients and controls and probably exert a protective effect. Population-based and intra-individual variability of the HLA complex could partially explain the different courses of COVID-19. Despite some limitations, our preliminary data shows that some HLA alleles may be associated with a more severe course of the SARS-Cov infection, while others could possibly be considered protective. This work was supported by grant KP06DK1/13, 2021, Ministry of Education and Science, Bulgaria.

**P17 | Association of TLR10 single nucleotide polymorphisms with hidradenitis suppurativa in a Caucasian Spanish population cohort**

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Hidradenitis suppurativa is defined as a chronic inflammatory condition affecting the pilosebaceous-apocrine unit, causing painful nodules and abscesses in different areas of the body. The pathogenesis of HS is still unknown, although increasing evidence suggests that the immune system plays an important role. To study the role of innate immunity, we analyzed several Toll-Like Receptor (TLR) functional single nucleotide polymorphisms (SNPs). Our objective was to analyze the role of several TLR functional SNPs in HS patients and healthy controls in a Caucasian population from Cantabria. Through a case-control study, we analyzed the allele and genotype distribution of the SNPs in 106 patients with HS and 278 age and sex-matched healthy control subjects for the following SNPs (TLR1 rs5743611 and rs4833095, TLR2 rs5743704 and rs5743708, TLR6 rs5743810, and TLR10 rs11096955, rs11096957 and rs4129009, by Real-Time PCR using a TaqMan assay. Obtaining statistical significant result for TLR10 SNPs. Subjects with the heterozygous genotype variant (T/G) for both rs11096955 and rs11096957 had an increased risk of developing HS (OR 1.741, 95% CI 1.106-2.742, p = 0.02 and, OR 1.734, 95% CI 1.103-2.725, p = 0.02 respectively). Likewise, for the heterozygous genotype (T/C) for rs4129009 (OR 1.644, 95% CI 1.049-2.578, p = 0.03). On the contrary, subjects with the homozygous genotype variant T/T for rs11096955 and rs11096957 were found to be protective for HS (OR 0.463, 95% CI 0.265-0.809, p = 0.008 and OR 0.296, 95% CI 0.296 0.878, p = 0.01 respectively). This pilot study could serve as an outline for future research that may help more accurately describe the pathophysiology underlying this disease and allow researchers to develop treatment strategies and clinicians to evaluate the risk of appearances in this pathology. Further studies are needed to understand well the implication of these SNPs in the pathogenesis of the disease.

**P18 | Haplotype frequencies and linkage disequilibrium between HLA-DRB1 and SNP -197 of IL-17 in Russian patients with rheumatoid arthritis living in Chelyabinsk region**

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Rheumatoid arthritis (RA) is a multifactorial chronic autoimmune disease with cytokine imbalance. The pro-
inflammatory cytokines TNF-α, IL-1B and IL-6 are the key cytokines which drive inflammation and the destructive process. Other cytokines such as IL-23, IL-17A also play crucial roles in the pathogenesis of RA. A lot of studies indicated that HLA-DRB1*04 is the main gene for susceptibility to RA. In addition to the HLA-DRB1 alleles, the role of non-HLA genes has been identified. The IL17 gene is located on chromosome 6. That is why the formation of HLA-DRB1~IL17 haplotypes is possible. The aim is to investigate linkage disequilibrium and HLA-DRB1~IL17 SNP -197 haplotype frequencies in patients with RA and healthy controls of a Russian population. DNA typing of HLA-DRB1 genes was performed in 90 RA patients and 116 healthy individuals (HC) of a Russian population by PCR-SSP using sets of Protrans (Protrans, Germany). SNP -197 G/A IL17 was detected by allele-specific PCR (“Lytech” Co. Ltd., Russia). The haplotype frequencies (HF) and linkage disequilibrium (D’) were analyzed using Arlequin 3.5. Association study p values were calculated by the exact two-tailed Fisher’s criterion, the criterion OR with 95% CI was established. In RA group the mutant allele -137*A IL17 was linked with HLA-DRB1*07 (D’ = 0.52; p = 0.007), DRB1*15 (D’ = 0.84; p << 0.001), DRB1*16 (D’ = 1.0; p = 0.02), DRB1*13 (D’ = 0.38; p = 0.08), DRB1*12 (D’ = 1.0; p = 0.1). Wild type allele -197*G IL17 was linked with HLA-DRB1*04 (D’ = 0.44; p << 0.001), DRB1*01 (D’ = 0.62; p = 0.001). The frequencies of HLA-DRB1*04- IL17-197*G (25% vs. 9%; p << 0.001; OR = 3.4 95% CI 1.9 -5.9); HLA-DRB1*01-IL17-197*G (14.4% vs. 9%; p = 0.09) HLA-DRB1*07-IL17-197*A (7.2% vs. 3.0%; p = 0.049; OR = 2.5 95% CI 0.98 - 6.4) were high in RA group. According to our data, the formation of haplotypes between SNP-197G/A IL17 and HLA-DRB1 is possible, some of them could be risk factors for RA.

The aim of the study is to determine the HLA-B allele frequencies in HIV-1 infected patients. HLA-B typing of 763 patients with confirmed HIV-1 diagnosis and 828 healthy unrelated individuals were evaluated retrospectively. Low-resolution HLA-B typing was performed using sequence specific oligonucleotide (SSO) polymerase chain reaction (PCR). The frequency of HLA-B allele distribution in the control and patient groups were calculated and presented as percentages with 95% confidence intervals calculated according to the Wilson method. Allele frequencies in HIV and control groups were compared with the Pearson’s Chi-square test or Fisher’s exact probability test. Benjamini-Hochberg correction was applied to p-values and a p-value less than 0.05 was considered significant. A total of 30 distinct alleles were identified in both groups. There was no difference between the two groups in terms of allelic diversity. The two most common alleles in the control and patient groups were HLA-B*35 (17.1% vs. 19.0%, respectively) and B*51 (12.8% vs. 15.3%, respectively). The alleles with a frequency of ≥5% in the control and patient groups were HLA-B*18 (6.8% vs. 6.4%, respectively) and B*44 (8.7% vs. 6.2%, respectively). Alleles with frequencies less than 1% in both groups were HLA-B*73, B*56, B*54, B*53, B*48, B*47, B*46, B*45 and B*42. The frequency of HLA B*57 in the control and patient groups was 1.6% vs. 1.8% respectively. The remaining alleles with frequencies between 5% and 1% were B*07, B*08, B*38, B*40, B*49, B*52, B*55, B*13, B*15, B*50, B*41, B*27, B*58, B*14 and B*39 in decreasing order. There was no statistically significant difference in terms of allele frequencies between the two groups. Although no disease-susceptible or protective alleles could be identified, comprehensive studies including clinical and demographic parameters are needed to investigate the effect of HLA-B on the course of HIV infection in the Turkish population.
been found. It is of interest to understand the ways in which genetic factors are realized in RA. The TNFRSF11B gene is of the greatest interest, since its protein products are involved in the maturation of osteoblasts. Currently, the study of polymorphisms in this gene in RA patients is still relevant. DNA typing of 91 RA patients and 154 healthy individuals was performed by PCR-amplification using a set of reagents for AS-PCR detection of G1181C polymorphism in the OPG gene ("Lytech" Co. Ltd., Russia). The detection was made by electrophoresis with UV-visualization of results by 3% agarose gel. Association study p values were calculated by the exact two-tailed Fisher’s criterion, the criterion OR with 95% CI was established. In the RA group, the heterozygous genotype 1181 G/C frequency was reduced compared with the healthy Russians of the Chelyabinsk region (41.7% vs. 56%, p = 0.034). The odds ratio indicates that this genotype could act as a protector of the development of RA (OR = 0.567, 95% CI [0.336; 0.958]).

According to the research data, RA is more common in women, and therefore it is relevant to search for additional risk factors separately in the RA group of women. As a result, we found out that the mutant genotype 1181 C/C could be considered as a biomarker of predisposition to the disease in women (31.0% vs. 14.9%, p = 0.02, OR = 2.63; 95% CI [1.149; 6.021]). Our research contributes to the development of ideas about the immunogenetic component of rheumatoid arthritis. In the future, scientific work on this topic will be continued in order to form a gene network of SNPs interactions in RA.

The Frequency of HLA-A, -B, -C, -DRB1 and -DQB1 alleles in Patients with Acute Lymphoblastic Leukemia and Acute Myeloid Leukemia

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The HLA system comprises the most polymorphic genes of the human genome. Several studies have shown association of ALL and AML with several HLA alleles. We aimed to observe HLA associations with AML and ALL. HLA-A, -B, -C, -DRB1, and -DQB1 were typed in 81 patients with ALL and 129 patients with AML. HLA typing using SBT/NGS was implemented. We found an increased frequency of HLA-A*02 (A*02:01, 82.93%), HLA-A*03 (A*03:01, 75.7%), HLA-A*24 (A*24:02, 89.80%), HLA-B*35 (B*35:03, 46.51%), HLA-B*18 (B*18:01, 94.12%), HLA-B*44 (B*44:02 53.85%), HLA-C*07 (C*07:01 65.31%), HLA-C*04 (C*04:01, 100%), HLA-C*12 (C*12:03, 64.00%), HLA-DRB1*11 (DRB1*11:01 50.00%, -DRB1*11:04, 47.83%), HLA-DRB1*04 (DRB1*04:01, DRB1*04:02, DRB1*04:03 23.53%), HLA-DRB1*15 (DRB1*15:01 56.00%), HLA-DQB1*03 (DQB1*03:01 70.51%), HLA-DQB1*06 (DQB1*06:02 34.21%), HLA-DQB1*05 (DQB1*05:02, 35.42%), in ALL patients. HLA-A*02 (A*02:01, 86.36%), HLA-A*03 (A*03:01, 70.00%), HLA-A*24 (A*24:02, 100%), HLA-B*35 (B*35:01, 39.29%), HLA-B*18 (B*18:01, 100%), HLA-B*44 (B*44:02, 58.33%), HLA-C*07 (C*07:01, 48.39%), HLA-C*04 (C*04:01, 100%), HLA-C*12 (C*12:03, 65.38%), HLA-DRB1*11 (DRB1*11:01 58.33%, (DRB1*11:01 36.11%), HLA-DRB1*04 (DRB1*04:03, 42.86%), HLA-DRB1*15 (DRB1*15:01 50.00%), HLA-DQB1*03 (DQB1*03:01 57.53%), HLA-DQB1*06 (DQB1*06:01 34.21%), HLA-DQB1*05 (DQB1*05:01 45.95%), in AML patients.

These results might suggest effects of the HLA alleles in the genetic susceptibility to develop ALL or AML.

Bioinformatics, Data Analysis in Immunogenetics

P22 | HLA-A*03:01 is associated with systemic side effects in COVID-19 vaccination

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In the last two years, billions of individuals worldwide have been safely vaccinated against SARS-CoV-2, the virus that causes COVID-19. However, a substantial number of people experience mild to moderate side effects, which may hamper vaccine and booster uptake; understanding the processes underlying differential responses to these vaccines can help to improve global vaccination efforts. Variation in HLA has been linked to disease outcome in COVID-19, and HLA-A*03:01 has previously been reported to increase risk for side effects following vaccination. Here, we expand on those findings, examining HLA...
variation for association with vaccine side effects in 6470 patients of European ancestry from the United States. In our cohort, ~30% of individuals experienced systemic side effects (e.g., fever, chills, headache) after their initial vaccination series, while that proportion climbed to >60% in individuals receiving booster doses. We confirm the association of HLA-A*03:01 with systemic side effects to COVID-19 vaccines, particularly the Pfizer-BioNTech vaccine (OR = 1.52 [95% CI 1.23-1.97], p = 0.002). We observed similar effect size of this allele in individuals reporting side effects from the initial series or boosters (OR = 1.25 [95% CI 1.15-1.53]; p<0.0001), but comparatively higher effect size in individuals who subsequently experienced breakthrough infections (OR = 2.11 [95% CI 1.12-4.31]; p = 0.04). Our results confirm prior reports regarding HLA association with vaccine side effects, and suggest that the immunopathology underlying the HLA-A*03:01 association with side effects may increase those individuals' propensity for breakthrough infections after vaccination. Our results highlight the need to explore the functional mechanisms underlying this association to improve vaccine design and implementation strategies against emergent SARS-CoV-2 variants.

P24  |  Statistical inference of immunogenetic parameters reveals an HLA allele associated with pediatric proteinuria

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Massive proteinuria is a major symptom of pediatric nephrotic syndrome, which is often associated with a decreased kidney glomerular filtration rate. African Americans (AA) exhibit an increased risk for developing chronic kidney disease (CKD) and predisposing genetic factors have previously been described. Here, we conducted the first genomic study of AA pediatric CKD to identify its underlying genetic determinants. DNA from 140 AA kids with CKD from the CKiD cohort was genotyped on Illumina Exome chips. After quality control and SNP imputation, 630,883 SNPs (minor allele frequency >5%) were tested for association with baseline uPrCR (urine protein/creatinine ratio in g/g) using multivariate regression models. Our analysis revealed 51 variants from 11 loci significantly associated with proteinuria (p<5x10^-8). We notably highlighted genetic variants within the 12q13.11 (p = 1.8 x 10^-9) and the 17q12 loci (p = 4.4 x 10^-8), which have previously been associated with adult CKD. Interestingly, we also identified novel associations with proteinuria within 1p13.3 (p = 1.1 x 10^-15) and 1q44 (p = 5.1 x 10^-13) loci, encoding for proteins implicated in autophagy and mature B lymphocytes differentiation, respectively. Moreover, we imputed 108 HLA alleles in high resolution from SNPs using the HIBAG R package and inferred HLA amino acids with Easy-HLA (hla.univ-nantes.fr). The strongest associations with proteinuria were found for HLA-C*07:04 (p = 3.5 x 10^-5, OR = 4.8) and the 95L and 156D HLA-C amino-acids (p = 2.67 x 10^-5, OR = 0.012).

P23  |  Attention based immune repertoire classification

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The immune memory repertoire encodes the history of present and past infections and immunological attributes of the individual. As such, multiple methods were proposed to use T-cell receptor (TCR) repertoires to detect disease history. We here show that a counting model outperforms all current leading algorithms. We then show that the counting can be further improved using a novel attention model to weigh the different TCRs. The attention model is based on the projection of TCRs using a Variational AutoEncoder (VAE). Both counting and attention algorithms predict better than current algorithms whether the host had CMV, and their HLA alleles. As an intermediate solution between the complex attention model and the very simple counting model, we propose a new Graph Convolutional Network approach that obtains the accuracy of the attention model and the simplicity of the counting model. The code for the models used in the paper is provided in: https://github.com/louzounlab/CountingIsAlmostAllYouNeed
Overall, this genomic investigation of proteinuria in AA children identified 11 statistically significant loci and highlighted a role for class I HLA molecules in the molecular pathogenesis. Further genetic and functional analyses focusing on these loci will enhance our understanding of molecular mechanisms underlying pediatric CKD.

**P25 | SHLARC imputation server: HLA imputation from SNPs made easy with a new website and a large reference panel**

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In the past decade, genotyping and sequencing technologies confirmed the important role of HLA in immune-related pathologies. To cut time and cost of HLA typing, inference algorithms, such as HIBAG, allow to predict HLA alleles using only SNPs and a reference panel. The SNP-HLA Reference Consortium (SHLARC) aims at strengthening HLA association studies by improving and facilitating HLA imputation. Here we developed a freely accessible website destined to the scientific community for HLA imputation available at hla.univ-nantes.fr. We aim at providing a reference panel containing more than 10,000 samples from a wide diversity of ancestries. Upon connection to the website, users will need to create an account. Then, users can start the HLA imputation process: (1) upload SNP data in the plink format, these data should at least contain SNPs within the MHC genomic region (chromosome 6: 29Mb to 34Mb); (2) select which gene(s) of interest to impute (by default HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQB1 are selected; with the possibility to add HLA-E, HLA-F, HLA-G, HLA-H, MICA, and MICB); (3) the data will then be automatically and safely uploaded to our server and the imputation process will start; (4) users will receive an e-mail when HLA imputed data are ready for download. In addition, we are developing future options. First, we plan to add the possibility of creating a reference panel matching the SNPs contained in the data to impute. Second, we plan to offer an ancestry selection choice based on users input or ancestry informed markers. Overall, this website will be an important tool for the scientific community interested in exploring disease HLA association in more diverse populations.

**P26 | Nanopore sequencing data analysis using a cloud computing service**

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Nanopore sequencing from Oxford Nanopore Technologies (ONT) became commercially available in 2016 and has since embarked a new era of long-read single molecule technology. The raw read analysis of ONT data is complex and can be time-consuming. It requires combination of hardware and software resources on different operating systems. In this study, we described the development of an analytic pipeline that leverages the scalability and flexibility of cloud computing power on Microsoft Azure platform for an automatic, totally hand-off and cost-effective pipeline for third-generation sequencing data analysis. This study involved firstly the selection of suitable virtual machine for computing resources to balance between the best performance versus cost effectiveness. Secondly, we developed the Docker containers to install open-source tools in the cloud computational environment. Lastly, we performed comparisons of HLA genotype concordance between the in-house manual method and the automated cloud-based pipeline. The automatic pipeline using the cloud computing reduced the analytic time of ONT data from 3 working days down to 3 hours. The analysis cost was estimated at $17.55 per run or $0.37 per sample. Furthermore, there was no difference in the HLA genotype results obtained from both data analysis pipelines. Lastly, all data were stored in a data center within Australia with upmost security. Overall, the workflow was shown to meet all the key imperatives for performance, cost, usability, simplicity, confidentiality and accuracy.

**P27 | GRMA—Graph based HLA-matching with mismatches**

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In this study, we described the development of an analytic pipeline that leverages the scalability and flexibility of cloud computing power on Microsoft Azure platform for an automatic, totally hand-off and cost-effective pipeline for third-generation sequencing data analysis. This study involved firstly the selection of suitable virtual machine for computing resources to balance between the best performance versus cost effectiveness. Secondly, we developed the Docker containers to install open-source tools in the cloud computational environment. Lastly, we performed comparisons of HLA genotype concordance between the in-house manual method and the automated cloud-based pipeline. The automatic pipeline using the cloud computing reduced the analytic time of ONT data from 3 working days down to 3 hours. The analysis cost was estimated at $17.55 per run or $0.37 per sample. Furthermore, there was no difference in the HLA genotype results obtained from both data analysis pipelines. Lastly, all data were stored in a data center within Australia with upmost security. Overall, the workflow was shown to meet all the key imperatives for performance, cost, usability, simplicity, confidentiality and accuracy.
HLA matching between patients and all candidate donors in Hematopoietic Stem Cell Transplants (HSCT) requires a comparison of large donor registries with possibly ambiguous patient typing. This often requires imputation of the patient and donor's HLA typing followed by the computation of the matching between the resulting high resolution typings of the patients and the candidate donors. While fully matched donors can be obtained in real time in most current matching algorithm, all mismatched donors often require a long time to find. We here present Graph based HLA matching with mismatches (GRMA) to detect donors with up to three mismatches in real time. GRMA is based on the following elements: (A) Pre-imputations of all donors, using a multi-race GRIMM imputation, B) Graph-based representation of the patients and donors typing, (C) In memory computation of the matching with efficient graph data structures, (D) Graph traversal based screening of non-candidate donors, limiting the search to a small subset of donors. The combination of these elements allows for real time (less than a second typically) detection of all donors up to three mismatches. These results can then be further filtered in real time for other factors such as age, gender, CMV, ABO matching and others.

P28 | Kidney transplantation follow-up: Personalized patient contextualization with a nearest neighbor approach

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In the past years, the healthcare field has been particularly involved in big data collection, including in solid organ transplantation. In an effort to improve personalized monitoring and outcome prediction in the kidney transplant field, the French KTD-Innov consortium was launched in 2018 in order to collect more than 1,000 clinical data and samples from kidney-transplanted patients. Moreover, 7,000 transplanted patients from 2000 to 2017 of the French historical database DIVAT were integrated in the analyses, including well-filled recipient and donor clinical data, as well as transplant characteristics. The objective is to project the biological values of a patient of interest (POI, KTD-Innov data) among the values of a reference population (POR, DIVAT data) selected with the characteristics of the POI to visualize the direction of a specific biological value as a function of follow-up time. 23 relevant variables were selected according to clinicians’ expertise (e.g., donor and recipient age, body mass index). One of the contextualization algorithms we used is the nearest neighbor approach. After applying a dimension reduction method, a weighted Euclidean distance was computed to select the closest patients that will compose the POR. After selection, growth-like curves were calculated and displayed to represent kidney function-specific biological levels (e.g., proteinuria) during follow-up. The POI can then be projected in this space, enabling fast and accurate contextualization. This method is implemented in a clinical decision support system to provide a personalized monitoring for kidney-transplanted patients with two other contextualization methods: a filter approach and another distance method with a hierarchical clustering algorithm. This application facilitates access to large amounts of data by allowing their visualization and comparison as a way to optimize medical care and guide clinical decisions.

P29 | HLA-net interactive interface: making big data small and accessible

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Big data are advantageous only when they can be readily useable and integrated with new data. Such purpose leads to the creation of an interactive tool that integrates the HLA data of the anthropology components from previous workshops (12th to 18th IHIWS) with the possibility to include the user’s own datasets and to access a number of analytical and visualization applications both for single
and multi-locus. This tool is the interactive HLA-net interface: i-HLA-net. The new tool allows one to visualize and obtain allele frequency distributions and other population genetic parameters for single and multiple populations, both in tabular, graphical and geographical map formats. Haplotype and linkage disequilibrium (LD) estimation and visualization are also included. In particular, the tool includes a much improved LD viewer allowing to compare LD patterns among several populations and using several measures of LD. It integrates most of the gene[rate] applications already available in the HLA-net platform linking them in a pipeline-like chain with the advantage for the user of no longer requiring upload and download of intermediate files. The outputs, be they tables, files, graphs, maps, are now more generic and easily defined by the user. The common and well-documented (CWD) catalogues are also available and linked to the other facilities that include binding motif logo charts and peptide binding profiles (as per MHCcluster) both for specific proteins and populations. The cornerstone of this new tool is a combination of two programming paradigms, reactive and functional, into an abstraction layer that also includes a hook for the public data. It is expected that the tool can be expanded to almost fully automatically curate new data and made it publicly available. While this new tool greatly facilitates everyone access to HLA workshop databases it also assists in curating new data, hence contributing both to more data and to more easily accessible data.

P30 | A large French genetic cohort to identify predictive molecular factors of chronic lung allograft dysfunction

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Chronic respiratory diseases are among the leading causes of morbidity and mortality worldwide with 3.3 million deaths each year. For patients with end-stage lung disease, the only viable option is lung transplantation (LT). However, the lung graft survival remains limited with only 50.6% survival at 5 years post-transplantation mainly due to the development of chronic allograft dysfunction (CLAD). CLAD is defined by a > = 20% decline in measured forced expiratory volume value from the reference and regroups 3 phenotypes: BOS (bronchiolitis obliterans syndrome) which is a predominantly obstructive ventilatory syndrome; RAS defined by a restrictive pattern; and a mixed obstructive and restrictive pattern. There is therefore an essential need for better understanding the molecular mechanisms involved in the CLAD phenotypes pathophysiology. COLT is a French cohort of LT created in 2009 in Nantes, which has included 2,117 patients from 9 transplant centers (Nantes, Bordeaux, Lyon, Paris, Marseille, Strasbourg, Grenoble, Toulouse and Suresnes). To supplement the rich clinical data collected for COLT (donor and recipient demographics and medical history, transplantation follow-up), we genotyped a subset of 392 LT donor-recipient pairs with the Affymetrix Axiom PRMA microarray (900K SNPs). Our genetic cohort represents an accurate snapshot of the entire COLT cohort as no variables (e.g., age, sex, initial respiratory disease, survival rate) significantly diverge from the global cohort (p>0.05). Overall, 54% were male, mean age at transplant was 47, the mean follow-up was 41 months and the mean survival rate was 3 years. We have built one of the largest genetic cohorts dedicated to LT comprising both donor and recipient large-scale genotypes. The integration of clinical phenotypes with genetic data will empower us to investigate HLA and non-HLA factors involved in CLAD phenotypes in the near future in order to improve the management and survival of lung transplanted patients.

