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Genomic loss of the patient-specific HLA has been described in previous single-center studies as a frequent mechanism of leukemia immune evasion and relapse. Here we present the first global collaborative study to investigate its incidence across transplant platforms.
Twenty-seven transplant centers from across the globe formed the HLALOSS consortium and collected a total of 634 cases of relapse after allogeneic HSCT from HLA-haploidentical relatives (29.3%), HLA-mismatched unrelated donors (MMUD, 25.9%), 10/10-matched unrelated donors (MUD, 35.8%), or unrelated cord blood units (UCB, 9.0%). Cases were analyzed using conventional HLA typing, the recently developed HLA-KMR assay (Ahci and Toffalori, Blood, 2017) or a novel Next-Generation Sequencing (NGS) method developed to cover all possible HLA-A, -B, -C, -DRB1, -DQB1 and -DPB1 alleles and to analyze multiple samples in a single run. To date, we analyzed 222 relapses, 127 of which using the newly developed HLA-NGS platform. This method resulted highly robust, reliable and sensitive: allowing with a minimum read-depth of 1000x the detection of up to 0.5% of target DNA. False positive reads for patient-specific HLA alleles were detected in 34/73 donor samples, but they were as low as 0.5% on average, and always restricted to one single exon of one or two loci. Ten relapses tested in parallel via HLA-NGS and HLA-KMR showed remarkable concordance between the two methods (R^2=0.86, p<0.0001). In the total 222 cases analyzed to date, we documented 35 HLA loss relapses, 27 of which after haploidentical HSCT (26.0% of relapses in this setting), 7 after MMUD HSCT (11.5%), 1 after 10/10 MUD HSCT (2%) and none after UCB HSCT. The present data, obtained from the largest collaborative study on the immunobiology of relapse to date, confirm the clinical relevance of HLA loss as a major mechanism of recurrence, including after HSCT from unrelated donors.

O7

UNRAVELLING DYNAMIC CHANGES IN THE MATERNAL IMMUNE COMPARTMENT AT THE MATERNAL-FETAL INTERFACE DURING PREGNANCY BY MASS CYTOMETRY

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Preserving the semi-allogeneic fetus during pregnancy relies on finely tuned maternal immune adaptations. To better understand the dynamics of maternal immune cell types at the maternal-fetal interface throughout gestation, a system-wide and in-depth approach was undertaken. Mass cytometry was applied to determine the composition of the maternal myeloid and lymphoid immune compartments from the beginning of pregnancy until parturition. Immune cell heterogeneity within leukocytes isolated from first trimester (6-13 wks) and term pregnancy decidua, as well as peripheral blood from the mother at time of delivery was visualized by high dimensional single-cell t-SNE and HSNE-based analysis tools starting with an overview level of all major immune cell lineages and zooming into distinct immune cell subsets. In a data-driven and unbiased manner, utilizing 39 immune cell markers simultaneously, we identified the presence of multiple innate lymphocyte subsets in the first trimester, decreasing towards the end of pregnancy, contrasting the dynamics of diversifying T cell subsets throughout gestation. Furthermore, several immune cell types residing in the decidua clustered completely separate from immune cell types in the peripheral blood, revealing a unique immune cell composition at the maternal-fetal interface. Separate clustering was also observed between different gestational ages. This study provides insight into how the local maternal immune landscape changes over the course of pregnancy and identified a specific immune cell subset composition in both the innate and adaptive immune compartment. Understanding the organization of immunity at the maternal-fetal interface and the changes that occur from beginning until the end of gestation leads the way in recognizing dysregulated immune mechanisms that drive pregnancy complications.