P31 | Protection of HLA-A and HLA-B epitopes in the context of platelet transfusions in haplo-identical hematopoietic stem cell allograft candidates

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The use of iterative platelet transfusion induces recipient immunization against HLA class I antigens. Unfortunately, in allogeneic hematopoietic stem cell transplantation (HSCT), a significant anti-HLA recipient immunization may be directed against one or more HLA
antigens carried by haplo-identical donor. The aim of our study is to define a platelet transfection strategy for patients awaiting haplo-identical HSCT by protecting corresponding HLA epitopes of the haplo-identical donor. 110 consecutive patients who underwent familial HLA typing for HSCT were selected. Verified HLA-A and HLA-B class I epitopes present in potential donors and must be protected against secondary immunization to platelets transfusion were identified on HLA Epitope Registry website. Then, we compiled a list of HLA class I antigens carrying these “protected” epitopes in order to avoid them in future platelet transfusion. We calculated the probability of finding platelet donors not expressing these HLA antigens (virtual PRA, vPRA) and then verified the availability of suitable platelet concentrates (PCs). On the 110 studied patients, 82 had haplo-identified donors with a mean of 6 verified mismatched HLA class I epitopes (0 to 13) leading to protect a mean of 7 HLA-A antigens (0 to 22) and 16 HLA-B antigens (0 to 49). Mean of vPRA was 80.2% (0 to 99.9%). This drastic selection has resulted in a very small number of PCs available in our regional transfusion center (mean of 1.6 PC; range 0 to 6) improved by a better availability at French national level (mean of 65 PCs; range 0 to 207). A vPRA of more than 96% was linked to less than 10 PCs available at the French national level. Protecting all verified mismatched HLA-A and HLA-B epitopes of the haplo-identical donor is possible upon initiation of an HSCT course in the recipient. However, this strategy requires setting up an upstream organization to found suitable PCs.

P32 | Defining and confirming novel HLA serological specificities from combinational analyses of single antigen bead and cell-based flow crossmatch assays

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HLA serological specificities were defined by the reactivity of HLA molecules with live cells with sets of sera and monoclonal antibodies. We proposed novel serotypes for 11 HLA loci in addition to the currently recognized 109 WHO assigned antigens, and initiated confirmation of the novel serotypes. B*48:02 was previously reported as B48. The single antigen beads (SABs), B*48:02 and B*15:03 (B72), with sera from sensitized patients showed concordant reactions but discordant with that of B*48:01 (B48). B*15:15 was previously reported as B62, but we computed as B75. All three B75 and 6 B62 SABs with sera from a sensitized patient carrying B*15:15 were negative and positive, respectively, confirming the validity of our serotype assignment. We confirmed the novel serotypes corresponding to alleles A*02:05, A*30:02, A*34:02, A*66:02, B*15:11, DRB1*04:02, DRB1*04:03, DRB1*13:03, DRB1*14:02, DRB3*02:02, DRB3*03:01 and DRB5*02:02. We analyzed median fluorescence intensity (MFI) values by a linear regression model using a known antigen as one variable (Xi) and novel serotype as another variable (Yi) with sera from >11,600 patients. High correlations (R² > 0.95) were observed when two proteins were considered as serologically identical: A*02:01/A*02:06, B*57:01/B*57:03, DRB1*01:01/DRB1*01:02, DRB1*04:01/DRB1*04:04, DRB1*04:01/DRB1*04:05, DRB1*14:54/DRB1*14:01, DRB1*15:01/DRB1*15:02 and DRB1*15:01/DRB1*15:03. In contrast, low correlations were observed when two proteins were recognized as different serotypes: A*34:01 vs. A*34:02 (R² = 0.69). Interestingly, we did not find differences between DR17 and DR18. The DR3 splits identified by cytotoxicity assays may correspond to DQ heterodimers associated with the common DRB1*03 alleles. We further confirmed unexpected discrepancies (e.g., A*11:01 vs. A*11:02) as negative using T- and B-cell flow crossmatch assays. The approaches and serologic analyses can be applied systematically to identify and confirm novel serotypes.

P33 | Significantly different HLA genotypes associations with multiple sclerosis suggest different pathophysiological underlying mechanisms

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Genetic association studies for multiple sclerosis (MS) have identified at least 13 alleles from 7 HLA genes, but the pathophysiological underlying mechanisms remain
unexplained. To provide leads for explanation, our objective was to identify genotypes with significantly different MS associations suggesting different mechanisms. We analyzed HLA data for 11,376 MS cases and 18,872 controls from the WTCCC dataset. HLA alleles were imputed using the HIBAG R package (HLA-A, -B, -DRB1 and -DQB1), or inferred with proxy SNPs (LTA: rs2229092, DQA1: rs9273912, DPB1: rs9277565). We tested multiple hypotheses in 20% of the data (exploratory sample) to select pairs of contrasting genotypes with significantly different odd ratios (OR) as non-overlapping 95% confidence intervals (CI), using Epitools R package. Those genotype pairs were tested for replication in the remaining data (80%). Confirmed pairs were tested in the global sample (100%). Our results for the exploratory sample show significant MS association for all 13 tested alleles, but HLA-DQB1*03:02, with similar OR as in Moutsianas. Out of the 32 resulting MS-genotypes, 24 were significantly associated with MS (p <0.05), among which we selected 5 pairs of contrasting genotypes. In the replication sample, all results were confirmed. Estimation for these genotypes in the global analysis were: HLA-DRB1*03:01/*non-MS: OR 1.13, CI 1.05-1.22, DRB1*15:01/*non-MS: OR 3.04, CI 2.85-3.23, DRB1*15:01/*15:01: OR 7.08, CI 6.20-8.11, HLA-DQA1 rs9273912-T/T: OR 0.42, CI 0.35-0.50, rs9273912-T/C: OR 0.72, CI 0.68-0.76, HLA-DQB1*03:01/*03:01: OR 0.46, CI 0.40-0.54, DQB1*03:01/*non-MS: OR 0.64, CI 0.72-0.81. In conclusion, we identified 3 HLA-DRB1 genotypes with significantly different MS associations, as well as 2 HLA-DQA1 and 2 HLA-DQB1 genotypes. Taking into account those 5 pairs of contrasting genotypes would provide leads to decipher MS susceptibility.

P34 | Data sharing, research evaluation and Open Science

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The growing movement of Open Science is entering research practices widely, reflected in institutional and funder requirements, for example, by the European Commission (EC). In the assessment of research, publications have traditionally been the major criterion. However, as many other research activities do exist such as creation of databases, data production, management and curation, a number of initiatives have been taken recently including the “Agreement of Reforming the Research Assessment” (Science Europe), and declarations by NASA and UNESCO. The re-use of data is an important activity and should be performed in compliance with international standards, personal data protection regulations and the FAIR (Findable/Accessible/Interoperable/Reusable) data principles. However, the lack of recognition of Open Science activities in career development is still an obstacle. The “Sharing Rewards and Credit Interest Group” (ShaRC IG) was created in the framework of the Research Data Alliance (RDA) and aims to promote academic crediting and rewarding mechanisms as regards Open Science. The group has conducted an on-line survey in order to explore how Open Science activities are or should be considered in research assessment, career development and funding of scientific projects. A total of 230 responses were received originating from 37 different countries, most of the respondents declared that their main job title was researcher and had between 10 and 20 years of experience in their field. The results will be compared with the survey published in March 2022 by the ON-MERRIT project (co-funded by the EC) with about the same number of responses. One of their hypotheses was that Open Science practices might increase the existing inequalities between research groups in rich and poor countries, if specific measures are not taken to avoid that. Hence, Open Science has to be implemented carefully, but the movement is irreversible and is a fundamental aspect of science today.

P35 | A retrospective study: the effect of sensitization events on anti-HLA antibody development

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HLA alloimmunization remains a critical factor affecting the success of kidney transplantation. Sensitization events such as blood transfusions, pregnancies, or organ transplants are the main reasons for developing anti-
HLA antibodies. In this retrospective study, we evaluated how sensitization affects the development of panel reactive antibodies (PRA) in solid organ transplant candidates. Anti-HLA class I/II antibody PRA screening-identification tests were performed on a total of 2532 renal transplantation candidates using Luminex assay (Lifecodes LifeScreen Deluxe Kit, Immucor Gamma, USA) kits. PRA-positive rates (HLA-class I and/or class II antibodies with median fluorescence intensity [MFI] values ≥1500) were analyzed for different sensitization events and sex. Sensitized and non-sensitized patients were compared for each sensitization event. The anti-HLA class I, II, and I+II positivity rates were 28.7% (p<0.001), 29.3% (p<0.001), and 38.1% (p<0.001) in patients with blood transfusion sensitization; 37.1% (p = 0.001), 36.1% (p = 0.005) and 48.4% (p<0.001) in patients with pregnancy sensitization; and 43.6% (p<0.001), 53.4% (p<0.001), and 61.8% (p<0.001) in patients with previous transplantation sensitization, respectively and these rates were higher than in non-sensitization patients. In conclusion, in patients awaiting kidney transplantation, blood transfusion, pregnancy, and transplantation-related sensitization have a significant impact on the development of HLA class I and class II antibodies.

**ABSTRACTS**

**P36 | A research tool to interrogate combined single antigen bead (SAB) files with donor and recipient HLA typing information to identify HLA mismatches and MFI levels of donor specific antibodies over time. Introducing the mismatch data aggregator (MDA) program**

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Research studies in the field of H&I require definition of HLA mismatches and donor specific antibody (DSA) levels. These can be time-consuming to obtain in a manner that is easy to analyze. The H&I laboratory at Cambridge University Hospitals (CUH), UK, have developed a program, the mismatch data aggregator (MDA), to aid with this. SAB data is exported from HLA Fusion into individual patient folders for use with the SAB Combiner (SC) program which combines all SAB MFI data for a patient. These SC files are used in conjunction with HLA typing information in the MDA to identify mismatches at HLA-A, -B, -C, -DRB1/3/4/5 and -DQ loci, including the date and MFI of the highest DSA, and listing out the MFI data for all dates where mismatches are identified. Initial validation revealed that the utility of the MDA was limited due to it being designed to identify DSA to high resolution HLA mismatches leading to some donor HLA mismatches being missed. If considering only the high-resolution data for these transplants, mismatches would be identified by the MDA, but no DSA data would be generated as the respective beads were not present on the SAB panel. Donor HLA types within the cohort with at least one high-resolution allele that was not represented on SAB included C*07:01, C*08:02, DRB1*08:03, DRB1*08:04, DRB1*13:02, DQB1*05:03 and DQA1*01:04. As an example, DSA in the cohort were identified at Cw7, where molecular DSA were missed due to the high-resolution mismatches being C*07:01. Therefore, a new version of the MDA was created that could also identify serological DSA. Additionally, the MDA did not originally identify mismatches at DRB3/4/5 so these were incorporated during the update. The new version of the MDA was then validated for research use. Instructions, rules and functional limitations were defined during this process. Full validation documents and the program are open access and available at https://github.com/ttyping/Mismatch-Data-Aggregator.

**P37 | Reshaping individuals’ rights in Big Data research: the contribution of data altruism**

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Due to the technical developments of Big Data, the European Union (EU) has engaged in profound reform to promote a responsible use of digital techniques in health in-line with EU values. Therefore, the EU has proposed regulations to maximize the use of health data in research putting citizens and patients at the heart and designing governance principles to ensure transparency and trust. Then, the recently adopted Data Governance Act (DGA) has proposed to implement the new concept of data altruism (also included in the proposal for a European Health Space). It will be crucial to support free data donation in a secure technical environment and will include individuals as stakeholders in the governance of
health data sharing. According to DGA/article 2 data altruism means “the voluntary sharing of data on the basis of the consent of data subjects to process personal data pertaining to them, or permissions of data holders to allow the use of their non-personal data without seeking or receiving a reward”. However, if this concept reflects the EU’s ambition to promote health data sharing on a large scale for the benefit of the society and of research, its implementation raises ethical and legal issues regarding individual's rights, especially for information and consent. Thus, does data altruism mean data donation and, as a result, a waiver of related individual rights? Is it a new fundamental right? What are the consequences for individuals’ autonomy and for their ability to control the data uses regarding GDPR requirements? Will it be feasible to draft a harmonized European consent form for data altruism? How can individuals reap the benefits of sharing their data? Will transparency about the use of their data be guaranteed? In this presentation, we will decipher the concept of data altruism from a legal and ethical perspective and analyze the consequences of its adoption on individuals’ rights and on research data sharing activities.

**P38 | A comprehensive statistical analysis to assess MFI values in HLA antibody screening by two commercial platforms**

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Mean Fluorescence Intensity (MFI) is an indirect measurement of HLA antibody titer by bead-array Luminex technique. MFI value strongly depends on the platform used, as well as other interfering factors. We present a comprehensive statistical analysis to compare two commercial kits. Thirty-five consensus, highly reactive (cPRA>50%) sera from Italian National EPT, were screened for HLA class I and II antibodies by One-Lambda (OL) and Immucor (IC) kits. Beads from two vendors were aligned for shared specificities and MFI values were compared side-by-side. For class I, 1171 beads were positive (MFI>1000) for OL and 1018 for IC. For class II, 654 for OL and 572 for IC. Statistical analysis were performed to assess correlation, agreement and best fitting cut-off for the two platforms. Strong correlation was shown by Pearson coefficient ($r = 0.93$ for class I and $r = 0.96$ for class II) and linear regression (95% confidence limits): class I, $y = 0.746x+167$ (DS = 0.006, 47); class II $y = 0.869x +51$ (0.005, 46). Agreement between measurements was graphically assessed by Bland-Altman plot. Mean difference between the vendors was for class I $MFI = 1282$ (DS 2680) and $MFI = 466$ (2088) for class II. A strong bias was confirmed for MFI values around cut-offs in use for positivity (1000) and permissivity (3000). ROC curves were used to confirm correlation (AUC) and define the best fitting cut-offs of the vendors. The OL cut-offs in use for positivity ($MFI = 1000$) and permissivity ($MFI = 3000$) were set and the IC MFI values that best predict these thresholds in terms of sensitivity, specificity and accuracy were assessed. AUC confirmed high correlation and best fitting MFI values are shown in table 1. In conclusion, MFI values measured by the two platforms showed a high correlation but a low agreement in absolute values and appropriate statistical analysis are needed to compare results and establish homogenous cut-offs.

**P39 | Work smarter not harder! A clinical tool to combine single antigen bead (SAB) files for patients allowing for data to be easily viewed and analyzed in the clinical laboratory. Introducing the SAB Combiner (SC) program**

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Analysis of patient single antigen bead (SAB) results is an integral role of the H&I laboratory in supporting transplantation. To ensure accurate analysis of an individual patient's HLA antibody profile, it is of help to be able to view together multiple SAB results in one file. The H&I laboratory at Cambridge University Hospitals (CUH), UK have developed and validated a program, termed the SAB Combiner (SC) to do this. Firstly, SAB data is exported from HLA Fusion into individual patient folders for use with the SC. The SC then combines all SAB MFI data for a patient into a single file sorted by HLA class, date and bead. During validation of the program the key issue identified in the SC was due to lot number changes in Luminex SAB, where some beads were added or discontinued. This created gaps in some of the patient combined files, so to prevent this, the SC was updated to fill gaps with an ‘x’. There were also some nomenclature differences between SAB lots, including HLA-C alleles.
being listed as both Cw* and C*, and splitting of DQA1 and DQB1 depending on whether a colon was used to separate first and second field. To account for these differences in Fusion exports, the SC was updated to convert any differences into standardized nomenclature. After completion of troubleshooting, a full validation of the SC was performed. Briefly, this involved checking SC data from 10 random patients for accuracy and completeness when compared with data files exported from HLA Fusion. If present, up to four files were checked per patient, two for SAB I and two for SAB II. A copy of the validation document and updated instructions for use can be found at https://github.com/ttyping/SAB-Combiner. The H&I laboratory at CUH use this program routinely to help in their interpretation of sensitized patients, it is open access and available to all.

P40 | Advyser solid organs software for accurate post-transplantation monitoring

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The Advyser solid organs software is a fast, exact, and easy-to-use medical device software for visualizing quantitative and qualitative monitoring of donor-derived cell-free DNA (dd-cfDNA) in the plasma of patients following kidney organ transplantation. Advyser can analyze millions of short DNA sequences in minutes and provide accurate results that enable early detection of transplant rejection. An early increase in dd-cfDNA is measured in patients who developed a graft reaction after kidney transplant. The dd-cfDNA displayed in the software is sequenced using NGS (Next Generation Sequencing) technology as part of the Devyser post-transplantation solution. Monitoring results are displayed in % dd-cfDNA quantified based on DNA markers for monitoring of cfDNA and screening of donor/recipient pre-transplantation samples. The analysis of the data starts with the conversion of the generated sequencing files into structured table files using multithreading to enable rapid conversion. A patient project is then created with screening data and successively added monitoring data. Visualized screening and monitoring results in the user interface alerting the user to possible sample mix-ups, low coverage or background noise and indicate possible double transplants. Filters in the different views allow the user to compare data in selected time periods. Finally, different report layouts and formats can be created. In addition, relevant data can be exported as XML to transfer the results to a laboratory management system. Overall, the steps in the software are designed for intuitive navigation. In addition, the clear presentation of data in the user interface and reports improves the understanding and communication of results and ensures a prompt and accurate diagnosis of patients to prevent allograft reactions at an early stage.

P41 | HLA-A~B~DRB1-DQB1 homozygosity among the Brazilian Bone Marrow Registry living in Rio de Janeiro State, Brazil

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The Brazilian Bone Marrow Registry (REDOME) counted 5,541,795 HLA typings in August, 2022. Since July 2021 new volunteers have been typed at allelic resolution for 6 HLA loci, but 3,583,504 entries only had HLA-A, -B and -DRB1 at medium or high resolution. Rio de Janeiro, localized in the Southeast region, contributed 4.6% of these donors. A possible cell source for regenerative medicine and testing for immune-mediated cell response is induced pluripotent stem cells (iPS), although HLA compatibility must be a preliminary condition. In order to constitute an iPS biobank we screened the Rio de Janeiro REDOME entries to determine the frequencies of possible donors for homozygosity and their haplotypes. The Brazilian Bone Marrow Registry (REDOME) counted 5,541,795 HLA typings in August, 2022. Since July 2021 new volunteers have been typed at allelic resolution for 6 HLA loci, but 3,583,504 entries only had HLA-A, -B and -DRB1 at medium or high resolution. Rio de Janeiro, localized in the Southeast region, contributed 4.6% of these donors. A possible cell source for regenerative medicine and testing for immune-mediated cell response is induced pluripotent stem cells (iPS), although HLA compatibility must be a preliminary condition. In order to constitute an iPS biobank we screened the Rio de Janeiro REDOME entries to determine the frequencies of possible donors for homozygosity and their haplotypes. The Brazilian Bone Marrow Registry (REDOME) counted 5,541,795 HLA typings in August, 2022. Since July 2021 new volunteers have been typed at allelic resolution for 6 HLA loci, but 3,583,504 entries only had HLA-A, -B and -DRB1 at medium or high resolution. Rio de Janeiro, localized in the Southeast region, contributed 4.6% of these donors. A possible cell source for regenerative medicine and testing for immune-mediated cell response is induced pluripotent stem cells (iPS), although HLA compatibility must be a preliminary condition. In order to constitute an iPS biobank we screened the Rio de Janeiro REDOME entries to determine the frequencies of possible donors for homozygosity and their haplotypes. The Brazilian Bone Marrow Registry (REDOME) counted 5,541,795 HLA typings in August, 2022. Since July 2021 new volunteers have been typed at allelic resolution for 6 HLA loci, but 3,583,504 entries only had HLA-A, -B and -DRB1 at medium or high resolution. Rio de Janeiro, localized in the Southeast region, contributed 4.6% of these donors. A possible cell source for regenerative medicine and testing for immune-mediated cell response is induced pluripotent stem cells (iPS), although HLA compatibility must be a preliminary condition. In order to constitute an iPS biobank we screened the Rio de Janeiro REDOME entries to determine the frequencies of possible donors for homozygosity and their haplotypes. Rio de Janeiro REDOME entries accounted 182,753 in medium and high-resolution typings for HLA-A, -B and -DRB1, 4887 also with DQB1 or C loci. We found 943 entries homozygous for HLA-A, -B and -DRB1 at the allele group level among Rio de Janeiro individuals (n = 317, haplotypes). We were able to contact individuals and include in our biobank 10 donor cells. Homozygosities were
P42 | Comparison of kit use and performance in UK NEQAS for H&I Scheme 3—HLA antibody specificity analysis

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Scheme 3 assesses participants’ ability to correctly determine the specificity of HLA antibodies in 10 samples annually. The scheme requires participants to report any antibodies detected at HLA-A, -B, -Cw with elective registration at DRB, DQB and DPB. An antibody is classed as ‘present’ if >75% participants report it as positive, ‘absent’ if >95% participants report it as negative or ‘negative’ if all participants report it as negative. Re-analysis of the samples reported in 2022–2023 was performed separating the results by commercial kit use. Of the 66 participants in the scheme only those participants that reported exclusively using One Lambda (OL) LABScreen (n = 39) or Immucor (Imm) Lifecodes (n = 14) single antigen kits were included in the analysis. Participants that used both kits and those that didn’t state a testing method were excluded. The separate analysis by commercial kit revealed a similar percentage of antibodies reaching consensus ‘present’ in both kits (CI OL 24.9%, Imm 21.7%; CII OL 8.1%, Imm 7.5%). There was less concordance in the definition of ‘absent’ antibodies (CI OL 62%, Imm 50.1%; CII OL 83.5%, Imm 75.5%). A greater percentage of class I antibodies were classed as not assessed compared with class II for both kits (CI OL 13.2%, Imm 28.3%; CII OL 8.4%, Imm 17.1%). This could be due to the smaller number of participants only using Imm kits. Average overall performance for detecting the ‘presence’ of antibodies was marginally higher for users of OL kits (CI OL 97%, Imm 87%; CII OL 97%, Imm 89%). For the confirmation of ‘absence’ of antibodies the difference in overall performance was also comparable between kit users (CI OL 98%, Imm 95%; CII OL 98%, Imm 96%). Overall performance of laboratories in scheme 3 is good. However, there are a proportion of HLA antibodies that do not meet consensus and not assessed; this remains even when participants that use the same testing methodology are compared.

P43 | An HLA-DRB1*03:105 allele in a patient awaiting a kidney donor in Colombia

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In Colombia, matching criteria for solid organ transplantation are established by the Coordinación Nacional de Trasplantes at the Instituto Nacional de Salud (INS). The HLA typing should be performed with an intermediate resolution technique. Results are entered in serological equivalency in the database (REDDATA), and once a potential donor is available, HLA matching is performed where HLA-DR has a higher matching score than HLA-A, -B or -DQ among other criteria. Here we are reporting a case, where HLA-DRB1 typing by Luminex and Sanger sequencing was HLA-DRB1*03:105. DNA was obtained and HLA-A, -B, -C, -DRB1 and -DQB1 typing was performed using Immucor kits. The two digit molecular typing results were (HLA-A*30,32, HLA-B*18,42, HLA-C*05,17, HLA-DRB1*03,10, HLA-DQB1*03,05. When results were going to be uploaded to REDDATA, HLA-DRB1 typing could not be uploaded since HLA-DRB1*03:105 did not have a serological equivalent based on the HLA Nomenclature dictionary, and REDDATA does not allow an HLA-DRB1*03 assignment. The HLA-DRB1*03:105 allele was reported in 2014 (IPD-IMGT/HLA Database, HLA11608 entry), without ethnicity or geographical location). This allele is found in allelefrequencies.net in two haplotypes from Chile, one of them with A*02, B*18, DRB1*03:105 and another A*29, B*18, DRB1*03:105. Our sample had an HLA-DQB1*03 allele (03:01) associated with the HLA-DRB1*03:105, unusual association for an HLA-DRB1*03 allele. Based on sequence alignment between HLA-DRB1*03:01:01, DRB1*03:02:01 and DRB1*03:105, we suggested that it could be registered in the national database (REDDATA) with a serological equivalence of HLA-DR17, a recommendation without other supportive evidence. This is the first time that an HLA-DRB1*03:105 is observed in Colombia. The problems arising from lack of serological equivalents for many HLA alleles, should prompt the upgrade the Colombian registry (REDDATA) to molecular based assignments and not serological assignments.
Transplantation of HLA or KIR mismatched allogeneic hematopoietic stem cells (HSCT) can lead to NK cell activation/inhibition or education/licensing and change anti-tumor immunosurveillance. In this study, we investigated a correlation between missing ligand recognition and the changed education status of donor NK cells in different constellations of KIRs and HLA cognate ligands in donor and recipient. N = 325, patients with acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphoblastic leukemia (ALL), myelodysplastic syndrome (MDS), or lymphoma receiving T cell-replate HSCT from HLA-matched or partly mismatched unrelated donors between 2002 and 2020 were included in this study. Missing ligand recognition was defined depending on the presence or absence of recipient HLA ligand for donor inhibitory KIR. Changed NK cell education status was defined by loss (downward resetting) or getting a new cognate pair of HLA-KIR (upward licensing) in a new HLA environment of the recipient. We found highly significant deterioration of disease-free survival (DFS, 17% vs. 63%, p = 0.000014, HR = 4.00, 95% CI 2.13-7.69) when at least 1 inhibitory KIR-HLA cognate pair was lost post-transplant. Simultaneously, we did not find any improvement of DFS when at least 1 donor ligand out of 3 (C1, C2, Bw4) was missing post-transplant (41% vs. 36%, p = 0.40, HR = 0.85, 95% CI 0.59-1.23). The missing ligand effect remained insignificant when two ligands were missing in the recipient for donor inhibitory KIR (DFS, 38% vs. 36%, p = 0.96, HR = 0.99, 95% CI 0.58-1.67). Since the effect of lost NK cell licensing seems to be reciprocal to missing ligands we excluded patients with DOWNWARD resetting and/or UPWARD licensing pairs. In the remaining patients, the missing ligand effect remained insignificant, similar to limiting the study sample to patients with AML and MDS. We conclude, that in malignant patients NK cell licensing effect is independent of the missing KIR ligand effect after HSCT.

The presence of Donor Specific-Antibodies (DSAs) may be of concern in both hematopoietic stem cell (HSCT) and solid organ transplantation. Strategies to desensitize patients with DSAs are effective except in the presence of high MFI values, that is, > 20.000, increasing the morbidity and mortality in those patients waiting for a HSCT. As an Ab-producing cell type, the plasma cell (PC) represents a logical target of therapy aiming at lowering DSAs. We here investigated the MFI variations in patients affected by multiple myeloma (MM) with anti-HLA Abs before the beginning of treatment, in order to extrapolate the effect of anti-myeloma drugs on anti-HLA Ab production. In particular, the role of a new class of drugs, the anti-CD38 monoclonal Abs (mAbs), is under investigated in this setting. This monocenter, prospective observational study “(My)eloma (T)herapy vs. anti-(H)LA antibodies”, MYTH) was approved by the local Ethics Committee. From June 2021, a total of 13 adult patients with diagnosis of MM were enrolled. One out of 13 (8%) was positive for an anti-DR12, with an initial MFI of 3890. During the treatment with Daratumumab-VMP, a reduction of MFI was observed in the first weeks, followed by a raise up to the pre-treatment levels. No emergence of new anti-HLA Abs was observed. MFI kinetics mirrored the schedule of treatment, with lower MFI values observed when the anti-CD38 mAb were administered at shorter intervals, indicating an effective, although short-term, inhibition of anti-HLA Ab production. The patient responded to treatment and is in very good partial remission at last evaluation. In conclusion, these data support the activity of anti-CD38 therapy on anti-HLA Ab production, suggesting the potential integration of such treatment into desensitizing protocols. Larger cohorts and more studies are warranted to
investigate the role of PC-directed therapy on anti-HLA Ab synthesis in vivo, in an attempt to improve the current desensitizing protocols.

P46 | Microchimerism levels of recipient cells: frequency and impact on hematopoietic stem cell transplantation outcome

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The value of information provided by chimerism status of patients following hematopoietic stem cell transplantation (HSCT) in selection and modification of post-HSCT treatment is indisputable. However, the level of detected recipient cells which has a clinical relevance is still a subject of investigation. First goal of the present study was to determine the rate of recipient cells occurrence at microchimerism level (<1%) in post-HSCT patient sample and investigate whether this rate is affected by factors such as conditioning regimen, HSC source, and patient/donor characteristics. Our second objective was to evaluate the impact microchimerism has on the relapse occurrence and overall survival (OS). Study included 105 adult patients diagnosed with AML and transplanted at UHC Zagreb, for whom chimerism was determined at one, three and six months after HSCT using KMRtrack method and KMRengine software (GenDx). Following results were obtained: patients who received myeloablative conditioning had a significantly higher rate of detected microchimerism at six months post HSCT as opposed to those with reduced intensity or non-myeloablative conditioning (p = 0.006); source of HSC, patient age, donor gender and age were not associated with microchimerism detection rate; there was no difference for one year OS nor relapse occurrence between patients with full donor chimerism (FDC) and those with microchimerism detected at one, three or six months after HSCT as opposed to those with reduced intensity or non-myeloablative conditioning; there was no difference for one year OS nor relapse occurrence between patients with full donor chimerism (FDC) and those with microchimerism detected at one, three or six months post HSCT; OS was significantly better (p<0.001) and relapse occurred less frequently (p = 0.002) among patients for whom last detected chimerism was either FDC or microchimerism in comparison to those patients with >1% of detected recipient cells. In conclusion, our results suggest that microchimerism levels of recipient cells in post-HSCT patient sample do not elevate relapse occurrence risk, but should be monitored closely for possible increase beyond 1% threshold, at which point relapse occurrence risk and worse OS prognosis should be considered.

P47 | Facilitation of stem cell transplantation in a highly sensitized AML patient through Imlifidase treatment

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An AML patient was referred to our laboratory for HLA typing in 2020. Two potential related donors were HLA typed and neither were fully matched. An unrelated donor search identified six potential 11/12 (one DPB1 mismatch) donors. A Cord Blood Unit (CBU) search found several CBUs with 6/8 matches suitable for transplantation. HLA antibody screening indicated the presence of class I and II HLA antibodies. After several months of treatment, the patient became highly sensitized with HLA antibodies against many common HLA antigens, including donor specific antibodies (DSAs) against the potential donor options. Due to difficulties obtaining verification typing samples and concerns about using CBUs, the Transplant Centre (TC) started Plasma Exchange (PE) to facilitate a haploidentical transplant from her daughter. PE had limited effect on reducing the DSAs that were present at >25,000 MFI. Dilution studies suggested that these antibodies were present at higher titers than were detectable by the identification kits. As the patient needed a transplant urgently, the TC applied to use Imlifidase on a compassionate basis. Imlifidase is derived from the IgG degrading enzyme of Streptococcus pyogenes (IdeS) and cleaves antibodies. It has been used in renal transplantation to allow transplantation of highly sensitized individuals who are unresponsive to other antibody removal therapies. The application was approved, and two doses were given in December 2021 followed by a graft from her daughter. Antibody testing indicated that DSAs had decreased post-treatment but, as the patient was unable to undergo further PE, they increased again. Unfortunately, the patient passed away through sepsis before engraftment could be detected. This case demonstrates the feasibility of using Imlifidase for
highly sensitized patients in the stem cell transplant setting. However, it also highlights the need to be able to continue antibody removal therapy post-transplant.

P48  |  Comparison of two methodologies for monitoring chimerism after allogeneic stem-cell transplantation: Next-generation sequencing (NGS) versus short-tandem repeats (STR)

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Chimerism analysis after allogeneic hematopoietic stem cell transplantation (allo-SCT) helps to monitor engraftment, and to predict relapse. We have evaluated the performance of next-generation sequencing (NGS) using AlloSeq HCT kit (CareDx) to monitor chimerism in comparison with STRs using PowerPlex 16HS kit (Promega). Seven artificial mixtures (50%, 25%, 5%, 2.5%, 1.25%, 0.63%, 0.31%) were used to detect the limit of detection (LOD) and accuracy. Quantification and clinical application analysis were performed on a total of 88 bone marrow clinical samples post-allo-SCT (days +28, +56, +100) from 27 patients with acute myeloid leukemia or myelodysplastic syndrome. A Bland and Altman analysis was applied to evaluate the correlation of the quantification of both techniques. Intra- and inter-experiment reproducibility was evaluated by duplicate samples (n = 4). Informativeness was assessed in 29 donor-recipient pairs (D/R). The analysis of artificial mixtures showed better LOD in NGS (0.3%) than in STR (2%), with high accuracy between the expected and observed results ($R^2 = 0.9989$). There was an excellent correlation of the quantification of the 88 clinical samples between both techniques, with Bland and Altman analysis showing a small bias (-0.3012). Informativeness was better for NGS than for STR in 27 D/R (100% vs. 95%). Reproducibility was similar for both techniques (SD ±0.63% NGS vs. ±0.42% STRs). A mixed chimerism was detected only by NGS in 6/88 samples, all below the LOD of STR. This allows to detect increasing recipient cells by NGS in 2/29 patients with complete chimerism by STRs, at a median of 21 days prior to relapse. Using the same quantity of DNA (10 ng), the NGS technology allows a reliable quantification of chimerism with better LOD and informativeness than STR. However, the NGS kit requires to accumulate 24 samples per run, and turnaround times and costs are higher.

P49  |  Loss of mismatched HLA haplotype after haplo-identical hematopoietic stem cell transplantation relapse

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Hematopoietic stem cell transplant (HSCT) with a fully HLA matched related donor is widely considered the gold standard treatment for many hematologic malignancies. In the absence of this option, a 12/12 CMV matched unrelated donor is usually the preferred donor of choice. Recent therapeutic discoveries such as the use of high-dose post-transplant cyclophosphamide (PTCy), have seen a growth in haploidentical HSCT, which has shown low incidence of graft-versus-host disease (GvHD), graft failure, and non-relapse mortality. Despite that, post-transplant relapse remains a common cause of treatment failure for many patients. One of the mechanisms behind this is the mismatched haplotype loss leading to evasion of immune surveillance. Here we describe the case of a 25-year-old male Hodgkin lymphoma patient, who received haplo-HSCT from his mother in 2017. Due to relapse in 2021, a second transplant from an unrelated donor was considered and post-transplant buccal swab samples were sent to our laboratory. HLA typing was performed at HLA-A, -B, -C, -DRB1, -DRB3/4/5, -DQA1, -DQB1, -DPB1 loci using GenDx NGSgo®-AmpX v2 kits utilizing Illumina Sequencers. The results obtained showed homozygosity at all HLA loci and loss of the mismatched HLA haplotype, due to possible contamination from cancer cells in the sample. A new set of buccal swab samples was requested from the transplant center, suggesting concentrating the swabbing on the
cheek area to try and minimize contamination. Samples were received during clinical remission, and the subsequent NGS typing showed a chimeric HLA type, indicating a mixture of somatic and donor-derived blood cells. The donor search was carried out using the pre-transplant HLA type, due to the difficulty in obtaining germ-line DNA for this patient. This study confirms the possible incidence of loss of mismatched HLA haplotype after haplo-HSCT relapse and highlights the importance of the sample timing and sampling technique in these patients.

P50  |  Detection of HLA Antibodies in potential haploidentical HSC transplant recipients

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The aim of our study was to determine the frequency of HLA antibody detection, including donor-specific antibodies (DSA), in potential recipients of a haploidentical HSC transplant. The study included 133 children (3 month to 17 years old), 227 adult patients (18–68 years old) planned for haploidentical HSC transplants. Both groups were dominated by males (68.4% and 59.0%, respectively) and patients with hematological malignancies and bone marrow failure syndrome (75.19% and 85.9%). HLA antibodies were determined using the Luminex bead assay using LIFECODES LifeScreen Deluxe, LIFECODES LSA CLASS I, II reagent kits. Positive criteria: MF I >750, MFI/LRA ratio>cut-off. In children, HLA antibodies were detected in 17.3% (n=23) of patients (MFI 772–20961): in 6% (n=8) - class I HLA antibodies (MFI 774–10747), in 3% (n=4) - HLA class II antibodies (MFI 4025–6433) and 8.27% (n=11) - HLA class I and class II antibodies (MFI 772–20961). In adults, HLA antibodies were detected in 31.7% (n=72) of patients (MFI 753–23471); in 7% (n=16) - class I HLA antibodies (MFI 1132–20359), in 10.13 % (n=23) HLA class II antibodies (MFI 832–15001) and 14.54% (n=33) - HLA class I and class II antibodies (MFI 753–23471). The identified HLA antibodies were against HLA-A, -B, -C, -DRB1, -DRB3, -DRB4, -DRB5, -DQA1, -DQB1, -DPB1. DSA HLA antibodies were detected in 6% (n=8) of children and 10.13% (n=23) of adult patients. The data obtained indicate that 17.3% and 31.7% among children and adults respectively of a haploidentical HSC transplant potential recipients have preexisting HLA antibodies. HLA antibodies of class I and II are determined with approximately the same frequency in adults; in children HLA antibodies of class I are more often determined. However, class I and class II HLA antibodies are most frequently detected in both adult and children group. Of the HLA-sensitized potential recipients of haploidentical HSC, 34.78% (among children) and 31.94% (among adults) have DSA antibodies.

P51  |  Assessment of chimerism by next-generation sequencing: A comparison to STR-PCR method

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Chimerism analysis enables the assessment of engraftment and minimal measurable residual disease (MRD) for early detection of relapse in patients post-hematopoietic stem cell transplantation (HSCT). The objective of this study is to demonstrate the utility and sensitivity of novel multiplex next generation sequencing (NGS)-based chimerism assay compared with the gold standard Short Tandem Repeats-PCR (STR-PCR) method. Five patients were evaluated for chimerism and MRD at various times post HSCT. DNA was extracted from peripheral blood samples and chimerism testing was performed by a multiplex NGS-based chimerism assay and compared with STR-PCR method. Limit of detection for complete chimerism by NGS and STR-PCR methods was 0.1% and 5% respectively. The NGS-based chimerism assay identified 9 informative markers that were used to detect and quantify MRD less than 5%. In addition, NGS-based chimerism assay detected a wide range (0.1% - 92%) of recipient mixed chimerism (Table 1). In contrast, STR-PCR method detected at least 95% donor DNA yet was unable to quantify MRD below 5%. The multiplex NGS based chimerism assay is more sensitive and may
allow for better quantitative monitoring of engraftment and MRD post HSCT compared with the gold standard STR-PCR method.

**P52 | Chimerism analysis using next-generation sequencing**

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Recipient–donor chimerism is routinely analyzed after allogeneic hematopoietic stem cell transplantation (HSCT) to monitor engraftment and graft rejection. For malignancies, chimerism can also be used to screen for disease relapse. Short-tandem repeat (STR) genotyping is widely used to determine the proportions of donor and recipient cells after HSCT. In this study, the Devyser Chimerism NGS kit in combination with a MiSeq System was introduced in our laboratory for monitoring HSCT. This system is a complete workflow solution for labs with a designed-for-purpose analytical software. It’s based on targeted sequencing of 24 indels through the human genome and measuring their allele frequency. After a first step of comparison, the NGS system has replaced the STR method in diagnostic routine of our center. In order to carry out an internal validation, peripheral blood and bone marrow samples, coming from external quality control and diagnostic patients, were tested with the two methods to evaluate the performance of the NGS kit. Results showed good correlation between STR-NGS techniques. Moreover, thanks to a greater sensitivity (0.1%), NGS method shows to detect previously mixed chimerism and allows early diagnosis of relapse. The Devyser NGS kit is in use at the Genetic Diagnostics laboratory of the AOU Careggi in Florence for the analysis of post-HSCT chimerism since January 2019. A total of 1847 samples of post-HSCT patients have been analyzed: 1140 from peripheral blood and 707 from bone marrow. In total, 254 recipient/donor pairs were studied. All of them show >2 informative markers, except 8 cases (related) with only 2 informative markers and one case with only one informative marker. The mean of informative markers is higher in patients receiving an unrelated donor (9 markers) than in patients with a related donor (5 markers). We propose to expand the use of this highly sensitive method for detection by applying it to specific cell lines for improving relapse prediction.

**P53 | Non-malignant hematological diseases treated by haploidentical hematopoietic stem cell transplantation: anti-HLA antibodies and graft failure**

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In solid-phase immunoassays (SPI) performed on a fluoroanalyzer (Luminex), a positive test for DSA is considered when MFI is above 1000; although rejection can occur at any DSA level for MFI>1000, the likelihood of developing GF increases as the MFI levels increase. As currently documented in several studies, the incidence of GF appears to increase with MFI levels above 5000. In the present study, using One Lambda reagents (Thermo Fisher, Canoga, USA) on a Luminex platform, we determined the presence of IgG anti-HLA Abs in the sera of 58 hHSCT pediatric patients, affected by non-malignant hematological diseases (NMD) at the Pediatric Hospital Bambino Gesù in Rome including hemoglobinopathies (20), immune deficiency (25), severe aplastic anemia-SAA (5), Fanconi anemia—FA (6) and adrenoleukodystrophy (2). We observed a high proportion of IgG anti-HLA antibodies among the patients (68%): 16% of patients experienced a GF (9 out of 58). Among the 9 patients with GF we found the presence of anti-HLA antibodies in 8 patients: 3 showed levels of anti-HLA antibodies with MFI values included between 1000 and 5000 and 5 patients showed MFI values higher than 5000. In 3 patients we found preformed DSA. Patients who showed MFI greater than 5000 underwent desensitization procedures.

**P54 | KIR genotyping of hematopoietic stem cell haploidentical donors: A single center experience**

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Allogeneic hematopoietic stem cell transplantation is a lifesaving therapy for patients with hematological malignancies. A fully matched HLA donor is the best option, but the likelihood of finding a matched sibling donor is around 30% and Matched Unrelated Donors (MUD) are only available within a median time of 2–3 months from the start of the donor search. The use of alternative transplants, as haploidentical donors, has increased markedly this past decade. There are still no strict criteria for selecting a donor; if there are more candidates the donors against whom the patient has DSA (Donor Specific Antibodies) should be excluded. Research on the importance of the KIR/HLA relationship in the field of transplantation is in progress. Several studies showed 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. Since April 2022, in agreement with our transplant centers, we have performed KIR genotyping on all potential haploidentical donors. We typed KIR genes on 70 suitable haploidentical donors for 38 patients with various hematological malignancies by PCR-SSO Luminex method using the KIR SSO Genotyping Test (One Lambda Inc., Canoga Park, CA). We evaluated: genotype (A/A or B/x), B Content score (0-4), also defining if a donor is Neutral, Better or Best through the Donor KIR B-content group calculator (https://www.ebi.ac.uk/ipd/kir/matching/b_content/), and the presence or absence of alloreactivity according to the KIR/KIR-L mismatch model in the GvH direction using the KIR Ligand Calculator (https://www.ebi.ac.uk/ipd/kir/matching/ligand/). We found the following results: 12 A/A and 58 B/x; 34 Neutral, 27 Better and 9 Best; 26 alloreactivity in GvH direction and 44 no alloreactivity. Our preliminary results suggest potential benefits performing KIR genotyping in routine work to choose the best donor.

Blood samples and buccal swabs are widely accepted sources of DNA from hematopoietic cell transplant patients and donors for HLA typing. Here, we compare sequence quality and HLA typing results obtained from blood and buccal starting material to determine if one sample type is preferable. Patients in remission from malignant disease (n = 47) and healthy volunteer donors (n = 58) were included in the study, with both blood and buccal material collected from 32 patients and 6 donors. DNA was extracted on an automated platform for all sample types and was sequenced for HLA-A, -B and -C and -E in a single library on the PacBio Sequel. PHRED scores and PacBio cluster diversity metrics (lower score indicates less cluster variation) were compared for each sample type. Sequences from patient buccal swab DNA had significantly higher PHRED scores versus patient blood (33.2 vs. 32.7, p<0.05) and lower cluster diversity (0.06 vs. 0.11, p<0.0001), indicating that patient buccal DNA samples yield sequences of higher quality with less background errors. No difference was seen in sequences from donor blood and buccal DNA samples. Overall, blood DNA samples generated better HLA typing results that can be automatically accepted (80.7% vs. 79.8%) and had less allele dropout (2.0% vs. 7.2%) than buccal DNA samples. Importantly, where both sample types were provided, all typing results were concordant between blood and buccal derived DNA. Four sequences with novel variation were observed in patient blood samples and were also seen in their respective buccal samples, confirming they were not cancerous mutations. Despite the higher quality of sequences derived from patient buccal DNA, buccal samples were more prone to PCR failure due to lower DNA quality and concentration versus blood (84.5% vs. 93.8%, p<0.05). Overall, there is no difference in HLA typing results for blood and buccal DNA samples for the same individual and both are reliable sources for identifying novel sequence variation.
A 10-year-old female patient, diagnosed with common variable immunodeficiency, a candidate for haploidentical hematopoietic stem cell transplantation (HSCT), was referred for histocompatibility evaluation with her parents as potential donors. High-resolution HLA typings were performed by next-generation sequencing (AllType Fastplex, One Lambda/Thermo Fisher, Canoga Park, CA, USA) and revealed a nucleotide substitution in HLA-DQA1 gene at codon 229 in exon 4, which changes CAA (Glutamine) to TAA, resulting in a premature stop codon. This mutation was also observed in her father’s typing, so the possibility of a somatic mutation event was ruled out. The patient has the following paternal and maternal haplotypes, respectively: A*03:01, B*39:01, C*07:02, DRB1*01:01, DQA1*01new, DQB1*05:02, DPA1*02:01, DPB1*13:01, and A*02:01, B*44:03, C*02:02, DRB1*07:01, DRB4*01:01, DQA1*02:01, DQB1*02:02, DPA1*02:01, DPB1*11:01. In order to determine whether the new variant in HLA-DQA1 interferes with the patient DQ5 protein expression, HLA serological typing by complement-dependent cytotoxicity was performed (Lambda Monoclonal Typing Tray, One Lambda/Thermo Fisher). The result revealed a normal expression of HLA-DQ2 protein but the HLA-DQ5 was not detectable, demonstrating that the new HLA-DQA1 allele, present in the paternal haplotype, leads to a non-functional DQ molecule. Although HLA-DQA1 typing is not mandatory for HSCT purposes, its expression can alter the HLA phenotype of the recipient or the donor, thus potentially inducing alloreactivity in the rejection or graft-versus-host direction. This case illustrates the importance of null alleles identification and the complete recipient and donor HLA typing to minimize potential risks for post-transplant complications. This novel allele is in the process of submission to IPD-IMGT/HLA Database.

P57  |  Haplidential stem cell transplantation in a patient sensitized with donor specific antibodies

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Recent advances in stem cell transplantation have overcome the limitation represented by the availability of HLA-identical donors, making this procedure possible for almost all patients. Haplidential donors are increasingly being considered as suitable in the absence of fully HLA matched donors or if transplantation is urgent. It is now well established that donor-specific anti-HLA antibodies (DSA) present in the recipient’s serum represent an important barrier to the engraftment of donor cells and are associated with an increased risk of Primary Graft Failure (PGF): patients with preformed DSA have a 7.47-fold increased risk of PGF compared with patients without DSA; moreover, the association between DSA and PGF appears to be unaffected by the antibody MFI cut-off, primary disease, graft type, source of HSCs or pre-transplant desensitization. Here we describe the clinical cases of a 59-year-old female patient with high-risk chronic myeloproliferative disease and no HLA-identical related or unrelated donors for whom the possibility of a haploidential transplant with either of her children was considered. As per our procedure and local recommendations, the presensitization status was evaluated by testing the serum with Luminex technology; the patient had DSAs directed towards the paternal haplotype shared by both potential donors. As per our protocol, crossmatches were performed with complement-dependent cytotoxicity and flow cytometry methods, both with negative results. In the absence of other donors, the transplant center started the desensitization protocol, and our laboratory performed a careful monitoring of the DSA: after two sessions of plasmapheresis and IVIg the DSA levels went to zero and the patient was successfully transplanted. This case shows how important is a close collaboration between the transplant center and the HLA Laboratory in the management of the most complex cases, to provide the best assistance to patients undergoing stem cell transplantation.

P58  |  PCR-associated HLA-C allele drop-out in two related samples typed by next-generation sequencing—A cautionary tale

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Currently, HLA typing is performed using PCR-based methods such as Next Generation Sequencing (NGS) and Sequence-Specific Primer amplification (PCR-SSP). However, it is well known that allele dropout may be observed when using PCR-based methods due to preferential amplification during the PCR reaction, as well as some mutations leading to single nucleotide polymorphism (SNPs) in primer binding sites. Here, we report a case of HLA-C allele dropout in two related samples (patient and mother) typed by NGS with a PCR-based commercial kit (GenDx AmpX v2). The initial HLA-C and -B typing was C*04:01:01, B*35:08:01:01, *39:06:02:01, for the patient and C*05:01:01, B*39:06:02:01, *44:02:01 for the mother. The rare HLA-B*39:06:02:01 association with both HLA-C alleles and subsequent haplotype comparison between patient and mother, led to suspicions of possible HLA-C allele dropout in both samples. Further investigation by PCR-SSP identified the presence of HLA-C*07 in both samples, confirming allele dropout in the initial NGS typing. The investigation into the allele dropout was performed in collaboration with GenDx. Our research group identified the missing allele as HLA-C*07:02:01:138 utilizing PacBio SMRT DNA sequencing. The investigation concluded that the probable reason for the previously unseen allele dropout was the presence of one or more SNPs in a possible reverse primer binding region (3´UTR) for the commercial primer kit. As the sequence for allele HLA-C*07:02:01:138 on the IPD-IMGT/HLA Database 3.49 did not include full 3´ UTR sequence, primer design could not account for the SNPs present in the likely primer region. An extended sequence for this allele has now been submitted to the database and the full gene sequence (5´UTR to 3´UTR) will soon be available. Useful discussions were had with our provider, GenDx, following the discovery of this anomaly. This case highlights the importance of enriching the IPD-IMGT/HLA Database to aid future primer design.