O8

LOSS OF HLA-A IN ACUTE MYELOID LEUKEMIA TRANSMITTED BY THE DONOR TO LIVER AND KIDNEY GRAFT RECIPIENTS

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We report transmission of acute myeloid leukemia (AML) from a 79-year old cadaveric female donor, deceased because of brain hemorrhage, to two kidney and one liver recipients, transplanted in October 2011. According to

Donor Safety Guidelines released by Italian Competent Authority, donor risk was standard. No clinical, imaging or laboratory records of the donor led to suspect AML. After the alert from nephrologists suggesting the possible transmission of donor-related AML in the 2 kidney recipients (R1, a male patient of 57 at the time of transplantation, diagnosed with AML in May 2013 and R2, a female patient of 50 at the time of transplantation, diagnosed with AML in June 2013), it was ascertained that also the liver recipient (R3) had died from AML complications in November 2012. R1 received treatment for leukemia, but died of complications in October 2012, while R2 went into complete remission until February 2017 when she relapsed. She then received an allogenic bone marrow transplantation but died of treatment-induced complications in December 2017. Retrospective analysis of mutations in the nucleophosmin (NPM1) and Fms-like tyrosine kinase 3 (FLIT3) genes was performed using the buffy coat from the donor and samples from R1 and R2 before and after kidney transplantation. NPM1 mutation was present in 17% of donor, in 49% of R1 and in 24% of R2 blood samples, respectively. The FLIT3 mutation was detected exclusively in R1 (44%), suggesting a distinct clonal evolution. Microsatellite analysis confirmed donor chimerism in circulating cells from R1 and R2 (83% and 29% donor DNA, respectively). Whole exome DNA sequencing was performed on the donor DNA, and on R1 and R2, prior and after kidney transplantation. Allele frequency distributions confirmed the different degrees of chimerism after transplant and enabled the detection of additional mutations. Exome sequencing data were also used to analyze genome wide copy number variation, which suggested copy number loss of the HLA-A region, in both R1 and R2, potentially providing a molecular mechanism for tumor escape. This is the first report of AML transmission following solid organ transplantation and NGS analysis highlights molecular mechanisms explaining evolution of the tumor and escape from immune response.

MHC Evolution, Anthropology & Population Genetics

O9

AGE-DEPENDENT CHANGES IN REGULATION OF HLA-DQ GENE EXPRESSION

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The effects of aging on the immune system are manifest at multiple levels that include reduced production of B and T cells in bone marrow and thymus, and diminished function of mature lymphocytes in secondary lymphoid tissues. As a result, elderly individuals do not respond to immune challenge as robustly as the young. Not only adaptive immunity, but also the innate immune response also declines with age. There are changes in innate cell numbers, with skewing of hematopoiesis towards the myeloid lineages. Ageing macrophages and dendritic cells display reduced phagocytic function and HLA class II expression. We analyzed peripheral blood leucocytes in 78 healthy unrelated donors and in 12 related members of several three generation families. The genotyping of HLA-DRB1, HLA-DQB1 and HLA-DQA1 was performed using PCR with sequence specific primers. The Real-Time PCR was applied to quantify differences in HLA-DQA1 and HLA-DQB1 gene expression. The Chromatin ImmunoPrecipitation (ChIP) assay was used to isolate chromatin fragments containing acetylated histone protein H3 associated with DQA and DQB promoter regions. We detected the activation marker (acetyl-H3) and the inhibition marker (trimethyl-H3K9). At both levels (mRNA transcription and histone modification), senescence of immune system has been characterized by the decreased gene expression of HLA-DQ alleles. The highest expression of DQ beta gene was detected in adulthood, the lowest one in seniors ($p=0.02$). Similar results were obtained also for DQ alpha gene. Moreover, the histone acetylation and methylation have shown the age specific level of histone modification of each allele. Besides non-relative individuals we studied also three-generation family, where we proved that one allele, transmitted from generation to generation, is differentially epigenetically modified in dependence on age of its carrier.

O10

THE DISTRIBUTION OF HUMAN LEUKOCYTE ANTIGEN A AND B GENES IN POPULATIONS IS STRONGLY SHAPED BY THEIR FUNCTIONAL RELATIONSHIP

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The remarkable polymorphism of the human leukocyte antigen (HLA) genes is usually seen as the result of a selective advantage due to the essential function of the HLA molecules in the presentation of pathogen-derived peptides to the