P59 | Two cases of HLA mistyping in patients with acute myeloid leukemia before transplantation

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Leukemia is a clonal disease; it develops from a single cell. Mutations lead to a maturation disorder and proliferation of blasts (non-functioning blood cells). These blasts leak into the blood and lead to a displacement of functioning hematopoietic cells. HLA molecular typing is performed on peripheral blood samples. In the two patients described, there were no visually visible signs of blast crisis. NGS whole gene typing revealed heterozygous results for all typed HLA loci (HLA-A, -B, -C, -DRB1, -DRB345, -DQA1, -DQB1, -DPB1). The first sample, a 61-year-old woman with an AML, showed an apparent new allele at HLA- (HLA-A*01:01, 11:new). This putative new allele showed a single nucleotide exchange in exon 1, position 56 (T>C). Sanger sequencing confirmed this single base mutation. Subsequent typing of the patient’s son revealed a common HLA-A*11:01 for the maternal haplotype. However, HLA typing of the patient’s buccal swab also revealed the common A*11:01. In the second sample, a 45-year-old woman who also had AML, a single nucleotide exchange (exon 4, position 791 C>T) resulted in an HLA-B*44:new, in addition to an HLA-B*07:02. Subsequent typing of a buccal swab revealed two common alleles, HLA-B*07:02 and HLA-B*44:03. HLA typing results from patients with hematological malignancies should be viewed critically. Homozygous results possibly due to LOH (loss of heterozygosity), apparent new alleles or even rare alleles should be checked by re-typing on a buccal swab or on a peripheral blood sample from patients in complete remission. HLA mistyping leads to incorrect donor identification, in one case to a time delay with detrimental consequences, in the worst case to a selection of a mismatched donor.

P60 | Transplanting across a donor specific HLA antibody in hematopoietic stem cell transplantation

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A 41-year-old female with acute myeloid leukemia was referred to our laboratory for hematopoietic stem cell transplantation. The patient had no suitable related donors and no HLA antibodies at time of referral. An unrelated donor search was initiated and there were no 12/12 matched unrelated donors identified. However, there were three HLA mismatched donors identified: Donor 1. Female (18), HLA-DQB1, DPB1 (permissive)
mismatch 10/12, CMV matched. Donor 2. Male (41), HLA-A, DPB1 (permissive) mismatch 9/12, CMV matched. Donor 3. Male (37) HLA-B, DPB1 (G group) mismatch, CMV mismatched. Due to transfusions with unmatched blood products the patient became highly sensitized by the time of donor selection. HLA antibodies were detected using LABScreen® Luminex HLA antibody detection and Identification kits (One Lambda, Inc). Donor specific antibodies (DSAs) against all potential unrelated donors were identified, with two of the three donors having multiple DSAs present. Further investigation to determine complement binding of the antibodies were carried out by Immucor LIFECODES LSA and C3d kits. The donor with a single DSA HLA-B61 MFI 8,629 proceeded to work up. The transplant center decided to reduce the patient’s HLA antibody levels by performing two cycles of plasma exchange (PEX). Samples pre- and post-PEX were tested and the HLA-B61 (B*40:06) antibody levels declined from 8,629 MFI considered an intermediate risk to 2,319 MFI considered low/standard risk to transplant. The patient was supported by PEX post-transplant and DSA dropped to levels below our positive cut-off to standard level of risk. The patient was transplanted successfully, and engraftment has been shown to be taking place.

P61  |  Case report of a patient with acute myeloid leukemia complicated by recurrent hemophagocytic syndrome with pancytopenia and sepsis

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Here we report the case of a 58-year-old woman with acute myeloid leukemia with myelodysplastic-related changes diagnosed in March 2022 and a history of anemia since adolescence, later complicated by combined autoimmune disease associated with neutropenia. The patient’s leukemia was assessed as low clinical risk and intermediate risk by the European Leukemia Net. The patient was indicated for induction therapy, which was complicated by the development of recurrent hemophagocytic syndrome with pancytopenia, febrile neutropenia, and sepsis in April. The pancytopenia was treated with massive hemotherapy, the hemophagocytic syndrome with intravenous immunoglobulins and corticosteroids, and hematopoietic stem cell transplantation (HSCT) had to be indicated. The hemotherapy was complicated by the development of strong complement-activating HLA antibodies, which led to a severe post-transfusion reaction in April. The patient was transfusion dependent from March to September and HLA crossmatched platelets were selected for hemotherapy from April to July and HLA crossmatched erythrocytes from May to July. In June, the patient underwent allogeneic HSCT from an unrelated, female, HLA-matched donor. Bone marrow was used as the graft and the transplantation was performed in a non-myeloablative regime. Granulocyte engraftment occurred on day +12, but platelet engraftment did not occur until day +57. Complete donor engraftment occurred already on day +14 post-transplantation, but gradually from day +75, there was a slight increase in autologous hematopoiesis. At present day +150, microchimerism is detected - up to 1% of the recipient genotype (0.488%). The patient is considered at risk and it is necessary to monitor the dynamics of cellular chimerism at more frequent intervals. Currently, the graft is functional after overcoming many complications, the patient is not dependent on transfusions and regularly attends the hematology outpatient clinic.

P62  |  Activities of the Macedonian Bone Marrow Donor Registry in the search and match process

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The Macedonian Bone Marrow Donor Registry (MBMDR) was founded in 2010 and immediately started its activities in recruiting voluntary donors. The search for unrelated matched donors (MUD) in the WMDA database for national patients has been done since 2012, with high-resolution 10/10 match as primary criteria for donor selection. Additional criteria are donor age (younger donors preferred), HLA-DPBI1 match, CMV status and ABO. One hundred and seventy one searches for national patients were performed in the period of 2012-2022. Patients were referred to international bone marrow transplant centers for HSCT, until 2018, when MUD transplantation was established as routine therapeutic procedure at the University Clinic for Hematology in Skopje. Out of 116 searches done in last five years, one
third had less than 10 matched unrelated donors in the WMDA database and eight patients had zero available donors. One of the reasons was presence of alleles with low frequencies in these patients such as B*39:10, B*51:08 and DRB1*08:04. Since the first MUD HSCT in 2018, a total of 42 procedures were completed. In 2022 the first and the only one for now HSCT from national donor for national patient. Most of the patients (33) had 10/10 donor, eight had 9/10 and only one had 8/10 matched donor. HSC were imported from Germany (26), Poland (7), Turkey (4), Israel (2), Italy (1), UK (1) and Greece (1). Most of the donors were male (33 out of 42) and 39 were younger than 39 years of age. We had performed 39 searches for international patients in our Registry and sent seven samples for verification typing. PBSC apheresis was completed for three donors from MBMDR and the HSC were exported to USA, Bulgaria and Australia. The work of the MBMDR is very valuable in the search and import of HSC for national patients with hematological malignancies as well as providing donors for international patients.

**P63 | HLA-A, -B, -C, -DRB1, -DRB3/DRB4/DRB5, -DQA1, -DQB1, -DPB1 haplotypes in families of patients awaiting allogeneic hematopoietic stem cell transplantation**

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Family pedigrees remain the gold standard for studying HLA haplotype segregation. The aim of our investigation was to study HLA-A, -B, -C, -DRB1, -DRB3/DRB4/DRB5, -DQA1, -DQB1, -DPB1 haplotypes in families of patients awaiting allogeneic hematopoietic stem cell transplantation (HSCT). All individuals were HLA-A, -B, -C, -DRB1, -DRB3/DRB4/DRB5, -DQA1, -DQB1, -DPB1 typed by AllType FASTplex NGS Assay (One Lambda, USA) and sequenced using MiSeq (Illumina, USA). Haplotypes were built by family segregation. The frequencies of haplotypes were calculated by direct counting. We characterized 354 different HLA-A, -B, -C, -DRB1, -DRB3/DRB4/DRB5, -DQA1, -DQB1, -DPB1 haplotypes in 180 subjects belonging to 110 families. The most common haplotype was A*03:01:01:01~B*07:02:01:01~C*07:02:01:03~DRB1*15:01:01~DRB5*01:01:01~DQA1*01:02:01:01~DQB1*06:02:06:416 Q~DPB1*01:03:01~DPB1*04:01P (it was identified 4 times). The second common haplotype was A*03:01:01:01~B*35:01:01:05~C*04:01:01:11~DRB1*01:01:01~DQA1*01:01:01~DQB1*05:01:05:263~DPB1*01:03:01~DPB1*04:01P (it was identified 3 times). These two haplotypes had no associations with other DPA1-DPB1 alleles. Among HLA-A, -B, -C, -DRB1, -DRB3/DRB4/DRB5, -DQA1, -DQB1 haplotypes A*01:01:01:01~B*08:01:01:01~C*07:01:01:16~DRB1*03:01:01~DRB3*01:01:02~DQA1*05:01:02~DQB1*02:01:01:02:163N was the most common, it was determined 6 times. This haplotype was associated with 6 different DPA1-DPB1 pairs: DPA1*01:03:01~DPB1*01:01P, DPA1*01:03:01~DPB1*02:01:02, DPA1*01:03:01~DPB1*04:01P, DPA1*01:03:01~DPB1*04:02P, DPA1*01:03:02~DPB1*04:01P, DPA1*02:01:02~DPB1*01:01P. The obtained data on haplotype frequencies in families of our patients in need of allo-HSCT can be used for reviewing and predicting haplotypes in clinical HLA genotyping and a search for unrelated HSC donors.

**P64 | HLA-DRB3/4/5 and HLA-DRB1 allele and haplotype frequencies in hematological patients planned for hematopoietic stem cell transplantation**

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HLA-DRB3/4/5 genes of HLA class II are closely linked to the HLA-DRB1 locus. Because they are in strong linkage disequilibrium, the HLA-DRB1 antigen determines the presence or absence of the HLA-DRB3/4/5 gene. The DRB1 gene is expressed in each HLA haplotype. DRB3, DRB4 and DRB5 gene can be expressed as second DRB gene, depending on which DRB1 allele is already present in a haplotype. Our transplant centers protocols take into consideration HLA-DRB3/4/5 genes of mismatched unrelated donor. The aim was to analyze the HLA-DRB3/4/5 and HLA-DRB1 allele and haplotype frequencies in hematological patients planned for HSCT. Study included 304 hematological patients. HLA typing was performed by high resolution PCR SSP (Olerup SSP). The allele frequencies were estimated using the ARLEQUIN v3.1. The most frequent were: DRB3*02:02 (30.2%), DRB3*01:01 (13%), DRB5*02:02 (13.4%), DRB4*01:03 (11.7%), DRB5*01:01 (8%). DRB3*01:01, DRB3*02:02 and DRB3*03:01 alleles were observed in haplotypes with
DRB1*03:01. DRB3*02:02 was present in the haplotypes with five identified DRB1*11 alleles and with DRB1*12:01, DRB1*12:02, DRB1*14:01, DRB1*14:04 and DRB1*14:54. DRB3*01:01 and DRB3*02:02 were present in haplotypes with DRB1*13:01, DRB1*13:03, DRB1*13:05. DRB3*03:01 was observed with DRB1*13:01. DRB4*01:03 was observed in the haplotypes with seven identified DRB1*04 alleles. With DRB1*07 were DRB4*01:01, DRB4*01:03, DRB4*01:03:01:02N. With DRB1*15:01, DRB1*15:07 was DRB5*01:01 and with DRB1*15:02 were DRB5*01:02, DRB5*02:02 and DRB5*01:10N. DRB5*02:02 was observed in the haplotypes with DRB1*16:01 and DRB1*16:02. Analysis of HLA-DRB1-DRB3/4/5 haplotypes distribution can be used in donor selection.

P65 | High-resolution HLA allele frequencies in unrelated populations determined by next-generation sequencing

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The HLA system is one of the most polymorphic genetic systems in the human genome. Knowledge of HLA allele frequencies is important for the transplantation medical field and also brings upon new data on the genetic diversity among the various human populations. Previous studies have shown the impact of next-generation sequencing (NGS) in HLA typing. The main purpose of the study was to determine the HLA-A, -B and -C allele and haplotype frequencies in the analyzed Caucasian cohort. It is the first major study on the analyzed HLA variants for the Romanian population. The current study includes 420 healthy unrelated Romanian individuals which underwent genetic testing for HLA-A, -B, -C and through NGS sequencing on an Illumina Miniseq platform. Detected sequences were determined by MIA FORA software algorithms. HLA testing resolution was up to 6 digits. The most frequently observed tested HLA alleles were HLA-A (A*02:01:01, 26.11%; A*01:01:01, 12.5%; A*24:02:01, 11.67%), HLA-B (B*18:01:01, 11.25%; B*51:01:01, 10.83%; B*08:01:01, 7.78%) HLA-C (C*07:01:01, 17.36%; C*04:01:01, 13.47%; C*06:02:01, 9.44%). The most frequent haplotypes involving the 3 tested HLA alleles have also been analyzed. The results of the current study reveal the frequencies of the various MHC class I alleles in the Romanian population, with important implications for both the clinical and research areas and also for a more accurate HLA-match donor identification. Further studies are needed for the confirmation of the results and for the future involvement in and support for the national transplantation programs.

P66 | The automation of DNA quantification and normalization workflow through the introduction of the MaxPrep Liquid Handler

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Anthony Nolan employs multiple downstream processes post-extraction that show optimal performance at specific DNA concentrations. This validation details the transition to an automated workflow for the quantification and normalization of DNA in the laboratories. The Promega MaxPrep Liquid Handler, and GloMax plate reader complete a platform that processes samples of many types, utilizing the QuantiFluor ONE dsDNA system for accurate quantification. Standard curve preparation and fluid transfers are performed on the MaxPrep into a 96 well plate. Resulting DNA fluorescence is read on the GloMax, and the concentrations extrapolated from this data. Normalization and/or sample transfer steps are then completed by the MaxPrep into designated labware, to complete the sample journey. This is facilitated by the in-built Portal Access Software, which shares tracking and essential data between all instruments. 279 DNA samples were quantified and/or normalized across 60 runs to statistically assess accuracy, inter-assay and intra-assay repeatability in raw fluorescence/concentration results. The data analysis performed in this validation demonstrated the excellent accuracy and precision achieved when using the Promega platforms to quantify and normalize clinical samples, supporting the automation of this technique. Repeatability and accuracy metrics are shown to routinely fall below 10% CV, ensuring
consistent sample quality to benefit Anthony Nolan's NGS platform. The results from this validation, combined with the intuitive Portal software for data management, facilitate the integration with Anthony Nolan’s LIM system for improved sample processing and tracking. Scientist time is more effectively managed and a controlled flow of samples through the laboratory ensures efficient processing. The automation of this process improves reproducibility and reliability and allows Anthony Nolan a greater preparedness for business growth and continuity.

**P67 | External proficiency testing offered by the HLA Department of the Institute of Hematology and Blood Transfusion in Prague, Czech Republic: Detection of HLA alleles associated with diseases**

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The HLA Department of the Institute of Hematology and Blood Transfusion in Prague, Czech Republic offers EPT “Detection of HLA Alleles Associated with Diseases”. This testing is included in the EPI list of EPT providers and the extended variants fulfill the criteria of EPI standards. For the Czech participants annual workshop focused on the problems of the EPT is organized in February-March. More of genetic laboratories which were not specialized for HLA are now testing HLA Alleles Associated with Diseases. The program is offered in three types: 1) Alleles of DQ loci associated with celiac disease (CD): DQA1*02, *03, *05, DQB1*02, *03:02. 2) B*27 (association with Morbus Bechterev and other rheumatoid autoimmune diseases) 3). DQB1*06:02 (association with narcolepsy). All types are offered in standard variant (5 samples) and extended variant (10 samples). Results are evaluated in terms of correct determination of predisposing alleles/allelic groups and the clinical interpretation. Every year we notice some problems with the detection of CD associated alleles and the interpretation of results. Otherwise there were no problems with HLA-B*27 and DQB1*06:02. This EPT is performed twice a year. Efficiency and number of participants increase every year. All genetic laboratories willing to participate in our EPT are welcome. Please contact the guarantor Milena Vraná (milena.vran@uhkt.cz) or our assistant Barbora Kinská (barbora.kinska@uhkt.cz). Supported by the Ministry of Health, Czech Republic - conceptual development of research organization (00023736, UHKT).

**Immunogenetics in Organ Transplantation**

**P68 | Adsorption with X-match cells and Elution (AXE) protocol testing to clarify HLA antibody reactivity in a highly sensitized patient**

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A 59 year-old male with two previous renal transplants was listed for a third transplant in 2021. The patient was typed as HLA-A2, 24; B7, 44; Cw5, 7; DR11, 16; DQ7, 5 by a low resolution rSSO (LabTypeTM SSO, One Lambda) method. Interestingly, while the patient typed as HLA-B7, class I HLA single antigen bead (SAB) testing (LABScreen SAB HLA class I, One Lambda) showed a weak reactivity against HLA-B*07:02 allele. In this study we illustrate a detailed histocompatibility workup leading to the correct characterization of antibody specificity in the patient’s sera. Extended high resolution HLA typing performed by NGS (AllType FASTplex, One Lambda) at the HLA-B locus revealed that the patient types as HLA-B*07:20, 44:02, confirming the antibody directed against HLA-B*07:02 allele is not autoreactive. Sequence alignment showed two positions, 163 and 167, where B*07:20 (163L +167S) differs from B*07:02 (163E +167W). No described eplet could justify the entire class I HLA antibody reactivity. Therefore, we used the adsorption with X-match cell and elution (AXE) protocol with a 163E+167W donor cell (B*07:02, 08:01) followed by SAB testing to confirm the antibody reactivity in the patient’s serum. Eluate results after adsorption confirmed the presence of antibodies reacting with all HLA alleles containing 163E+167W polymorphism, including B*07:02. In addition, there was a reactivity with some HLA alleles expressing 163L+167W polymorphism. This reactivity could be explained by a 163E/L eplet. The AXE protocol allowed to demonstrate that the B*07:02 specific antibody, although weak, is real and probably due to exposure to HLA-B7 antigen in the second kidney donor (HLA-A2, 3; B7, 18; DR11, 15). This case emphasizes the importance of detailed histocompatibility testing and analysis, including high resolution typing, eplet analysis, sequence alignment and adsorption/elution in aiding with the interpretation and assignment of HLA antibody specificities in highly sensitized patients.
Desensitization protocols can improve access to transplantation for highly sensitized patients (HSP), who face a significant immunological barrier in finding a compatible kidney donor. Imlifidase, a bacterial protease, is a novel agent that cleaves all IgG subclasses, leading to a rapid antibody level decline and inhibition of IgG-mediated immune response. Here, we report the first HSP treated with imlifidase in Greece. The patient is a 49-year-old hypersensitized male (cPRA = 99%) who acquired priority for a deceased donor kidney after his HLA incompatible living donor donated a kidney to the national transplant waiting list (combination of list exchange and desensitization). Fourteen days later, the HSP received a renal graft from an ABO-identical deceased male donor of the same age. The patient had pre-formed anti-HLA-DR11, -DR14, -DR52, -DQ5 donor specific antibodies (DSA) with a cumulative Mean Fluorescent Intensity (MFI) value of 35735 and positive B-flow crossmatch prior to transplantation. Samples before (0h) and after imlifidase administration (2h, 4h, 6h) were sent to the Immunology laboratory for single antigen bead analysis and CDC and Flow crossmatches. At 2h post-imlifidase administration, all anti-HLA antibody specificities were detected with an MFI < 1000 and the positive B-flow crossmatch converted into negative. Cold ischemia time was 13 hours. The patient experienced delayed graft function. From day 7 post-transplant creatinine levels started to decrease and renal graft function continued to improve, despite the fact that all DSA rebounded on day 9. At the last follow up visit (day 212), the patient had stable renal graft function with a creatinine level of 0.93 mg/dl. Desensitization with imlifidase is a promising therapeutic strategy that can lead to a rapid decrease of anti-HLA IgG antibodies and crossmatch conversion within hours, thus enabling HSP to undergo kidney transplantation with minimal risk of hyperacute rejection.

Mismatched donor major histocompatibility class I chain-related gene A (MICA) has been reported to contribute to immune response following heart transplantation (HTx). For this study we used a cohort which consisted of 30 heart transplant recipients and their donors. Patient samples were sent to our Centre for post-HTx monitoring of HLA donor-specific antibodies (DSA). Retrospectively, we analyzed recipient-donor MICA allele mismatching and incidence of developing anti-MICA DSAs. Next generation sequence-based typing of MICA was carried out using NGSgo®-AmpX MICA, MICB commercial kit (GenDX). Detection of anti-MICA antibodies was performed using LIFECODES LSA MIC single antigen kit (Immucor). A total of 20 different MICA alleles were identified. The most frequent MICA among recipients was MICA*002:01 allele with a frequency of 24.1% while MICA*008:01 allele was the most common among donors with a frequency of 28.9%. The MICA*002:01 allele was found to be more frequent in heart transplant recipients ($p = 0.032, OR: 3.804, 95% CI: 1.151-12.578$). No MICA allele mismatches were observed for 3/30 (9.9%) donor-recipient pairs; one allele was mismatched for 11/30 (36.7%) and both alleles were mismatched for 16/30 (53.4%) pairs. Among MICA mismatched donor-recipient pairs, donor-specific anti-MICA antibodies were detected in 5/27 (18.5%) recipients. The most prevalent antibody specificity was MICA*008 (present in all five anti-MICA DSA positive recipients). This is in concordance with the most frequent mismatched allele group MICA*008 (34.3%) in the cohort. All anti-MICA DSA positive recipients were negative for anti-HLA antibodies. Our preliminary data indicate that MICA ought to be considered as transplantation antigens and developing anti-MICA antibodies should be taken into consideration during post-HTx monitoring.
P71  |  Efficacy and limits of anti-Rituximab antibodies use in cross-matches for patients treated with Rituximab prior to kidney transplantation in Grenoble University Hospital

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Rituximab (RTX), an anti-CD20 therapy, is commonly given prior to ABO or HLA incompatible kidney transplantation and its use in combination with Imlifidase in desensitization protocols is currently discussed. When used before transplant, RTX can interfere with lymphocytotoxicity and flow cytometry cross-matches (XMs) by inducing false positive results on B-lymphocytes. To get rid of this interference, we use an anti-RTX mouse monoclonal antibody to neutralize the RTX in sera of treated patients. In most cases, the amount of anti-RTX used is sufficient to clear the interference, but some XMs remain positive in patients who have had a very recent injection of RTX. The aim of this work was to define the serum concentration of RTX for which our protocol no longer counteracts RTX interference and therefore better predict the sera for which the protocol may not prevent false positives. A computer query of all XMs made with anti-RTX was performed in our database from 2017 to 2022. The results of XMs before and after anti-RTX, the posology and dates of RTX injections and immuno-adsorptions were collected. RTX-containing sera were retrieved from our biobank and sent to the pharmacology laboratory for mass spectrometry dosing. During the follow-up, 70 patients had a XM with anti-RTX for ABO and/or HLA incompatible kidney transplantation. A review of the data and RTX dosing is underway to determine in what % anti-RTX failed to negate the positive XM, and whether this remaining positivity was due to a high concentration of RTX in the serum or to the presence of anti-HLA antibodies specific of the donor. Finally, the impact of immunoabsorption sessions on sera RTX concentration is also being studied. To date, no such studies have been conducted to assess the efficacy and limits of anti-RTX on XMs. Recently, Imlifidase got the European use authorization for desensitization. In this context, the question of RTX use to avoid the rebound of HLA antibodies is of major importance.

P72  |  Quantification of plasma and urine Annexin-V positive microparticles as biomarkers in a Donor Specific Antibody positive kidney transplant population

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Renal transplantation (RT) is the best treatment for end-stage chronic kidney disease (CKD). In this study we were interested in the Annexin-V positive microparticles (MPs) profile in urine and plasma of RT patients to investigate whether MPs could be a biomarker of renal rejection. We included 62 RT patients: 30 with stable renal function, 15 with graft dysfunction due to fibrosis, 9 with donor-specific antibody (DSA) Antibodies Mediated Rejection (ABMR) and 8 with T-Cell Mediated Rejection (TCMR). As control cohorts we included 32 healthy volunteers, 47 and 45 patients with stages II and III CKD and stages IV and V, respectively. For each patient, we analyzed urinary and plasma annexin-V positive MPs levels by flow cytometry (Cytoflex, Beckman Coulter) with quantitative method (MP-Count Beads-Biocytex), targeting immune (CD19, CD3, CD16, CD56), endothelial (CD144), platelet (CD41), glomerular (podocalyxin), tubular (aquaporin-1), and renal regeneration (CD133) subpopulations. Total urinary MPs concentration (/μL) was similar between RT patients with stable graft function (2964 ± 2779/μL) and healthy controls (3477 ± 2863/μL) (p < 0.05). About RT patients with DSA positive ABMR compared with RT with stable renal function, we showed the following non-statistically significant trend: decrease of total, podocalyxin and CD133 urinary MPs. On the other hand, regarding total and subset plasma MPs, we failed to show a distinct profile between our patient populations. Total urinary MPs concentration (/μL) was similar between RT patients with stable graft function (2964 ± 2779/μL) and healthy controls (3477 ± 2863/μL), and lower in CKD cohorts according to the decrease of renal function (995 ± 1010/μL at end-stage) (p < 0.05). About RT patients with DSA positive ABMR compared with RT with stable renal function, we showed the following non-statistically significant trend: decrease of total, podocalyxin and CD133 urinary MPs. On the other hand, regarding total and subset plasma MPs, we failed to show a distinct profile between our patient populations. In this study we were able to identify a pattern of Annexin-V positive urinary MPs in a CKD cohort. Some urinary MPs subsets (podocalyxin, CD133) seem to be interesting to study in a RT cohort with DSA positive.
ABMR. However, our results must be confirmed by a larger scale study.

P73 | Antibody monitoring in highly sensitized kidney transplant candidate with preformed donor specific antibodies and desensitized with Imlifidase before kidney transplantation. First case in Italy

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A 43-year-old highly sensitized (cPRA 99%) woman, with lack of vascular access for hemodialysis, was selected to receive desensitization with Imlifidase prior to kidney transplantation from deceased donor. Antibody identification of the recipient showed the presence of 9 preformed donor specific antibodies (DSAs). The patient was treated with Imlifidase and monitored every two hours, up to 10 hours prior to transplantation. Imlifidase is a cysteine protease which cleaves all four IgG in F(ab)’ 2+Fc fragments incapacitating pathogenic antibody. The presence of DSAs was determined by testing the patient’s serum against a panel of fluorescent beads coated with single-HLA antigen preparation (Lifecodes Single Antigen, Immucor Transplant Diagnostic Inc., Stanford, USA) detected by X-MAP Luminex Technology. The level of MFI of preformed DSAs decreased immediately already 2 hours from the infusion. Our laboratory performed daily monitoring of the patient’s sera after kidney transplantation. DSAs remained absent until 6 days after transplantation and from day 7 we observed a rebound effect with the appearance of de novo DSAs. Moreover, preformed DSAs showed higher MFI than baseline. Rescue treatment with Eculizumab, intravenous immunoglobulin (IVIG), Thymoglobulin and plasmapheresis was essential to promptly treat early antibody mediated rejection (AMR). Post-transplant antibody monitoring was necessary to understand the best therapeutic approach to avoid allograft loss. Imlifidase is a new approach to desensitize high immunological risk recipients on waiting list to immediate remove antibody before transplantation, especially for patients with positive crossmatch. Our data confirm the absence of DSAs after Imlifidase infusion. The assay used by our laboratory did not detect any interferences due to cleaved IgG. Several studies are needed to minimize rebound effect of DSAs thus ensuring a better outcome for this category of patients and improving graft function and survival.

P74 | Donor specific HLA-DP antibodies in a highly sensitized kidney transplant recipient—A case report

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HLA-DP antibodies have been shown to be formed in 63% of highly immunized (HI) and 89% of kidney retransplant patients, which can be explained by a lower linkage disequilibrium between DQB1 and DPB1 compared with other HLA loci. Although HLA-DP donor-specific antibodies (DSA) have been associated with antibody-mediated rejection (AMR), their clinical significance remains controversial. To add to the current body of evidence, we report the case of a patient highly sensitized (vPRA 95%) with frequent HLA antigens during pregnancy and transfusions. Mismatched DPB1*03:01 with the child resulted in the development of a broad spectrum of HLA-DP antibodies detected by bead array technique (LABScreenSingleAntigen/OneLambda), including DPw13 (MFI 7800). The antibody epitope prediction algorithm (www.epregistry.com.br/) showed that 84 DAEV epitope was present in all detected antibody specificities. The DPA1 alleles were fully matched between mother and child. All but HLA-DP unacceptable antigens were entered into the Eurotransplant Network Information System. Due to lack of offers, a kidney carrying DPw13 was transplanted resulting in an immediate increase in DPw13 DSA (MFI 18900). The patient was desensitized by plasmapheresis and intravenous immunoglobulins and received induction with lymphocyte depleting antibodies. A surveillance kidney biopsy 14 days after transplantation revealed early acute AMR (C4d positive). The patient received steroid pulses and an additional cycle of plasmapheresis and intravenous immunoglobulins. Treatment with eculizumab was started. Allograft function gradually improved (serum creatinine 125–150 micromol/l and eGFR 32–41 ml/min/1.73m2). After 10 months, the patient was DSA negative, and the level of donor derived cell-free DNA determined by NGS (AlloSeqcfDNA/CareDx) was not increased (0.36%). This case report provides evidence that desensitization protocols may help overcome the incompatibility barrier with HLA-DP antibodies in deceased-donor kidney transplantation.
High resolution HLA typing in kidney transplantation increases the access to transplant in highly sensitized patients

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Renal transplantation is the best curative therapy for chronic kidney disease. One of the most relevant parameters for successful kidney transplantation is HLA compatibility. The development of new high resolution (HR) HLA typing technologies, such as next-generation sequencing (NGS), has allowed us to easily type HLA loci to protein level, allowing us to optimize HLA compatibility between donor and recipient. A pre-sensitized 65-year-old woman, diagnosed with chronic kidney disease secondary to p-ANCA+ vasculitis, was selected for kidney transplant from a deceased donor. HR HLA typing of the recipient was: A*11:01, 32:01; B*15:01, 51:01; C*03:03, 15:02; DRB1*04:01, 04:04; DRB4*01:01P; DQA1*03:01, -; DQB1*03:02, -; DPB1*04:01, -. Patient pre-transplant panel reactive antibody (PRA) was 100%, calculated by Single Antigen bead assay, including anti-DRB1*04. The HR typing of the deceased donor was: A*03, 23; B*15(63), 50; C*04, 07; DRB1*04:03, 04:05; DRB4*01:01P; DQA1*03, -; DQB1*03 (8), 04; DP1*01:03P, -; DPB1*02:01, -. The evaluation of HLA compatibility at low resolution indicated that donor and recipient shared DRB1 locus, but the recipient had IgG anti-DRB1*04 antibodies (MFI: 5773) and was initially considered incompatible. However, the pair was re-evaluated by HR typing and protein level for Single Antigen. Only antibodies against DRB1*04:02 were detected, which were not present in the donor typing without any other antibody targeting any DRB1*04 molecule. The cytotoxic dependent complement cross-match was negative and she received the kidney graft without showing any early post-transplant complication. This case highlights the importance of including HR HLA typing in kidney transplantation in order to optimize HLA compatibility and the use of antibody detection panels to distinguish between allele at HR level, which could be especially relevant for highly pre-sensitized patients.

HLA-C, -DQ and -DP relevance in cPRA calculation before and after kidney transplantation

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Introduction of the Luminex single antigen assay has improved the detection of HLA unacceptable antigens. The calculated PRA (cPRA) level represents the percentage of organ donors that express unacceptable antigens. The aim of this study was to analyze the importance of HLA-C, -DQ and -DP typing in PRA calculation. This retrospective study was an analysis of 117 anti-HLA positive sera from kidney transplant candidates (74) or recipients (43), tested using a SAB Luminex assay. HLA antigens with MFI > 1000 were assigned positive. To calculate cPRA in HLA fusion software, we used 3 populations of donors: 482 donors were typed for HLA-A, -B, and -DR, 77 donors were typed for HLA-A, -B, and -DQ and 136 donors were typed for HLA-A, -B, -C, -DR, -DQ, and -DP. cPRA were assigned respectively PRA_donors, PRA_donors_DQ and PRA_controls. HLA typing was performed using PCR-SSP or PCR-SSO (One Lambda). We calculated C, DQ and DP added PRA as follows: C-PRA = PRA_controls – PRA_donors, DQ-PRA = PRA_donors_DQ – PRA_donors, and DP-PRA: PRA_controls – PRA_donors_DQ. Locus-added PRA medians were compared according to kidney transplantation history. The frequencies of HLA-C antibodies were 29.5% in pre-transplant sera and 44% in post-transplant sera (p = 0.36). There was no significant difference between C-PRA pre- and post-transplantation (11.6% vs. 3.64%, p = 0.43). The frequencies of HLA-DQ antibodies were 52% in pre-transplant sera and 100% in post-transplant sera (p = 0.00001). DQ-PRA median levels were 20.1% in non-transplant patients and 53.2% in transplant patients (p<0.001). HLA-DP antibodies were detected in 47.6% of pre-transplant sera and 35.7% of post-transplant sera (p = 0.37). DP-PRA median level was significantly higher.
in non-transplant patients when compared with transplant patients (4.41 % vs. 0%, p = 0.01). By the present results, we provide recommendations to include HLA-DQ and HLA-DP typing in donor population used for PRA calculation.

P77 | Selection of blood collection tube is crucial for accurate quantification of dd-cfDNA following solid organ transplant

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To ensure accurate relative quantification of donor derived cell free DNA (dd-cfDNA) in patients following solid organ transplant, the plasma needs to contain minimal amounts of genomic DNA. If patient genomic DNA is present in the cfDNA sample, the relative amount of dd-cfDNA will be diluted and could result in false negative results. This study presents results of a comparison of different blood collection tubes and their performance in quantification of dd-cfDNA. Blood from four healthy donors was collected in either K2 EDTA or STRECK Cell-Free DNA blood collection tubes. A secondary genotype was added either into whole blood or isolated plasma to mimic dd-cfDNA. Storage conditions tested up to five days were for blood, RT and +4°C, and two additionally storage conditions -18°C and -80°C were performed for isolated plasma. Tested timepoints of storage were 1, 2, 3, 4, 5 days for whole blood and 1, 3, 5 days for isolated plasma. Extraction of cfDNA was performed using QIAsymphony DSP Circulating DNA Kit (Qiagen). All cfDNA samples were analyzed with Agilent TapeStation using the Cell-free DNA ScreenTape and Devyser’ NGS assay. TapeStation analysis of cfDNA showed an increase of genomic DNA in K2 EDTA tubes as early as day 1 after collection. The relative percentage of dd-cfDNA decreased from 0.38% to 0.26% after one day of storage in RT and was below the preliminary quantification limit of 0.1% of the NGS assay after two days of storage at RT. No effect of the integrity of cfDNA or percent dd-cfDNA was observed after five days of storage using STRECK Cell-Free DNA collection tubes. Storage conditions of plasma for five days did not affect the integrity, purity or percent dd-cfDNA regardless of blood collection tube. Results of this study show the importance of suitable sample collection tubes and storage conditions when analyzing cell free DNA. If K2 EDTA collection tubes are used, the plasma needs to be isolated immediately after blood collection.

P78 | The prozone effect - solving discrepancies in antibody testing in two cases

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The Luminex single antigen bead (SAB) test has become the most sensitive tool for identification of antibodies to HLA molecules. Despite the great advantage of this assay, false negative results at high antibody titer due to a prozone effect can be obtained. Within our Laboratory, HLA antibody testing is performed combining two methods, Luminex along with complement dependent cytotoxicity (CDC). We present two cases, separately for class I and class II HLA antibodies, in which the prozone effect was observed. In case 1, a 62 year-old-female, hematological patient refractory to platelets, was antibody screened by CDC and Luminex SAB tests (Immucor, SA1). By CDC, serum was tested as native, diluted 1:2 and treated with dithiothreitol (DTT). By Luminex, serum was in native state and diluted 1:8. CDC cross-match (XM) with potential platelet donors was performed. In case 2, a 27-year-old-male was monitored after living kidney transplantation. Serum was tested by CDC (native, 1:2 diluted and DTT-treated) and Luminex methods (Immucor, SA1,SA2) and in addition the XM with his mother’s T and B cells was performed. In case 1, CDC was positive with 70% PRA. XM results with donors having HLA-A2 were positive as well, although Luminex SA1 was A2 completely negative. Due to such a discrepancy, we applied serum 1:8 dilution and performed Luminex retesting. After that MFI values for A2 reached 20,000. In case 2, donor specific antibody (DSA) to HLA-DRB3*02:02 was detected with MFI 4000. Considering XM with mother’s B cells was positive with such a low MFI value, we diluted the serum to 1:8. After repeated testing, MFI value increased to 16,500. These examples support the conclusion that is good to combine two methods, and serum dilution when needed, in order to maintain sufficient sensitivity and, simultaneously, to avoid false negative results due to prozone effect.

P79 | Impact of SARS-CoV-2 on HLA serological phenotyping level in southern Portuguese solid organ donors

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ABSTRACTS
**P80 | HLA-A, -B and -DRB1 distributions among end stage renal disease patients in the Turkish population**

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HLA molecules play a key role in transplant medicine and disease pathogenesis, being a useful tool in predicting disease progression and identifying potential solid organ donors (SOD). The Coronavirus disease 2019 (SARS-CoV-2) pandemic had a huge worldwide impact, which strongly affected the activity of different transplant programs. So far, it has been shown that HLA type may be a crucial differentiator between individuals who have varying occurrence, morbidity, and mortality response to SARS-CoV-2. In this work, we investigated if differences in the frequency of SOD HLA alleles, were impacted during SARS-CoV-2 pandemic.

We performed a retrospective file audit of all HLA-typings done in 2 subsets of SOD pre-pandemic period (pp) (n = 379) and pandemic period (pp) (n = 351), collected in equivalent timeframes. We discuss data for the major HLA-A, HLA-B, HLA-C, and HLA-DRB1 allele groups at serological phenotyping level. Overall, there was a 7% SOD decrease in the pp. Considering both periods, the most common allele groups were HLA-A2, HLA-B35, HLA-Cw7, HLA-DR7 and HLA-DQ2. For the pp group, the most common allele groups were HLA-A2, HLA-B35, HLA-Cw7, HLA-DR13 and HLA-DQ2, while in the pp group the most common alleles were HLA-A2, HLA-B44, HLA-Cw7, HLA-DR4 and HLA-DQ2. When comparing both populations at the serological phenotyping level an increased in relative frequency was found for 10, 12, 8, 8 and 2, and a decreased was found for 10, 24, 8, 6 and 5 for HLA-A, -B, -C, -DR and -DQ, respectively. The significant variation within the HLA frequencies between the different pre-pandemic and pandemic groups highlights the value of population-specific HLA-typing. Furthermore, the identification of different frequencies among both populations will impact in patients HLA compatibility with SOD thus impacting their transplantability.

The aim of the study is to determine the HLA-A, -B and -DRB1 associations in the Turkish population among end stage renal disease (ESDR) patients who are transplant candidates. HLA-A, -B and -DR typing results of 1147 patients with ESRD and 447 unrelated healthy individuals were evaluated retrospectively. Low-resolution HLA typing was performed using sequence specific oligonucleotide (SSO) PCR. Allele distribution in the control and patient groups were calculated as frequency and percentages with 95% confidence intervals according to the Wilson method. Allele frequencies in ESDR and control groups were compared with Pearson's Chi-square test or Fisher's exact test. Benjamini-Hochberg correction was applied to p values and a p-value less than 0.05 was considered significant. There were no differences between the two groups in terms of allelic diversity for HLA-A, -B and -DRB1. The most common HLA alleles in healthy control and ESRD groups were A*02 (21.6% vs. 22.3%), A*24 (17.6% vs. 15.6%), A*03 (11.4% vs. 12.3%) and A*01 (9.4% vs. 9.9%) for HLA-A; B*35 (16.3% vs. 17.0%), B*51 (14.3% vs. 12.5%), B*44 (8% vs. 8%) and B*18 (6.6% vs. 6.93%) for HLA-B; DRB1*11 (22.3% vs. 22.3%), DRB1*04 (13.4% vs. 11.2%), DRB1*15 (11.2% vs. 9.6%) and DRB1*13 (10.51% vs. 10.07%) respectively. When the allele frequencies between the two groups were compared HLA-A*25 was observed higher in the ESRD group than the control group (33 vs. 1; 1% vs. 0.1%, respectively) and the difference was statistically significant (adjusted p-value = 0.006). No statistically significant difference was found for HLA-B and HLA-DRB1 allele frequencies between the two groups. Thirty patients with A*25 allele were evaluated and no common clinical feature other than ESRD was found. Studies with larger number of patients and controls are needed to present the possible relation of A*25 and ESDR in our population.

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**P81 | Sensitization of patients in need of kidney transplantation with HLA antibodies in the Republic of Kazakhstan**

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In the Republic of Kazakhstan, a waiting list is maintained and the level of HLA antibodies is determined quarterly by three laboratories, the results of which are entered into a single waiting list program. The objective to evaluate the HLA-A, -B, -C, -DRB1, and -DQ2 antigen frequencies and to determine the correlation with HLA antibodies in patients waiting for kidney transplantation. In this study, we collected DNA samples from 447 unrelated healthy volunteers and 206 end stage renal disease (ESRD) patients in the Republic of Kazakhstan. HLA-A, -B, -C, -DRB1 and -DQ2 typing was performed using sequence specific oligonucleotide (SSO) PCR. Allele distribution in the control and patient groups were calculated as frequency and percentages with 95% confidence intervals according to the Wilson method. Allele frequencies in ESDR and control groups were compared with Pearson's Chi-square test or Fisher's exact test. Benjamini-Hochberg correction was applied to p values and a p-value less than 0.05 was considered significant.
of the study was to identify patients with a positive status of HLA antibodies on the waiting list using Luminex technology. At the present time the most sensitive method is microsphere technology. All samples were screened for the presence of HLA antibodies using Luminex-based analysis using Labscreen Mixed kits and LABScreen PRA class I & II kits (One Lambda). Samples showing MFI greater than 1000 were identified as positive. All samples were divided by percentage into 3 groups of recipients: with low (up to 30%), with high (from 30% to 50%), and with very high sensitization (50% and above). 2053 samples of blood sera of patients from the waiting list were examined. Of the 2053 samples tested, 80.3% (n = 1646) tested negative for HLA antibodies and 19.7% (n = 404) tested positive. Out of 404 positive samples (n, %), 114/28% have a positive result in both classes, 262/65% samples are positive only in class I, 28/7% in class II. All samples that showed a positive result for class I and II antibodies were tested with LABScreen PRA. The level of HLA antibodies ranged from 2 to 97%. Of the 376 sera that showed a positive result for class I HLA antibodies, the separation of the recipient’s sera showed the following (n, %): 202/53 with low, 76/20.2 with high, and very high serum sensitization 98/26. According to class II, there were 142 positive sera in total. Of these, 51/36 sera in the first group, 31/22 sera in the second, and 60/42 sera in the third. The specificity analysis revealed the presence of antibodies against antigens of HLA-C, -DQ, -DP loci. As a result, the country specificity analysis found the presence of antibodies against antigens of HLA-C, -DQ, -DP loci. As a result, the country

MHC Evolution, Population Genetics

P83 | Differences in Allele Frequencies in the MICA and MICB genes between Sardinian and Caucasian/European Population

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MICA and MICB genes are non-classical MHC class I genes, located close to the HLA-B locus. Both genes are highly polymorphic and interact with NKG2D receptor

P82 | Transition from One Lambda to Immucor single antigen bead assays for HLA antibody assessment facilitates access to kidney transplantation in highly sensitized patients: a single-center experience

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Hyper-sensitized patients (with virtual panel reactive antibody [vPRA] ≥ 80%) have restricted access to kidney transplantation. Solid phase single antigen bead (SAB) assays are used to assess the anti-HLA immunization profile of kidney transplant candidates. We investigated whether the SAB kits from One Lambda and Immucor had an impact on vPRA of hyper-sensitized patients on waiting list. Transition from One Lambda to Immucor assays in our center took place in November 2020. In August 2021, we implemented a procedure aiming to revise the immunological profile of all patients registered on the kidney waiting list before November 2020, with vPRA ≥80%. Thirty-four patients were included in our study. If a significant discrepancy between the historic profiles tested by One Lambda and the more recent profiles tested by Immucor was revealed, the most relevant sera obtained before November 2020 were retested by Immucor. HLA specificities identified by One Lambda but no longer by Immucor on at least two sera were removed from the unacceptable antigens list. Median vPRA assessed with One Lambda vs. Immucor were 97.8% (ranges: 80–100) versus 92.7 (ranges: 0–100) (p = 0.002), respectively. After immunological revision, 55% (n = 19) showed a decrease in vPRA (median drop of 18.8%, p<0.001) while 45% (n = 15) had no modification. Six patients received a kidney graft carrying an HLA antigen previously listed as unacceptable with One Lambda and removed after the review. After a median follow-up of 90 (ranges: 28–353) days, all have an acceptable graft function without immunological complication. Our data suggest that the Immucor SAB test showed better specificity and was associated with a decrease in vPRA in a significant number of hyper-sensitized patients on waiting list, thereby increasing their chances of being transplanted. Small sample size and short follow-up are important limitations to our study.
which plays an important role in immune surveillance by activating NK cells and co-stimulating T cell subsets. Recently, several studies have documented MIC allele frequencies within different populations and the haplotype associations between MICA and HLA-B, which are in linkage disequilibrium. We examined 300 Sardinian individuals using an NGS method and compared their MICA and MICB allele frequencies to those of other populations. A total of 590 MICA alleles and 598 MICB alleles were detected on this study. In our population, the most prevalent MICA alleles were MICA*002 (18%), MICA*001 (17%) and MICA*008 (13%). All 107 MICA*002 alleles found were MICA*002:01; while among the 74 MICA*008 alleles, 69% were MICA*008:01, 26% MICA*008:04, and only 5% were MICA*008:02. Together with these three alleles, the MICA*018, *016, *004, *009, *012, *011, *007, *049 alleles make up a total of 95% of the alleles found. The most common MICB alleles, accounting for 98% of the total, were MICB*005 (53%), MICB*002 (24%), MICB*004 (11%) and MICB*008 (10%). Other alleles found showed a frequency of ≤2%. In contrast to MICB frequencies, MICA alleles differ significantly from those of other populations. Some studies show that the MICA*008, MICA*002 and MICA*004 alleles represent more than 50% of the total, and MICA*008 is described as the most common (25%–55%). Instead, in our series, MICA*008 does not reach 15% of the alleles found. (Pc < 0.00001). The allelic distribution of MICA in the Sardinian population may be associated with an increased risk of rejection in kidney transplants, as well as contributing to the high incidence of several autoimmune diseases observed in the region.

P84 | Distribution of HLA-DRB3 alleles in Spanish population

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DRB3 is the most polymorphic DRB gene after DRB1 with 446 reported alleles on the IPD-IMGT/HLA Database. DRB1*03, 11, 12, 13, 14 alleles are usually associated with DRB3. The two most frequent DRB3-associated haplotypes in the Spanish population include DRB1*03 and are the following: the ancestral haplotype 8.1 (A*01~B*08~C*07~DRB1*03~DQA1*05~DQB1*02) and the A*30~B*18~C*05~DRB1*03~DQA1*05~DQB1*02 haplotype with frequencies of 2.9% and 2.3% respectively. However, less is known about the DRB3 encoded on these haplotypes at protein level. The aim of this study was to investigate the distribution of DRB3 alleles in the Spanish population, and especially in the two most frequent DRB3 haplotypes. 318 volunteer bone marrow donors carrying DRB3 were full HLA typed using commercial next-generation sequencing (NGSgo®-MX11-3, GenDx) and the MiniSeq (Illumina). Data were analyzed according to NGSeinge® software and versions from 3.45 to 3.49 of the IPD-IMGT/HLA Database library. DRB3*02:02:01 was the most common allele (56.3%) in our cohort, followed by DRB3*01:01:02 (37.4%) and DRB3*03:01 (12.3%). We also analyzed DRB3 distribution in donors carrying DRB1*03:01 and lacking other DRB1 alleles associated with DRB3. Two DRB3 alleles were detected in this sub-group: DRB3*01:01:02 (49.3%) and DRB3*02:02:01 (50.7%). Interestingly, in DRB1*03:01 group all HLA-B*08 positive donors showed DRB3*01:01:02 allele, while all HLA-B*08 negative donors and 77.3% of HLA-B*18 positive donors carried DRB3*02:02:01 allele. Our results describe DRB3 polymorphism in Spanish population and indicate that DRB3 proteins encoded on established haplotypes are not always the same. Therefore, our data highlight the importance of including DRB3 locus in histocompatibility studies in order to improve donor-recipient matching for transplantation.

P85 | Aggressive behavior in Italian children with ADHD in the middle of COVID-19 pandemic: Preliminary data on MAOA gene polymorphisms involvement

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Attention Deficit Hyperactivity Disorder (ADHD) is characterized by oppositional, defiant, disobedient, disruptive and also aggressive behavior. Many genes are involved in
its onset, particularly dopaminergic pathway genes. Moreover, genetic predisposition to aggression appears affected by the polymorphic genetic variants of the serotonergic system, among which, functional polymorphisms in monoamine oxidase A (MAOA). The risk of contracting coronavirus infection may arouse in some people severe emotional distress characterized by symptoms of fatigue, guilt, and aggression. A survey on the psychological impact of COVID-19 pandemic in Italian families of children with neurodevelopmental disorders such as ADHD showed how children have been particularly affected by the emergency. The aim of this study was to determine whether polymorphisms at the MAOA gene are associated with increased or reduced susceptibility to develop ADHD. Therefore, the variants rs6323, rs58777457 and rs1137070 of the MAOA gene were evaluated by SBT in 35 children (mean age 10.257 range 6-16) with ADHD and 27 healthy individuals. Our analysis allowed us to identify the G/G genotype of the variant rs6323 (Arg297Arg) was significantly associated with increased risk of ADHD ($p = 0.015$). Allele G indicates higher levels of the enzyme, while the T allele indicates lower levels of enzyme production. When compared in patients, the G allele was associated with higher anger ($p$-value = 0.01) and might cause aggressive behavior in males. Our study shows that defining a genetic profile of ADHD can provide important information on the etiopathogenesis of the disease and help identify the best therapeutic option for patients with this disorder.

P86 | Haplotypes MICA-129Met/Val and HLA-B in the Russian populations of the Chelyabinsk region of 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. In the current study we got haplotype frequencies and linkage disequilibrium HLA-B~MICA-129Met/Val in the major populations of Chelyabinsk region—the Russians. A group of 100 unrelated normal healthy Russian blood donors living in the Chelyabinsk region (Russian South Urals) were included in population study. Their belonging to the Russian ethnicity was determined by their family history at least three generations and their speaking Russian. HLA-B genes were sequenced on a MiSeq platform using reagent kit HLA-Expert (DNA-Technology LLC, Moscow, Russia), while MICA typing used PCR-SSP. Population genetic analysis included tests for Hardy-Weinberg equilibrium (HWE), haplotype frequencies and linkage disequilibrium using Arlequin 3.5. We obtained the following results: haplotype HLA-B~MICA-129Met/Val (haplotype frequencies %; linkage disequilibrium D$^2$): B*07:02~Val (14.5%;1), B*08:01~Val (6.5%;1), B*13:02~Val (6%;1), B*14:02~Met (1.5%;1), B*15:01~Val (9%;1), B*15:16~Met (0.5%;1), B*18~Met (5.5%;1), B*27:05~Met (6%;1), B*35:01~Met (8%;0.92), B*35:01~Val (0.5%;0.92), B*35:03~Val (1%;−0.24), B*38:01~Met (2%;1), B*39~Met (4%;1), B*40~Val (5%;1), B*41:01/02~Val (3.5%;1), B*44~Val (7%;1), B*51:01~Val (6%;0.78), B*51:01~Met (0.5%;−0.78), B*52:01~Val (2.5%;1), B*57:01~Met (3.5%;1). Only three allelic families HLA-B*15, B*35, B*51 change isoform of MICA-129, which may pose a potential threat of mismatch during transplantation.

P87 | Study of HLA-B51 and HLA-B27 antigen expression in non-infectious uveitis

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Uveitis represents a group of heterogeneous clinical entities characterized by a multitude of etiologies including: idiopathic, infectious, inflammatory or solely ocular. The aim of this study was to describe the etiological, clinical and paraclinical characteristics, as well as the therapeutic and evolutionary characteristics of uveitis of our patients and the contribution of HLA (B27, B51) typing in the diagnosis and the prognosis of uveitis. It was a retrospective descriptive study of 31 cases of non-infectious uveitis, followed in the internal medicine department of Military Hospital of Tunis. A serological study of the expression of the HLA-B27 and -B51 has been realized among our patients through a technique of complement-dependent microlymphocytotoxicity in immunology department. In our series, the uveitis was more often anterior in 42% of
cases. It was unilateral in 77.4% of cases and acute in 93.5% of cases. The systemic etiology was found in 45.2% of patients. HLA typing showed a significant association between the anterior uveitis and the HLA-B27 ($p = 0.04$). This association is more marked in patients with ankylosing spondylitis (AS) ($p = 0.01$). 22.5% of cases were positive for HLA-B51. Furthermore, positivity of this antigen is statistically correlated with chronic uveitis ($p = 0.01$). Based on our results, we suggest that HLA typing is of limited utility in the diagnosis (positive and etiologic) and prognosis of non-infectious uveitis. However, search for HLA-B27 may help to positively diagnose and guide the etiological research in the case of anterior uveitis associated with AS.

P88 | Possibility of linkage disequilibrium between SNP-197 of IL17 and HLA class I and II in the Bashkir Chelyabinsk region

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The HLA genes are located on the short arm of human chromosome 6. The MHC class I and II molecules are the most immunogenic antigens, recognized during rejection of an allogeneic transplant. The strongest determinant is HLA-DR, followed by HLA-B and -A. These 3 loci are the most important for matching donor and recipient. Some autoimmune disorders are linked to specific HLA alleles. The distribution of different HLA structures is not the same and depends on ethnicity. The IL17 gene is located on chromosome 6 (6p12). The aim is to investigate linkage disequilibrium between SNP -197 IL17 and HLA class I and II. A group of 65 unrelated Bashkir healthy blood donors living in the Chelyabinsk region were included in population study. Their belonging to the Bashkir ethnicity was determined by their family history for at least three generations. DNA typing of HLA genes was performed by PCR SSP using sets of Protrans (Germany). SNP -197 G/A IL17 was detected by allele-specific PCR (“Lytech” Co. Ltd, Russia). The haplotype frequencies (HF) and linkage disequilibrium (D’) were analyzed using Arlequin 3.5. We obtained the following results. The frequency distribution of SNP -197 G/A of the IL-17A gene is characterized by a rather low frequency of the allele with a replacement and its heterozygous genotype. In the Bashkir population the linked inheritance of the -197*A IL-17A polymorphism with HLA-DRB1*15, -A*03 and -DQB1*06:01 genes and -197*G IL-17A with the HLA-A*02 allele was revealed. According to our data, the formation of haplotypes between SNP-197G/A IL17 and HLA is possible. The relevance is associated with the need for a population analysis of HLA and associations with other genes, such as IL17, in various ethnic groups, and in using the data obtained to study the genetics of HLA-associated diseases, for anthropological research.

P89 | Distribution of the MHC patterns of Mexican Mestizo populations from the states of Durango versus Oaxaca and Cdmx

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NGS molecular HLA information reveals the structure and history of admixed populations. The ethnic and genetic composition of the different regions in Mexico differs greatly, because of the distinct Indian groups settled and Europeans established. The Durango samples from Northwest Mexico were compared with those in Mexico City (the Highlands) and Oaxaca (Southwest), to understand their different HLA diversity and origins. DNA was extracted from peripheral blood of the three Mestizo groups: 90 from Durango, 77 from Mexico City, and 112 from Oaxaca. The Maxwell16 instrument was used for extraction and NGS typing on a MiSeq performed for 11 HLA loci. For the Durango samples, One Lambda and the TSV Software v3.1 were used MIFAORA NGS Kit (Immucor, Inc.) with the FLEX v3.0 alignment software, for CDMX and Oaxaca. Statistical analysis was done with the BIGDAWG software; $p<0.05$ was considered significant. The most frequent alleles in Durango are A*02:01, A*24:02, B*51:01, B*40:02, C*04:01, C*03:04, DRB1*08:02, DRB1*07:01, DQB1*03:02, DQB1*03:01. In CDMX they are A*02:01, C*04:01, B*35:01, DRB1*08:02, DQB1*03:01. The prevalent haplotype in Durango is A*02:01~B*14:02~C*08:02~DPA1*01:03~DPB1*04:01, DQA1*01:01~DQB1*05:01~DRB1*01:02, (HF = 0.0167). Two significant haplotypes were found, with a lower frequency in Durango: A*02:01~B*07:02~C*07:02~DRB1*1501~DQA1*01:02~DQB1*06:02 ($p = 0.03$) versus CDMX; and A*68:03~B*39:05~C*07:02~DPA1*01:03~DPB1*01.
P90 | Recombination between HLA genes in two siblings

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Allogeneic hematopoietic stem cell transplantation remains an important treatment option for hematopoietic disorders. Comparison of recombination proportions with the corresponding physical distances provides evidence for the existence of hot spots of recombination; the recombination rate is expected to be 1% per megabase. Here we will present a case study of a Macedonian family including a patient suffering from acute lymphoblastic leukemia. For an HLA matched donor search, the patient and the whole family were typed by an SSO method. Further analysis was performed by next generation sequencing (NGS) for eleven loci and additionally HLA-E, -F, -G and -H loci. The results confirmed our earlier typing of the classical HLA loci and additional HLA-E, -F, -G and -H loci. The results confirmed our earlier typing. Recombination between maternal haplotypes is not that rare and has been detected in our laboratory several times. However, the finding of two recombinations in one family might indicate an enhanced susceptibility for recombinatorial events.

Psoriatic arthritis (PsA) is an inflammatory rheumatic disease belonging to the family of spondyloarthritides. It is a multifactorial disease whose genetic determinism is still poorly understood. Angiotensin-converting enzyme (ACE) is a key enzyme involved in the regulation of inflammatory signal transduction pathways. The insertion/deletion (I/D) polymorphism of intron 16 of the ACE gene determines plasma and tissue levels of ACE, particularly in synovial fluid, and is considered to be one of the candidate genetic markers of susceptibility to Ankylosing Spondylitis. We were interested in studying this polymorphism as a susceptibility factor to PsA in the South Tunisian population. Our study population was composed of 31 patients from Southern Tunisia followed for PsA and meeting the CASPAR 2006 criteria. Our control population consisted of 115 unrelated healthy individuals. For both groups, ACE genotyping was performed by an amplification technique using specific primers (SSP-PCR). The statistical study was performed using R. The results of molecular genotyping of the ACE I/D polymorphism allowed us to determine the three genotypes (II, ID, and DD) of both patients and controls populations. Analysis of genotype frequency distributions revealed a statistically significant difference between the two groups ($p = 0.0131$). The frequencies of DD and II genotypes were respectively 77.4% and 6.45% in the patient group and 47.8% and 12.2% in the control group. Analysis of the allele frequency distribution of the D allele revealed a statistically significant difference between the patients and controls groups (85.5% vs. 67.8%, $p \leq 0.01$). In our study, we found an association between
the I/D polymorphism of intron 16 of the ACE gene and PsA in the Southern Tunisian population. The DD genotype and the D allele were associated with PsA. On the other hand, I allele seems to have a protective role against this disease.

P92 | Association between migraine and HLA-B and HLA-DRB1 gene polymorphisms in a southern Croatia population

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Migraine is one of the most widely distributed neurovascular conditions characterized by wide range of disease severity and complex genetic background. As one possible genetic factor in migraine pathogenesis, some previous research addressed HLA complex - a highly polymorphic gene system which plays a key role in human adaptive immune responses. In this study we aimed to examine the associations of HLA-B and HLA-DRB1 genes with migraine or headache in population-based settings. The case group consisted of 46 self-reported headache or migraine patients in the southern Croatia region. Samples from both patients and healthy blood donors used as control group were genotyped for HLA-A, -B and -C genes. Class II loci are covered for HLA-DRB1 and DQB1, and exons 2-5 for HLA-DPB1. Besides these novel alleles, an additional 102 alleles were observed only once. Looking at their statistical significance by Fisher’s exact test. Compared with controls, no significance in age distribution was found, while the group of patients showed a slightly increased number of women than healthy subjects. A couple of HLA-B alleles showed significant differences when compared between the patient and control groups: HLA-B*15 was increased in patients, while surprisingly, HLA-B*35 showed a significant decrease in people suffering from migraines. Some differences were also noticed comparing HLA-DRB1 allele frequencies in both groups - HLA-DRB1*11 was significantly higher in migraine group while HLA-DRB1*13 showed only mild (though not significant) decrease. Several previously published research addressed possible HLA loci association with migraine pathology in Italian, Taiwanese and Brazilian populations, albeit with different outcomes from the results of our research, which is presumably due to large HLA system polymorphism and population specificities. Further investigations in larger clinic-based migraine patients cohorts subdivided according to latest classification criteria are needed to confirm these findings.

P93 | Next-generation sequencing reveals and validates HLA polymorphism among Croatsians

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Implementation of next generation sequencing (NGS) in a routine clinical HLA laboratory typing brings extra information but also challenges in data analysis. First, 1268 samples were typed in our Tissue Typing Centre at University Hospital Centre Zagreb, with NGSgo-MX6-1 (GenDx) for HLA-A, -B, -C, -DRB1, -DQB1 and -DPB1 loci, revealing 19 novel HLA alleles. Among them, 9 (47.4%) were single amino acid substitution variants when compared with the most similar allele (HLA-A*01:200, A*02:836, A*02:1079, B*08:251, B*18:169, B*40:517, C*04:477, C*05:276 and DPB1*1146:01). Four alleles (HLA-B*15:18:08, B*18:37:02, B*27:02:06 and C*12:03:80) had silent substitution with no change in amino acid sequence and remaining six alleles (31.6%) showed only intronic mismatches to their most similar allele (HLA-A*01:200, A*02:836, A*02:1079, B*08:251, B*18:169, B*40:517, C*04:477, C*05:276 and DPB1*1146:01). Four alleles were observed only once. Looking at their status in the CIWD catalogue (version 3.0.0.), compiled from data provided according to latest classification criteria are needed.
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The beta chain of the HLA-DR molecule is encoded by multiple genes, and depending on the DRB1 allele, a second DRB gene can be encoded on the haplotype. DRB1*04, *07 or *09 alleles have been associated with DRB4, which counts 223 reported alleles in the IPD-IMGT/HLA Database, 25 of which are considered nulls. DRB4*01:03:01:02N is one of these un-expressed alleles which is frequently accompanied by DRB1*07:01~DQB1*03:03, but it has been also unusually reported with DRB1*04. Recently, the first case of association between DRB4*01:03:01:02N and DRB1*04 in a Spanish solid organ donor was published; however, we were unable to determine the protein level of DRB1*04. The aim of the study was to investigate the association between HLA-DRB4*01:03:01:02N and DRB1~DQB1 haplotypes in a Spanish population. 252 volunteer bone marrow donors positive for DRB4 were full HLA typed using commercial next-generation sequencing (NGSgo®-MX11-3, GenDx) and the MiniSeq (Illumina). 27 donors (10.7%) carried DRB4*01:03:01:02N allele, and 21 of them (77.7%) showed the well-established association with DRB1*07:01~DQB1*03:03 haplotype. In three (11.1%) donors we were unable to determine the HLA class II haplotype, due to the presence of a second DRB4 associated allele. Interestingly, in 3 (11.1%) donors DRB4*01:03:01:02N allele was observed as part of the DRB1*04:02~DQB1*03:02 haplotype. No cases of DRB4*01:03:01:02N~DRB1*09 haplotype were detected. Our results confirm the presence of the unusual DRB1*04~DRB4*01:03:01:02N association in a Spanish population and indicate that this null allele is not only encoded on the haplotype DRB1*07~DQB1*03. Moreover, our data suggest that DRB4*01:03:01:02N could be included in DRB4*01:03:01:02N~DRB1*04:02~DQB1*03:02 haplotype in a Spanish population.

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The HLA system is highly polymorphic and 33,000 alleles are described to date. However, the serological equivalents of some of these alleles are not yet known, which is useful for transplanted patients with HLA antibodies. The aim of this study, initiated by the SFHI (Société Francophone d’Histocompatibilité et Immunogénétique) in 2020, was to fill some gaps in serological assignments. All French HLA labs were invited to send their results of HLA typing performed by NGS (2-field) on their stem cell and organ recipients and donors performed between 2016 and 2020. The frequency of HLA alleles was then calculated to define the alleles of interest (frequency >0.01%). Serological expression of these alleles was studied using the One Lambda monoclonal typing trays, by 5 centers. Nearly 80,000 typings were collected from 18 labs. We focused on the following 24 alleles: 8 HLA-A (HLA-A*01:25, 02:286, 11:29, 23:17, 26:12, 26:27, 32:26, 68:24), 5 HLA-B (B*18:18, 39:31, 39:42, 51:193, 56:55), 6 HLA-DRB1 (DRB1*01:77, 04:38, 04:92, 11:12, 11:24, 11:28) and 5 DQB1 (DQB1*02:04, 03:09, 03:19, 05:05, 06:11). For eleven of them less than 2 donors were available. Thirteen antigens were studied among them which 6 clearly have a same serological expression as the closest most common allele, 6 alleles have a weak to normal expression with some allo-antisera. One has no serological expression at all. Interestingly, the allele A*32:26 was not expressed on the cell surface on 4 different cells in 2 labs, despite the nucleotide substitution in exon 3 (codon 163, ACG>AGG, Threonine > Arginine) did not suggest such a result. This should be taken into consideration for
virtual cross-match with a patient or a donor HLA-A*32:26. To conclude most of the tested alleles had the expected phenotypic expression probably because only one substitution was observed for 75% of them, even if this mutation occurred in exon 2 or 3 (83%).

**P96 | Distribution of MHC-C encoded C1 and C2 epitopes and inhibitory KIR repertoire potential in West-African chimpanzees and humans**

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In humans, in particular HLA-C has evolved to function as ligand for killer cell immunoglobulin-like receptors (KIR), expressed on NK cells and subsets of T cells. All HLA-C allotypes possess a C1 or C2 epitope, which are discriminated by the presence of asparagine (N) or lysine (K) at amino acid position 80, respectively, and can be specifically recognized by lineage III KIR. In contrast, only a minority of the HLA-A and -B allotypes carry a specific KIR target epitope that is recognized by NK cells. As such, the regulation by inhibitory KIR is mainly dictated by HLA-C. All chimpanzee Patr-C allotypes also have a C1 or C2 epitope. In contrast to humans, chimpanzees have a more diverse lineage III KIR repertoire recognizing C1 or C2, with a stronger binding affinity. In the present study, the distribution of C1 and C2 epitopes and the inhibitory KIR repertoire have been investigated in 25 founder animals of a West-African chimpanzee population. A high allele frequency of C2 bearing Patr-C allotypes was observed in this panel. The majority of the animals are homozygous C2/C2 (n = 11), or heterozygous C1/C2 (n = 10), and only a few animals were homozygous for C1/C1 (n = 4). Similarly, chimpanzee subspecies of other geographic origins were found to display a high allele frequency for C2-positive allotypes. In contrast, most human populations show a higher allele frequency of C1 carrying HLA-C allotypes. Exploring the inhibitory KIR repertoire in the same cohort of chimpanzees revealed that the majority of the animals have haplotypes encoding two KIRs having C2-avidity, either in combination with 0, 1, or 2 KIRs with C1-avidity. These data suggest that in chimpanzees a C2-mediated KIR interaction seems to be most prominent and was likely enriched by natural selection.

**P97 | Identification of 8-Digit HLA-A, -B, -C, -DPA1, -DPB1, -DQA1, -DQB1 and -DRB1 allele and haplotype frequencies in a South Tunisian population**

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The development of next-generation sequencing (NGS) methods for HLA genotyping has already had an impact on the scope and precision of HLA research. We performed HLA NGS in a south Tunisian population to estimate HLA-A, -B, -C, -DPA1, -DPB1, -DQA1, -DQB1 and -DRB1 allele and haplotype frequencies up to an 8-digit resolution, which might be useful for an extended application of HLA results. In this study, allelic resolution HLA typing was obtained for 136 unrelated volunteers from South Tunisia. The data were produced by high-throughput NGS. All samples were genotyped for 8 HLA loci, namely HLA-A, -B, -C, -DPA1, -DPB1, -DQA1, -DQB1 and -DRB1. There were 40 different alleles of HLA-A, 79 of HLA-B, and 51 of HLA-C. HLA-A alleles showing frequencies of ≥10% were A*02:01:01, A*01:01:01 and A*24:02:01. For HLA-B, HLA-B*08:01:01, B*50:01:01, B*13:02:01, B*49:01:01 and B*07:02:01 were the more frequent alleles. The most common individual HLA-C representative was the HLA-C*06:02 (16.54%) allele, followed by HLA-C*07:01 (11.4%) and HLA-C*04:01 (9.14%). Class II loci had 43 different alleles of DPA1, 108 of DPB1, 39 of DQA1, 41 of DQB1 and 69 of DRB1. Among DRB1, HLA-DRB1*07:01:01 (13.51%) and HLA-DRB1*03:01:01 (7.00%) were most commonly present. In the end, our study has identified for the first time the frequencies alleles of HLA-DPA1, DPB1 and DQA1 in a healthy
population from southern Tunisia. In conclusion, these new data can be used as representative Tunisian data for further disease-related HLA type analysis.

P98 | Endoplasmic reticulum aminopeptidase gene polymorphism and susceptibility of psoriatic arthritis in a south Tunisian population

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Endoplasmic reticulum aminopeptidase (ERAP1) belongs to the M1 family of zinc-dependent metallopeptidases, involved in the final processing of HLA class I ligands and with a significant influence in the stability and immunological properties of HLA class I proteins. An association of single-nucleotide polymorphisms (SNPs) rs27044 (G/C) and rs30187 (A/G) of the ERAP1 gene with Ankylosing Spondylitis has been reported in the literature. We were interested in studying these polymorphisms as factors of susceptibility to Psoriatic arthritis (PsA) in the South Tunisian population. Our study population was composed of 36 patients from southern Tunisia followed for PsA and meeting the CASPAR 2006 criteria. Our control population was formed by 119 unrelated healthy individuals. For both groups, the genotyping of the rs27044 and rs30187 SNPs of the ERAP1 gene was performed by an amplification technique using specific primers (SSP-PCR). The statistical study was performed using the R language. For both patient and control populations, the results of molecular genotyping allowed us to determine the genotypes (GG, GC, and CC) for SNP rs27044 and the genotypes (AA, AG, and GG) for SNP rs30187 of the ERAP1 gene. For SNP rs27044, the frequencies of CC and GC genotypes were respectively around 50% and 16.7% in the patient group and in the range of 51.8% and 9.82% in the control group. For SNP rs30187, the frequencies of AA and AG genotypes were respectively in the range of 50% and 16.7% in the patient group and in the range of 51.8% and 9.82% in the control group. No statistically significant difference was shown between the two groups (p>0.05). In our work, the study of rs27044 (G/C) and rs30187 (A/G) polymorphisms of ERAP1gene in patients with PsA in Southern Tunisia showed no difference with the control population.

P99 | Distribution of the HLA-DPA1 and -DPB1 alleles in a South Tunisian population

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The purpose of this work was to establish for the first time the distribution of HLA-DPA1 and DPB1 alleles in a south Tunisian Population. DPA1 and DPB1 frequencies were determined in 136 unrelated and healthy samples. Typing was performed by NGS. Forty three different alleles of DPA1 and 108 of DPB1 alleles were observed with a heterozygosity index of 0.92 and 0.97, respectively. The DPA1 alleles 01:03 (gf=0.59) and 02:01 (gf=0.2638), were most frequent in South Tunisian population, followed by 02:02 (gf=0.048), 01:12 (gf=0.037) and 03:01 (gf=0.0184), whereas 01:04 and 02:07 were present only once. HLA-DPBI*04:01 predominates in South Tunisian population (gf=0.277). Other frequent HLA-DPB1 alleles were: DPB1*02:01, DPB1*104:01 and DPB1*04:02, (gf=0.165; 0.088; 0.080, respectively). Rare alleles (gf≤0.03) were DPB1*06:01, DPB1*14:01, DPB1*05:01, DPB1*09:01, DPB1*11:01, DPB1*15:01, DPB1*16:01, DPB1*105:01, DPB1*131:01 and DPB1*131:01. Knowledge of HLA-DPB1 and DPA1 frequencies in South Tunisian population is important for accurately interpreting HLA typing data especially in solid organ transplantation.

P100 | Detection of HLA-A and HLA-J haplotype diversity from next-generation sequencing data in commercially available samples

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HLA-J is located in the MHC centromeric to HLA-A. With increasing evidence to suggest matching of patients
at the haplotype level improving transplant outcomes, and recent evidence suggesting HLA-J may possess transcriptional activity, current IPD-IMGT/HLA Database naming still assumes pseudogene status for HLA-J. This study aims to investigate potential haplotype associations between HLA-A and HLA-J. Enrichment was performed on 216 International Histocompatibility Working Group and 96 UCLA DNA exchange gDNA libraries using AlloSeq Tx17 (CareDx) targeted hybrid capture technology with additional HLA-J probes. Sequencing was performed on Illumina next-generation sequencing instruments, and typing was completed using AlloSeq Assign analysis software. Haplotype analysis was conducted using HLA-J and HLA-A sequencing data in Arlequin v3.1 software. Sequence alignment and phylogenetic analysis were conducted using Clustal Omega v1.2.2 and Mega v11.0.11 software, respectively, and compared with putative HLA-A serological groups. Haplotype frequency estimation of HLA-A and HLA-J from 188 IHW samples and 78 UCLA samples revealed 96 possible haplotypes. Previous analysis of HLA-J coding sequence phylogeny identified at least three broad phylogenetic groups. When the haplotypes estimated were reviewed with the phylogenetic groups and HLA-A serological groups, HLA-A and HLA-J linkage disequilibrium was evident for haplotypes associated with A1, A3, A9, A11 serologically grouped alleles. Greater variability was shown with the haplotypes associated with A10, A19, A28. This study demonstrates evidence for haplotype diversity between HLA-A and HLA-J. With increasing evidence for protein expression and haplotype associations, the need to reconsider the nomenclature of HLA-J is evident. Overall, our analysis indicates linkage disequilibrium between HLA-A and HLA-J, which provides further evidence towards haplotype-based matching.

P101 | Association between HLA and SARS-COV-2 infection in Mexican Mestizos

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The Acute Respiratory Syndrome caused by the new coronavirus described in Wuhan, China in 2019 is a viral, respiratory multifactorial infectious disease, which presents different stages depending on genetic and environmental factors that influence severity. As December 19, 2022, 653,192,573 COVID-19 cases worldwide and over six million deaths; 330,795 occurred in Mexico, were reported. Our aim was to analyze the contribution of HLA in Mexican patients infected with COVID-19, categorized in different clinical subgroups. A total of 114 COVID-19 patients and 164 healthy controls, all of them Mexican Mestizos from the highlands, were included in the study; RNA columns were used for extraction, and real-time PCR method was performed for the virus identification. DNA was isolated with the Maxwell16 system and 11 HLA loci were typed using NGS (CareDx, Immucor, and One Lambda). The subjects included: 22 asymptomatic, 86 symptomatic and 109 who were previously vaccinated. We compared controls versus positive patients; versus symptomatic; vaccinated negative versus vaccinated positive; controls versus asymptomatic; asymptomatic versus symptomatic individuals. The significant high risk alleles were A*29:02 (OR = 3.95), B*45:01 (OR = 6.92), C*03:04 (OR = 2.24). DPB1*03:01 (OR = 3.17) is a susceptibility marker in vaccinated and unvaccinated patients. The latter is prevalent in Hispanics, Russia, Finland, Spain and the United Kingdom. DQA1*02:01 (p = 0.009, OR = 1.96; DQB1*02:02 (p = 0.009, OR = 2.13) was a susceptibility marker in infected patients who were vaccinated. This is prevalent in Argentina, Brazil, Algeria, Australia, Canada, and China, while high-risk B*45:01 and C*03:04 are prevalent in India, Israel, Eastern Europe, and Mediterranean countries. Protective alleles where DRB1*04:01, A*02:01, DQB1*03:01 and DPB1*02:01. These data are relevant to prioritize vaccination, according to the HLA profile in Mexicans, therefore these data are relevant for the epidemiology of COVID-19.

P102 | HLA-C allele-sharing associated with high viral load (HIV-1 RNA) increases the risk of HIV-1 transmission among heterosexual serodiscordant couples in Nigeria

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Few studies have documented serodiscordant couples sharing HLA-B alleles and increased risk of HIV-1 transmission. This study aimed to determine the sharing of
Unravelling the architecture of Major Histocompatibility Complex class II regions in rhesus macaques was readily unraveled by the line-up of a whole genome long read sequencing strategy (Oxford Nanopore Technologies) in concert with adaptive sampling. Most likely this pipeline is able to resolve compound genome areas in humans and non-human primate species but may be, for example, practical as well in the fields of live-stock management and conservation biology. The data emphasize that our approach facilitates the refinement of an animal model essential to study human biology and disease.

Identification of a rare association between DRB1*01:01 and DRB5 using and an NGS method

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We describe a rare linkage disequilibrium between HLA-DRB1*01:01:01 and HLA-DRB5*01:01:01 identified by next-generation sequencing (NGS) in an European unrelated donor found during our routine activity. Linkage disequilibrium between HLA-DRB1, DRB3/4/5, and DQB1 has been well described. The alleles encoding DRB1*01, *08, *10 are normally not linked with any DRB3/4/5 genes. We present a rare association between HLA-DRB1*01:01:01 and HLA-DRB5*01:01:01 in an European unrelated donor. We typed HLA-A, -B, -C, -DRB1/3/4/5, -DQA1 and -DQB1 alleles at high resolution in 1882 potential unrelated donors. Typing was carried out with different molecular techniques, initially both with SBT (Invitrogen, Brown Deer, WI; Atria, Abbott Park, IL) and XR PCR-SSO, using Luminescent technology (One Lambda Inc.), subsequently with NGS (Thermo Fisher - One Lambda Inc., Canoga Park, CA) once it was available in our routine activity. Of the unrelated subjects previously typed at DRB1, DRB3/4/5, and DQB1, 307/1882 expressed at least one DRB1*01 allele. The most frequent HLA-DRB1*01 alleles are *01:01 = 181/307, *01:02 (121/307) and *01:03(5/307). Of the 307 individuals who have the HLA-DRB1*01 allele only one of the 307 has the HLA-DRB5*01:01 allele in linkage with the HLA-DRB1*01:01:01 allele. The complete HLA typing was as follows: A*03:01:01, 32:01:01; B*07:02:01, 49:01:01; C*07:01:01, 07:02:01; DRB1*01:01:01,11:01:01; DRB3*02:02:01; DRB5*01:01:01;
The etiology of the rarely described association between DRB1*01 and DRB5 is currently unknown. Future studies are required to further elucidate the correlation of HLA-DRB1*01 and DRB5.

P105 | The genetic impact of changes in mating patterns driven by post-war relocation of population and economic development

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Cyprus is an island in the far eastern side of the Mediterranean Sea which became a precious prize for many conquerors. Due to its rich history of rise and fall of empires, invasions, slavery, trade and migrations, the Cypriot population has interacted with many other cultures throughout history. Cyprus faced a war, tourist development, an overseas educational wave as well as an increase in mixed marriages. Such interactions have resulted in the gradual transfer of genetic material causing a highly admixed Cypriot population. During the 1974 war, one third of the Cypriot population was relocated from small agricultural villages to large refugee communities, causing a spread among the rest of the population. This is reflected by a significant decrease of the homozygosity of HLA alleles and haplotypes. In the early eighties and thereafter, Cyprus experienced an economic expansion and a significant development of the touristic industry, leading to tourists, multiple times the size of the population, visiting the island every year. Additionally, due to the lack of local universities on the island at the time, nearly 80% of the high school graduates travelled abroad for study (mostly Greece and UK). This resulted to nearly a third of the marriages on the island to be among a Cypriot and a foreigner, in most cases without historical and geographic relations with the indigenous population. These mixed marriages are reflected in significant changes in the haplotype frequencies of the Cypriot population. A cohort of approximately 200,000 tissue typed samples, including about 4,000 cord blood samples, were analyzed for haplotype frequency determination and cumulative incidence. Samples were categorized by decade of birth, taking into consideration certain life events. Common haplotypes in the Cypriot population were identified helping in the documentation analysis of the genetic drift phenomenon.

P106 | New HLA alleles identified in the admixed Brazilian population

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HLA genes are highly polymorphic and show variability among different populations. Databases of HLA alleles in the Brazilian population are lacking, leading to serious limitations in studies aiming to identify HLA alleles that may predispose to disease. The HLA region was sequenced in 301 healthy individuals from different regions of Brazil, most of whom were born in the southeast region of Brazil. We sequenced 12 HLA Loci (HLA-A, -B, -C, -DRB1, -DQB1, -DPB1, -DQA1, -DPA1, -DRB3, -DRB4, -DRB5, -G) using NGSgo® panels (GenDx) and MiSeq Sequencer (Illumina). The data were analyzed with NGSengine v.2.16.2 software (GenDx) and IPD-IMGT/HLA Database 3.38.0 as reference. We obtained results with eight-digit precision; but we restricted to four-digit to compare with published data. The most common HLA alleles identified in Brazilian individuals was: A*02:01 (0.23); A*01:01 (0.12); A*24:02 (0.10); B*51:01 (0.11); C*07:01 (0.17); C*04:01 (0.15); G*01:01 (0.77), G*01:04 (0.14); DPA1*01:03 (0.71); DPA1*02:01 (0.20); DPB1*04:01 (0.28); DPB1*02:01 (0.18); DPB1*04:02 (0.15); DQA1*05:01 (0.27); DQA1*01:02 (0.20); DQA1*03:01 (0.15); DQA1*01:01 (0.15); DQA1*02:01 (0.10); DQB1*02:01 (0.20); DQB1*03:01 (0.18); DQB1*05:01 (0.13); DRB1*07:01 (0.11); DRB3*02:02 (0.22); DRB3*01:01 (0.12); DRB4*01:01 (0.24). Brazilians have an admixed genome, with different proportions of European, sub-Saharan African, and Native American ancestries. We estimated the average European ancestry per individual studied as 76%. Thus, it was no surprise that the frequency of HLA-A, -B, -C, -DRB1 was similar to that of European
Comparision of two single antigen bead assays for detection of anti-HLA antibodies and evaluation of their reactivity with complement binding

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We investigated differences in the detection of anti-HLA antibodies (abs) between two single antigen bead methods: Immucor (IC) and One-Lambda (OL). Moreover, we evaluated complement binding capacity of detected abs by each method. Sera from 97 kidney transplanted patients with positive panel reactive test (> 5%) were analyzed with IC and OL Luminex assays for anti-HLA class I and II abs. Complement binding was tested with C3d for IC and C1q for OL. Numbers of positive specificities (MFI > 1000) between IC and OL were compared, as well as percentage of binding complement specificities with each method (MFI > 500). The OL assay detected more positive specificities than IC, both for class I and II abs. Complement binding was tested with C3d for IC and C1q for OL. Numbers of positive complements specificities (MFI > 1000) between IC and OL were compared, as well as percentage of binding complement specificities with each method (MFI > 500). The OL assay detected more positive specificities than IC, both for class I and II abs.

Impact of TNF-alpha gene polymorphisms on the risk and clinical manifestations of Ulcerative Colitis

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Ulcerative colitis (UC) is a chronic, relapsing inflammatory bowel disease affecting the rectum and the colon continuously. Immune dysregulation caused by genetic and/or environmental factors has a central role in the etiology of the disease. Tumor necrosis factor alpha (TNF-alpha) is an important member of the cytokine family responsible for the inflammation seen in bowel diseases. Anti-TNF-alpha molecules have represented, until recently, the most potent agents available for the treatment of these disorders. The aim of this study was to evaluate the association of TNF-alpha rs1800629 (-308G/A) and rs361525 (-238G/A) polymorphisms with susceptibility of UC and its clinical manifestations. The two promoter single nucleotide polymorphisms (SNPs) were genotyped in 83 UC patients (49M/34F) and 160 healthy controls (84M/76F) of Romanian origin using TaqMan Allelic Discrimination Assays (Real-time PCR System, Applied Biosystems by Thermo Fisher Scientific, USA). 16 patients were recorded with extraintestinal manifestations (EIM). The software packages DeFinetti and PLINK v 1.07 were used for genetic association tests. Controls and patients were in Hardy-Weinberg equilibrium for the investigated SNPs. The distribution of alleles and genotypes showed similar frequencies in cases and controls for the two SNPs. The minor alleles were significantly over-represented in the subgroup of patients with EIM: 31.2% versus 7.5% in patients without EIM, \(p = 0.001\), OR 5.54 for 308*A and 9.3% versus 0.7% in patients without EIM, \(p = 0.04\), OR 13.55 for 238*A.
associations were found in the studied group. Our results show that TNF-alpha gene polymorphisms influence the phenotype of ulcerative colitis in Romanian population.

**P109 | Comparative analysis of HLA-haplotype distributions in two Slavic populations**

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The aim of the study was the comparative analysis of HLA-haplotype frequencies in Russians and Belarusians. The study included 1563 Russians living in Saint-Petersburg and 2420 Belarusians living in Republic of Belarus. HLA typing was carried out by PCR-SSP and PCR-SSOP. The determination of haplotype frequencies was performed by Arlequin program ver. 3.5. with using the EM algorithm. 1126 haplotypes were determined. The most common HLA haplotypes were: A*02~B*07~C*07~DRB1*03~DQB1*06 (0.0387), A*03~B*13~C*06~DRB1*07~DQB1*06 (0.0298), A*02 ~B*07~C*07~DRB1*15~DQB1*06 (0.0225), A*02~B*07~C*07~DRB1*15~DQB1*06 (0.0212), A*02~B*18~C*07~DRB1*11~DQB1*03 (0.0127), A*02~B*15~C*03~DRB1*04~DQB1*03 (0.0191), A*25 ~B*18~C*12~DRB1*15~DQB1*06 (0.0115), A*02~B*41~C*17~DRB1*13~DQB1*03 (0.0112), A*30~B*13~C*06~DRB1*07~DQB1*02 (0.0097). 1365 HLA haplotypes were determined in Russians. The most common haplotypes were: A*01~B*08~C*07~DRB1*03~DQB1*02 (0.0445), A*03~B*07~C*07~DRB1*15~DQB1*06 (0.0330), A*02~B*13~C*06~DRB1*07~DQB1*02 (0.0294), A*02~B*07~C*07~DRB1*15~DQB1*06 (0.0194), A*03~B*35~C*04~DRB1*01~DQB1*05 (0.0179), A*02~B*18~C*07~DRB1*11~DQB1*03 (0.0159), A*25~B*18~C*12~DRB1*15~DQB1*06 (0.0141), A*02~B*57~C*06~DRB1*07~DQB1*03 (0.0118), A*11~B*35~C*04~DRB1*01~DQB1*05 (0.0112), A*23~B*44~C*04~DRB1*07~DQB1*02 (0.0109), A*02~B*27~C*02~DRB1*16~DQB1*05 (0.0105). Seven of the most common haplotypes in Russians and Belarusians coincide and are close in frequency. At the same time the frequencies of haplotypes A*02~B*57~C*06~DRB1*07~DQB1*03 and A*02~B*27~C*02~DRB1*16~DQB1*05 are significantly higher in Belarusians (0.0118 vs. 0.0049; 0.0105 vs. 0.0038; p = 0.02). The frequency of haplotype A*02~B*41~C*17~DRB1*13~DQB1*03 is considerably higher in Russians (0.0112 vs. 0.0052; p = 0.02). Our study demonstrates both similarities and differences of HLA haplotypes frequencies in Russians and Belarusians which are Slavic populations close in origin.

**NK cells and KIR**

**P110 | Selective HLA haplotype loss and immunological escape of NPM1 + AML**

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Here we present a case of AML with specific loss of an HLA haplotype with high affinity for peptides derived from mutated nucleophosmin (dNPM1) as a mechanism of immune escape. The patient was a 47-year-old, 46XX, NPM1/FTL3 + AML, candidate for allogeneic bone marrow transplant. NGS HLA typing at diagnosis on peripheral blood (85% blasts) showed homozygosity of all classic and non-classic class I loci, heterozygosity of class II loci. Confirmatory typing on buccal swab and peripheral blood at complete remission: heterozygosity of all class I loci, heterozygosity without variations of copy number. A SNP array showed two balanced clones, one with loss of the whole HLA region, the other of only HLA class I genes. dNPM1 is a common driver AML mutation, giving raise to highly immunogenic peptides. We used IEDB software to test affinity of dNPM1 peptides for lost and kept HLA alleles. Lost HLA alleles have a much higher affinity for dNPM1 peptides than the counterpart kept ones (particularly A*11). All data support loss of presentation of highly immunogenic dNPM1 peptides to T cells as a mechanism of immune escape. We tested if specific loss of HLA ligands for inhibitory KIR could potentially trigger NK
cytotoxicity. The patient’s KIR profile showed a cenA/telA homozygous genotype with no activatory genes and NK cells educated by the presence in wild type of HLA ligands for all expressed inhibitory KIRs (2DL3, 3DL1, 3DL2). HLA loss does not reduce NK potential inhibition of mutated blasts.

P111 | HLA-E and its NKG2 receptors in graft-versus-host disease

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Graft-versus-host disease (GvHD) is a serious complication after allogeneic hematopoietic stem cell transplantation (HSCT). The non-classical HLA-E molecule is a common ligand for NKG2A and NKG2C NK cell receptors, which may also be found in its soluble form (sHLA-E) in the patient’s sera. In this study we aimed to assess whether polymorphisms in genes coding for HLA-E and both receptors, as well as sHLA-E serum concentration, may be associated with the risk of GvHD development. Genotyping was performed for 177 HSCT recipients and 104 donors. NKG2A rs7301582 and HLA-E rs1264457 single nucleotide polymorphisms were assessed using LightSNiP assays. The NKG2C 16bp deletion was detected by PCR-SSP. Serum sHLA-E levels 30 days after transplantation were measured by ELISA. Genotyping studies revealed that the NKG2A rs7301582 CC homozygous genotype prevailed among HSCT recipients when compared with the healthy donors (RR = 2.41, p<0.001). No other significant differences between recipients and donors were detected. Recipients who developed acute GvHD grades II-IV were more frequently characterized with presence of the NKG2C gene deletion in comparison to those without acute GvHD symptoms or diagnosed with mild grade I (52.4% vs. 20.9%, OR = 2.652, p = 0.029). Serum sHLA-E level was lower in patients who developed chronic GvHD compared with those lacking chronic GvHD symptoms (6.33 vs. 11.65 pg/mL, p = 0.033). As compared with other HSCT recipients, patients with acute GvHD grades II–IV characterized with lower sHLA-E levels (8.036 vs. 11.073 pg/mL, p = 0.081). These results imply that NK2A rs7301582 CC homozygosity may be associated with susceptibility for hematological disorders while deletion within the NKG2C encoding gene may act as a prognostic factor of severe acute GvHD occurrence. Furthermore, lower serum HLA-E concentrations seem to affect the development of chronic GvHD. This study was supported by the project No. 2018/31/B/ NZ2/03065 from the National Science Centre (Poland).

P112 | The extent of non-expressed KIR3DL1 alleles in a French population

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Different KIR3DL1 alleles are expressed at different levels on Natural Killer (NK) cell surfaces. In particular, the non-expressed KIR3DL1*004 allele appears common in Caucasian populations. However, the overall distribution of non-expressed KIR3DL1 alleles remains poorly documented in European populations. In a cohort of French blood donors (N = 278), we compared the distribution of expressed and non-expressed KIR3DL1 alleles using a next-generation-sequencing (NGS) technology combined with multi-color flow cytometry. We confirm the predominance of the non-expressed KIR3DL1*004 allele. Using allele-specific constructs, the phenotype and
the function of the uncommon KIR3DL1*019 allotype was characterized. Although poorly expressed on NK cell surfaces, KIR3DL1*019 is retained within NK cells where it confers missing-self recognition of the Bw4 epitope. Non-expressed KIR3DL1 alleles could therefore bias the functional KIR+ NK cell repertoire and influence the outcome of Hematopoietic Stem Cell Transplantation.

P113 | Allele frequencies for three framework Killer cell Immunoglobulin Like Receptor genes in the Western Australian population

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The Killer-cell Immunoglobulin-like Receptors (KIR) are cell surface proteins controlling the functions of Natural Killer cells and some T cells. 16 genes coding for inhibitory and activating receptors are located on chr19 and show variability in terms of gene content, copy number and nucleotide polymorphism. KIR is implicated in the outcome of hematopoietic stem cell and solid organ transplantation, control of viral infection and pregnancy. Gene content has been studied extensively however, recently more focus has been applied to allelic variation. With the use of NGS and long read length sequencing technology, high resolution typing of the KIR genes is feasible allowing for precise elucidation of KIR alleles. We have developed sequencing of KIR genes using an amplicon sequencing workflow on the long-read length Oxford Nanopore Technology platform. We have applied this workflow to a population of 130 healthy Western Australians to derive KIR allele frequencies. We report here the frequencies of the framework KIR genes KIR3DL3, KIR2DL4 and KIR3DL2. Sequence data was analyzed to two field resolution. Alleles count and frequency estimations were calculated in the R environment for statistical computing and visualization (v4.1.2). A total of 44, 9 and 21 distinct alleles were identified for KIR3DL3, KIR2DL4 and KIR3DL2 respectively. Allele frequencies ranged from 0.4% to 17.7%, 0.8% to 19.6% and 0.4% to 16.9% in KIR3DL3, KIR2DL4 and KIR3DL2 respectively. A total of four KIR3DL3 alleles, six KIR2DL4 alleles and six KIR3DL2 alleles had a frequency greater than 5% ([KIR3DL3*00206, 5.8%; KIR3DL3*00901, 8.8%; KIR3DL3*00011, 14.2% and KIR3DL3*00301, 17.7%] [KIR2DL4*00101, 17.8%; KIR2DL4*00010, 17.3% and KIR2DL4*00501, 19.6%] [KIR3DL2*00301, 4.8%; KIR3DL2*00501, 8.1%; KIR3DL2*00011, 11.5%; KIR3DL2*00103, 11.9%; KIR3DL2*00701, 16.2%; and KIR3DL2*00201, 16.9%]).

P114 | Allele-level characterization of KIR gene polymorphism in healthy elderly from four populations - Bulgarian, Romanian, Polish and Turkish

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Killer-cell immunoglobulin-like receptor (KIR) polymorphism has an impact on NK cell functions in normal and pathological conditions. We assume that the study of the population-specific KIR polymorphism in the context of successful ageing will contribute to our knowledge of immune dysfunction in the elderly. Therefore, in the “Immunogenetics of Ageing” project within the 18th International HLA and Immunogenetics Workshop, we aimed to assess the impact of KIR genes in healthy ageing by applying a next-generation sequencing (NGS) approach. Two main data sets from four populations (Bulgarian, Romanian, Polish, and Turkish) were collected: unrelated, healthy elderly individuals (age 65-99 years) and young controls (age 18-35 years). Preliminary results showed that KIR2DS2*001:01:08 (OR: 2.76, p = 0.005) was observed significantly more frequent among healthy elderly individuals from the Bulgarian population when compared with young controls. Furthermore, in elderly Bulgarians, a slightly increased frequency was found for the KIR3DL1*005:01:03 allele (OR: 3.23, p = 0.047). Moreover, in elderly Romanians, the inhibitory KIR alleles KIR3DL1*015:02:14 (OR: 8.09, 420 ABSTRACTS
KIR genotyping provides several layers of complexity: copy number variation, multiple haplotypes, sequence polymorphism and sequence homology. This makes data generation and analysis for allele-level typing challenging. One example is a highly homologous 3kb sequence at the 3’ of the KIR2DS3 and KIR2DS5 genes. This region causes only 5% of the 3’ reads to be uniquely mapped to either KIR2DS3 or KIR2DS5 when analyzing short reads (Illumina 2 × 151bp) in NGSengine software (GenDx) using separate libraries for KIR2DS3 and KIR2DS5 based on IPD-KIR version 2.10.0. When run on a long read sequencer (PacBio Sequel Ile), both loci were fully covered and typeable, as NGSengine can locus-assign long reads based on the 5’ part of the gene. The read-assignment issue for short reads was solved by creating a combined IPD-KIR library for KIR2DS3 and KIR2DS5. This allowed NGSengine to classify reads to both loci without discarding homologous reads. For the artificial locus named “KIR2DS35” in NGSengine, alignment, phasing and typing proved possible. Concordance with results obtained with PacBio using independent amplicons of the same samples was high. A limitation of this strategy for short reads is not being able to type samples carrying >2 alleles of 2DS3 and 2DS5. However, the expected frequency of these genotypes is low (1.66%) and not identified in the sample panel tested. As allele-level typing was not possible using short read technology for these loci without this adapted IPD-KIR library, this limitation is accepted. Co-located KIR loci 2DS3 and 2DS5 are highly homologous in the 3’ end of the gene. Combining the loci into a single KIR2DS35 locus for short-read analysis purposes allows for analysis in NGSengine. For long read data, separate KIR2DS3 and KIR2DS5 libraries can be applied, with the benefit of being able to type >2 copies of KIR2DS3 and KIR2DS5.

**P115 | Short and Long Read sequencing data analysis of co-located Killer-cell Immunoglobulin-like Receptor genes 2DS3 and 2DS5**

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Chronic myeloid leukemia (CML) is a hematological malignancy characterized by the increased and unregulated growth of myeloid cells in the bone marrow and the accumulation of these cells in the blood. Killer-cell immunoglobulin-like receptors (KIRs) are cell surface molecules expressed on Natural Killer (NK) cells. These receptors can be inhibitory or activating and the balance between their signals could lead to either triggering or blocking of NK-cell activity. A lack of activation of NK cells could explain the default of clearance of the leukemia cells in CML patients. KIR3DS1 is the only activating receptor with three extracellular domains. HLA-Bw4 with an isoleucine in position 80 (Bw4-80I) is a potential ligand for KIR3DS1. In this study, we investigate the relationship of KIR3DS1 and Bw4-80I with leukemia development in comparison to a control group. The subjects studied consisted of 16 patients diagnosed with treatment resistant CML and 126 healthy individuals. KIR3DS1 typing was done by PCR-SSP (polymerase chain reaction with sequence specific primer). The controls were typed for HLA class I using Next-generation sequencing (NGS). Then, the HLA-Bw4 genotype was deduced using the IPD-IMGT/HLA Database. HLA-Bw4 typing for CML patients was done using 2PCR-SSP reactions (Bw4-80I and Bw4-80T). 18.7% of leukemia patients and 23.8% of controls expressed the KIR3DS1 allele. The frequency of HLA-Bw4 was 68.7% (90.9% Bw4-80I) in CML patients and 63.5% (71.2% Bw4-80I) in controls. Co-expression of KIR3DS1 and HLA-Bw4-80I was found in 12.5% of patients and 10.3% of controls. No significant differences were noted in the distribution of KIR3DS1 and HLA-Bw4 between CML patients and controls. The frequency of Bw4-80I was higher in CML patients than in the controls without being significant. A larger sample of patients and controls with the inclusion of patients responding to treatment is necessary.

**P116 | KIR3DS1/HLA-Bw4 in Tunisian patients with chronic myeloid leukemia**

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P117  |  KIR3DS1/HLA-Bw4 distribution in the Tunisian population

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Killer-cell Immunoglobulin-like Receptors (KIRs) represent a family of activating and inhibitory receptors expressed on natural killer (NK) cells that shape and regulate NK cell functions. Within the 17 described human KIRs, KIR3DS1 displays some unique features such as being the only activating receptor with three extracellular domains. Recent genetic and functional studies have suggested that certain HLA-Bw4 molecules containing an isoleucine in position 80 (HLA-Bw4-I80) are a potential putative ligand for KIR3DS1. Recent studies have associated the presence or absence of KIR3DS1 with the occurrence and outcome of some malignancies, autoimmune diseases, and graft-versus-host disease (GVHD) as well as a potential protective role of KIR3DS1 in combination with HLA-Bw4-I80 in the context of HIV-1 infection. In this study, we analyzed the KIR3DS1/HLA-Bw4 distribution in the Tunisian population as a first step to better understanding the role of KIR3DS1 and its association with human disease. Our study population consisted of 126 healthy individuals typed for HLA class I using next-generation sequencing (NGS). We completed KIR3DS1 typing using PCR-SSP and deduced HLA-Bw4 genotype using the IPD-IMGT/HLA Database. Thirty individuals (23.8%) expressed the KIR3DS1 allele. Eighty individuals (63.49%) expressed the HLA-Bw4 alleles among which 57 (71.25%) expressed HLA-Bw4-I80. Co-expression of KIR3DS1 and HLA-Bw4-I80 was found in 13 individuals (10.31%). The KIR3DS1 allele expression is not frequent in the Tunisian population while the HLA-Bw4 is very frequent. These results can serve as controls for future studies.

New Technologies & New Approaches in Immunogenetics

P118  |  A modern twist on compatibility assessment in the histocompatibility and immunogenetics laboratory

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The Complement Dependent Cytotoxicity assay (CDC assay) was first introduced for histocompatibility testing in 1966. Superseded by the more sensitive flow cytometry assay, its use has declined. However, the CDC assay provides an understanding of antigen-antibody interaction truly representative of the cell surface antigens in their native configuration. It provides valuable data for immunological risk assessment in antibody incompatible transplantation. We have developed a CDC equivalent assay that uses an Image Cytometry Platform (ICP) to provide a value for percentage cell death with cells stained using the nucleic acid stains Acridine orange/Propidium Iodide. The ICP is a multifunctional hybrid platform for cell counting, imaging and cytometry that combines the microfluidic advantages of a flow cytometer and the imaging analysis of microscopy. Splenic lymphocytes from 10 deceased donors were incubated with serum samples from 6 patients with well characterized HLA specific antibody profiles plus control samples. The testing was performed in parallel using CDC and the ICP assay. The percentage cell death from the ICP assay was converted into the equivalent IHW score to allow for direct comparison with the CDC assay. Weighted Kappa comparison of the results from the two assays gave a kappa of 0.707 with a 95% confidence interval of 0.635 to 0.779 which is interpreted as a substantial agreement. There were 7 crossmatches designated an IHW score of 2 on the ICP platform that scored negative on the CDC. A further 2 were negative on the ICP assay and scored an IHW of 2 on the CDC assay. These differences are at the level that is considered borderline for positivity. The ICP assay eliminates background interference from red cells and other debris that introduce subjective variation into the CDC assay.

P119  |  The NanoTYPE experience: nanopore sequencing as a new tool for HLA laboratories enabling routine and urgent high-resolution typing

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In recent years next-generation sequencing (NGS) has replaced Sanger sequencing as the gold-standard for
high-resolution HLA typing as it offers multiple benefits over traditional sequencing methods, including increased throughput, whole-gene or near-whole-gene characterization, shortened turnaround time, and reduced ambiguity. However, while available NGS platforms (Illumina and Ion Torrent) can provide these benefits they are unable to sequence very long fragments, resulting in cis-trans ambiguities. Furthermore, a longer turnaround time (2–3 days) and a need to batch multiple samples per run, for cost effectiveness, hinders the use of this method for urgent sample or for laboratories with lower throughput. DNA sequencing through nanopores, as introduced by Oxford Nanopore Technologies (ONT) on the MinION platform, can offer a solution to these limitations of traditional NGS. Omixon has designed the NanoTYPE workflow for high resolution HLA typing which utilizes the ONT sequencing platform. The assay uses a multiplexed long-range PCR for 11 loci paired with rapid library preparation, nanopore sequencing, and a dedicated software for data-analysis. Between January and June 2022 both NanoTYPE workflows were tested with more than 600 samples typed externally as part of the NanoTYPE Beta study. We will be presenting some of the data obtained by labs related to sequencing quality and concordance of typing results.

P120 | Long read phased sequencing of HLA class I and II genes using MinION Sequencing

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The ability to generate long reads for HLA genotyping provides the benefit of phasing across exons and resolving phasing ambiguities observed with short read chemistry. The suitability of the R10.4/Kit 12 chemistry for routine HLA genotyping was assessed by testing a panel of 46 samples with known HLA genotypes using full gene amplification assays. Library preparation and sequencing were performed as per the manufacturer’s protocol. The results were assessed against sequencing metrics and HLA genotyping concordance. Samples were tested over two sequencing runs, with ~18 hrs of sequencing time and generated 6.5-7 Gb of data (~1M reads) consistent with expected outputs reported by ONT. The raw read and consensus read accuracy was 97.6% and 98.2% respectively, compared with 94.8% and 95.7% from the previously available R10.3 flow cell. These results are consistent with those published by ONT approaching that of other NGS platforms. A significant improvement in both the average noise and SN (delta signal-to-noise) ratio was observed in the R10.4 data compared with the R10.3 data resulting in better heterozygous base calls. Homopolymer and systemic inversion errors seen in the R9.4 and R10.3 data were significantly lower in the R10.4 data. ONT sequencing of full gene amplicons provided accurate and higher resolution HLA genotyping as the long reads provided the ability to phase across exons thereby resolving common phasing ambiguities observed using short read chemistry. Of the 369 loci analyzed, all alleles were resolved and concordant with previous results to a minimum of 3-field typing resolution. Intron sequence could be analyzed for all loci except DRB1. The results presented here confirm the suitability of the R10.4/Kit 12 ONT workflow for HLA genotyping.

P121 | Results of the 6 month post-transplant surveillance in patients transplanted with preformed donor-specific anti-HLA antibodies (DSA) by adding donor-derived cell-free DNA (ddcfDNA) testing

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Transplant with preformed DSA increases the risk of rejection. Despite limitations, we monitored these patients’ follow-up with DSA and surrogate markers of renal function. We hypothesized that ddcfDNA testing will be useful for the monitoring and early intervention. We measure ddcfDNA (AlloSeq cfdNA, CareDx) in patients with preformed DSA at 15 days, 1, 3 and 6-months post-transplant (posTx). Here we present an analysis of preliminary data including nine patients. Three patients showed dd-cfDNA values >1% at 15 days posTx (1.6 to 7.3%). One of them, with high MFI (>10000) DSA (anti-DP2 and DP3), had elevated
Evaluating recent nanopore sequencing chemistries for rapid and conventional HLA typing

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Oxford Nanopore Technologies (ONT) sequencing has potentially very rapid sequencing making it the only viable approach for SBT in the context of solid organ transplantation. In addition, the technology is financially accessible, has a small logistical footprint and has excellent scaling capacity, making it very appealing for clinics on a limited budget. The primary challenge with this technology is the relatively poor signal-to-noise ratio compared with Illumina, especially in the context of homopolymers. ONT has a history of iterating and improving their kits and chemistry and recently R10.4.1 MinION and Flongle flow cells were introduced to replace R10.4 MinION and R9.4.1 Flongle flow cells. In this study we compared performance of R10.4.1 to R10.4 MinION and R10.4.1 to R9.4.1 Flongle flow cells. DNA isolated from 58 genetically diverse cell line samples of the Genetic Testing Reference Material Coordination Program (GetRM) HLA58 panel was used (Coriell Institute). Pre-type information was available up to four fields from GenDx NGSgo-MX11-3 amplicon sequencing on an Illumina MiSeq. Amplicons were generated covering all exons for 11 loci (NGSTurboAmp) and libraries were created using a custom rapid prep keeping long amplicons intact (NGSTurboPrep). Data was generated on a MinION Mk1C or a GridION sequencing device, using high accuracy basecalling followed by analysis in NGSengine Turbo. Data was scored for two-, three- and four-field concordance with pre-types. Homopolymer performance was assessed based on homopolymer length. Meta data was generated for each chemistry and flow cell for a high level comparison. HLA typing concordance was high for all chemistries, except R9.4.1 Flongle. We revealed differences between the MinION and Flongle flow cells when using identical chemistries that should be taken in consideration for experimental design. We identified reproducible patterns that carry important information that help decipher homopolymer lengths.
96.7%, DQB1: 96.6%, DQA1: 89.9%, DPB1: 96.6%, DPA1: 99.3%, DRB345: 100%. Alleles incorrectly identified were: C*07:01:01 (BS), DRB1*04:11:01, DRB1*04:03:01, DRB1*06:06:02 (BS), DRB1*07:01:01, DRB1*11:01:01, DQB1*02:02:01, DQA1*03:01 (3 PB), DQB1*03:02 (BS), DQA1*02:01:01, DQA1*05:01:01, DQA1*05:03:01, DQA1*05:05:01 (5 PB), DQA1*01:02:01, DQA1*02:01:01 (6 PB) and DQA1*05:05 (BS), DPB1*02:01:19, DPB1*18:01:01 (4 PB), DPA1*02:01:08.

As the typing success depends on the accuracy of the base calling, we tested the ability of NanoTYPER to correct the results by modifying the high accuracy (HA) model, to the super accuracy (SA) model. The SA model corrected the ambiguities at the cost of reducing the number of reads. Even with these changes, alignment errors still need to be improved in NanoTYPER, mainly in DRB1*04 and DQA1*05 alleles, and in homopolymer regions. Finally, we tested 17 samples that were ambiguous in typing by short reads, and all results were resolved on the nanopore platform. In conclusion, the nanopore platform offers fast and accurate technology for high-resolution HLA typing.

P124 | Evaluation of the Magelia for automated purification of Caredx® Alloseq HCT kit libraries in the context of post-hematopoietic stem cells transplantation chimerism assessment

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New innovative techniques, such as next-generation sequencing (NGS), have revolutionized the quantification of chimerism. The AlloSeq HCT NGS contains all the reagents necessary for multiplex amplification of 202 loci and indexing for up to 96 samples. Once all sample libraries have been generated, magnetic bead cleaning followed by quantification and sequencing on MiSeq are performed. The Magelia’s unique core technology brings unparalleled sensitivity through reaction miniaturization, for highly efficient, full automation of diverse workflows, even on challenging, low input samples. The purpose of our evaluation was to compare the results obtained from a pool of samples treated with the Magelia platform with those obtained from a manual purification. We have selected 24 samples with various values of chimerism quantification. At the end of the PCR, two sets of samples were chosen for comparison between Magelia and manual purification. Manual purification was performed as a single pool of 24 samples. Purification in the Magelia was performed individually for each amplicon. The latter were eluted in 1.5 μL to constitute a pool with a final volume comparable to that of a manual treatment. This was equivalent to individual runs of 8 patients in the platform. After purification, the 2 pools of libraries were injected on the different flow cells. The metrics for Magelia and manually treated libraries were very similar, that is, the concentrations of the libraries were 1.62 and 1.42 nM, respectively. Similarly, the bioanalyzer analysis showed that the Magelia® purified library showed a slightly better yield. The chimerism results were comparable for all the samples tested with the HCT Software. The duration of the automatic protocol was comparable to the manual conditions (40 min). These first results are very encouraging and the performance of Magelia would allow the entire protocol to be automated.

P125 | Evaluation of the HISTO TYPE Rainbow kit from BAG DIAGNOSTICS at the HLA EFS laboratory in Marseille

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The HISTO TYPE Rainbow kit allows the genotyping of 11 HLA loci: HLA-A, -B, -C, -DRB1/3/4/5,-DQA1,-DQB1,-DPB1 and -DPA1 at low resolution in an emergency context. The principle of the method is based on the use of primers specific to HLA allele sequences (PCR-SSP) coupled to Taqman probes allowing the multiplexing of 5 reactions per well including an internal amplification control. This method requires the use of a qPCR instrument with 5 fluorescence channels, as CFX touch from Biorad. The kit allows a unitary typing. It is composed of a ready to use mix the Plex mix. The amount of DNA required for one test is 10-20 ng. A fluorescence
measurement is performed in real time during amplification and as an endpoint at the end of the reaction. The preparation, reaction and interpretation duration was nearly 1h15. A positive reaction is determined by the ratio between the specific fluorescence and the fluorescence of the internal control. The Plextyper software allows automatic analysis of the results. A total of 76 samples were tested, 32 controls and EQA and 44 samples from the laboratory routine. This sample panel contained DNA transmitted from EEQs (n = 21) and DNA from different biological sources: whole blood (n = 42) and saliva (n = 13), extracted by different extraction methods as Quickgene (n = 9), Chemagic (n = 30) and Labturbo (n = 16). The specificities of the selected samples were representative of the most frequent HLA alleles in homozygous and heterozygous combination. The results obtained for heterozygous and homozygous typing were all 100% concordant, except for a discrepancy identified for an A*34:01 homozygous result falsely assigned A*34, A*68. An adjustment of the threshold of the probe was performed to correct this misassignment. Because of its ease of use and the performance of the reagents and software tested, the HISTO TYPE Rainbow kit is suitable for typing the 11 low-resolution HLA loci in an emergency context.

P126 | Open Science in human immunogenetics; challenges and pathways

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Open Science has many features and, besides common values and practices, differences exist according to disciplines and communities. The culture of sharing data, protocols and experiences gives a specific role to the immunogenetics community in this movement. After a period of declarations of principles, international recommendations such as those of UNESCO (2021), and institutional positioning, we are now in an era where, beyond the Open Access to publications, policies of Open Data and implementation of FAIR principles require education, training, and practical tools. Beyond technical aspects, Open Science is a challenge as it regards society involvement, data protection, intellectual property rights, research career and research responsibilities. In this context several initiatives are of interest for the immunogenetics community: 1. the European Open Science cloud, EOSC Future project, funded by the European Commission (EC) has organized in collaboration with the Research Data alliance (RDA), a network of ambassadors in different domains, the author being one of them for immunogenetics and health ethics; various tools are made available; 2. Another EC funded project, FAIRPlus, has produced a Cookbook with recipes addressing technical and regulatory aspects in making data FAIR, an analysis of the values underpinning the FAIR principles and a training online program on FAIR; 3. National initiatives exist such as the French National Plan for Open Science with its 4 pillars (Open publications, data, software, Open Science training and assessment); 4. Groups from international organizations are actively exploring the ways Open Science activities are valued as research outputs, for example, RDA-ShARC (Sharing research and credit) group. We propose to present a map of initiatives and tools pertinent for the immunogenetics community and to invite some of its members to express their views through semi-structured interviews during or after the 2023 EFI Conference.

P127 | Identification of the novel HLA-DPB1*02:01:68 allele in a Greek individual

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Next Generation Sequencing (NGS) is a high-throughput methodology that performs a deeper interrogation of the genomic sequences for the HLA genes allowing the identification of novel HLA alleles. The novel HLA-DPB1*02:01:68 allele was observed in a Greek 27-year-old male patient candidate to Hematopoietic Stem Cell Transplantation (HSCT), during routine typing for HLA class I and II by NGS. The name DPB1*02:01:68 has been officially assigned by the WHO Nomenclature Committee for Factors of the HLA System in September 2022. The genomic DNA was isolated from peripheral blood using automatic extractor (Maxwell Promega). HLA genotyping using commercial locus-specific primers supplied by CareDX (AlloSeq Tx17 kit), on the Illumina MiSeq platform was performed. Libraries were quantified by the Qubit 1X dsDNA High-Sensitivity Assay Kit (Thermo Fisher Scientific). The raw sequencing data (FASTQ files) were analyzed by AlloSeq Assign analysis software Tx17.1 v1.0.3 (CareDX) with references from the IPD-IMGT/
HLA Database v3.47.1.2. The DNA sequence of the novel HLA-DPB1*02:01:68 allele (GenBank AN OP019279, IPD-IMGT/HLA SN HWS10062200) differs from the closely related HLA-DPB1*02:01:02:01 by one single nucleotide substitution (T>C) in exon 3 at gDNA position 10216, codon 179c GAT>GAC, resulting in a synonymous mutation coding for Aspartic Acid. In order to confirm the presence of the novel HLA-DPB1*02:01:68 allele, HLA genotyping using commercial NGSgo®-MX6-1 kit (GenDx) was also performed. Sequencing was carried out on MiSeq system. The obtained sequencing data were analyzed using NGSengine software v.2.21.0 (GenDx) with the IPD-IMGT/HLA Database v3.47.1.2. The extended HLA genotyping for the patient was HLA-A*02:01:01, 24:02:01; HLA-B*08:01:01, 51:01:01; HLA-C*07:02:01, 14:02:01; HLA-DRB1*01:01:01, 03:01:01; HLA-DRB3*02:02:01; HLA-DQA1*01:01:01, *05:01:01; HLA-DQB1*02:01:01, 05:01:01; HLA-DPA1*01:03:01, *02:01:02; HLA-DPB1*01:01:01, *02:01:68.

Identification and characterization of six novel HLA alleles by next generation sequencing in Spanish population during the last year

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HLA high-resolution typing by next-generation sequencing (NGS) has benefit histocompatibility labs since it improves HLA gene coverage, obtains phased sequences, reduces ambiguities and permits the identification of new HLA alleles. HLA typing of new volunteer bone marrow donors with NGSgo®-MX11-3 (GenDx) and MiSeq (Illumina) was performed. Data were analyzed using NGSmengine software. We discovered HLA-B*49:78 which showed six differences from B*49:01:01:01: three in exon1 and three in non-coding regions (5’UTR and intron 1). Changes in exon 1 were located in genomic positions (gp): 5 (G>T) (codon -23: CGG>CTG; replacement of Leucine -23 to Arginine), 11 (C>T) (codon -21: ACG>ATG; change Methionine -21 to Threonine), and 15 (A>T) (codon -20: GCA>GCG; synonymous). Changes in non-coding regions were located in gp -61 (T>G), -18 (A>G) and 89 (C>G). DQB1*03:493 presented a single change to DQB1*03:02:01:01 at gp 68 (C>A), codon 174 (GAT> GAC), causing the replacement of Alanine -10 to Glutamic acid. DQA1*01:04:07 had a synonymous substitution in exon 2, gp 3907 (C>T), codon 21 (CCC>CCT), and an insertion in intron 1 from gp 3356:1 to 3356:4 (A was inserted four times), compared with DQA1*01:04:01:02. DQA1*02:01:09:01 displayed a synonymous difference at gp 4779 (T>C), codon 174 (GAT> GAC), compared with DQA1*02:01:01:01. DQB1*03:01:01:47 showed a single change in intron 1 (gp 364: A>G) compared with DQB1*03:01:01:19. DQB1*03:01:01:48 presented a single change in intron 1 (gp 1235: T>A) compared with DQB1*03:01:01:19. NGS has allowed the in-depth description of new HLA polymorphisms, even outside the peptide-binding region. Therefore, the complete characterization of HLA genes is needed in order to clarify the role of other polymorphic regions and its possible clinical relevance in transplantation outcome.

Identification of the novel HLA-A*01:426 allele in a Greek individual

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The HLA genes are the most polymorphic of the human genome, and novel HLA alleles are continuously identified by next-generation sequencing (NGS) technologies. The novel HLA-A*01:426 allele was observed in a Greek 21-year-old male bone marrow donor registry candidate to Hematopoietic Stem Cell Transplantation (HSCT), during routine typing for HLA class I and II by NGS. The name A*01:426 has been officially assigned by the WHO Nomenclature Committee for Factors of the HLA System in November 2022. Genomic DNA was isolated from peripheral blood using an automatic extractor (Maxwell Promega). HLA genotyping using commercial NGSgo®-MX6-1 kit (GenDx) on the Illumina MiSeq platform was performed. Libraries were quantified by the Qubit 1X dsDNA High-Sensitivity Assay Kit (Thermo Fisher Scientific). The raw sequencing data (FASTQ files) were analyzed by NGSmengine analysis software v.2.21.0 (GenDx).
with references from the IPD-IMGT/HLA Database v3.47.1.2. The DNA sequence of the novel HLA-A*01:426 allele (GenBank AN: OP712622, IPD-IMGT/HLA SN: HWS10064231) differs from the closely related HLA-A*01:01:01:01 by one single nucleotide substitution (G>A) in exon 4 at gDNA position 1617, codon 199a GCC>ACC, resulting in the amino acid change of Alanine (A) to Threonine (T). In order to confirm the presence of the novel HLA-A*01:426 allele, HLA genotyping using commercial locus-specific primers supplied by CareDX (AlloSeq Tx17 kit) was also performed. Sequencing was carried out on MiSeq system. The obtained sequencing data were analyzed using AlloSeq Assign Tx17.1 v1.0.3 (CareDX) analysis software with the IPD-IMGT/HLA Database v3.47.1.2. The extended HLA genotyping for the bone marrow donor was HLA-A*01:426, 32:01:01; HLA-B*35:01:01, 37:01:01; HLA-C*04:01:01, 06:02:01; HLA-DRB1*03:01:01G, 11:04:01G; HLA-DRB3*02:02:01; HLA-DQA1*05:01:01, 05:05:01; HLA-DQB1*02:01:01, 03:01:01; HLA-DPA1*01:03:01; HLA-DPB1*02:01:02, 04:01:01.

**P130 | Identification of the novel HLA-A*02:09:01:04 allele in a Greek individual**

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The introduction of Next generation sequencing (NGS) technology provides for high throughput HLA allele typing and is defining the frontier of a new “Gold Standard” technology for HLA sequencing. The novel HLA-A*02:09:01:04 allele was observed in a Greek 36-year-old female, who was typed for HLA class I and II by NGS as control individual in a disease association study. The name A*02:09:01:04 has been officially assigned by the WHO Nomenclature Committee for Factors of the HLA System in November 2022. The genomic DNA was isolated from peripheral blood using an automatic extractor (Maxwell Promega). HLA genotyping using commercial NGSSgo®-MX6-1 kit (GenDx) on the Illumina MiSeq platform was performed. Libraries were quantified by the Qubit 1XdsDNA High-Sensitivity Assay Kit (Thermo Fisher Scientific). The raw sequencing data (FASTQ files) were analyzed by NGSengine analysis software v.2.21.0 (GenDx) with references from the IPD-IMGT/HLA Database v3.47.1.2. The DNA sequence of the novel HLA-A*02:09:01:04 allele (GenBank AN:OP795782, IPD-IMGT/HLA SN:HWS10064239) differs from the closely related HLA-A*02:09:01:01 by three single nucleotide substitutions (SNS) in intron 5; One SNS (T>G) at gDNA position 2263; One SNS (A>T) at gDNA position 2266; One SNS (C>T) at gDNA position 2268. In order to confirm the presence of the novel HLA-A*02:09:01:04 allele, HLA genotyping using commercial locus-specific primers supplied by CareDX (AlloSeq Tx17 kit) was also performed. Sequencing was carried out on MiSeq system. The obtained sequencing data were analyzed using AlloSeq Assign Tx17.1 v1.0.3 (CareDX) analysis software with the IPD-IMGT/HLA Database v3.47.1.2. The extended HLA genotyping for the Greek individual was HLA-A*02:09:01:04, 26:01:01; HLA-B*35:01:01, 58:01:01; HLA-C*03:02:02, 04:01:01; HLA-DRB1*03:01:01, 13:02:01; HLA-DRB3*02:02:01, 03:01:01; HLA-DQA1*01:01:01, 05:01:01; HLA-DQB1*02:01:01, 06:04:01; HLA-DPA1*01:03:01; HLA-DPB1*02:01:02G, 04:02:01G.