**ORAL SESSIONS**

**O-1**

**IMPACT OF HLA-E*01:03 ALLELE CARRIER STATUS AND NKG2C GENE DELETION ON THE RISK OF CMV INFECTION AFTER RENAL TRANSPLANTATION**

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CMV infection (CMVi) causes serious complications after transplantation. Among HLA molecules the non-classical HLA-E has the ability to bind peptide sequences of the CMV virus. In this context it is of note that (i) allelic variants of HLA-E display substantial differences in peptide binding and that (ii) a gene deletion of the activating NKG2C receptor occurs in about 4% of the population, which might impair HLA-E specific NK cell responses towards CMV. Thus, the question emerged whether HLA-E and NKG2C polymorphisms play a role in CMVi after renal transplantation (Tx) and may be useful for further identification of patients at CMVi risk. 192 living kidney pairs were typed for HLA-E and NKG2C deletion using PCR method. Clinical data (focusing on CMVi within the 1st year after Tx) were associated with typing results. 20 Tx recipients had CMVi during the 1st year independent of the CMV risk status (high risk n=14). With regard to recipient HLA-E polymorphism we found a significantly increased incidence of CMVi among HLA-E*01:03 allele carriers (homozygous or heterozygous) compared to HLA-E*01:03 negative recipients (p=0.0086; OR=9.66 [95% CI: 1.62-102.6]). The same results were obtained for CMV high risk patients (n=14; p=0.036; OR=8.412 [95% CI: 1.29-96.8]). Regarding NKG2C deletion, a tentative association with CMVi in recipients was found (p=0.065; OR=2.2; 95% CI 0.93-5.4).

Multivariate-Cox analysis including CMV high risk status, ATG-induction therapy, recipient HLA-E*01:03 and NKG2C deletion carrier status, revealed HLA-E*01:03 carrier status to be an independent prognostic risk factor along with known risk factors CMV and ATG-induction therapy. No association was observed between donor HLA-E or NKG2C polymorphism and CMVi. The strong association of HLA-E*01:03 with CMVi in recipients indicates that HLA-E typing may be a useful method to identify recipients at risk for CMV infection.

**O-2**

**SELECTIVE IMMUNOMODULATORY EFFECT OF MTOR INHIBITION ON NAÏVE AND VIRUS-SPECIFIC CD8+ T CELLS DRIVES IMMUNE APPLICATION BEYOND ITS ROLE AS IMMUNOSUPPRESSANT**

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Cytomegalovirus (CMV) infection and reactivation remains one of the most important complications in transplantation medicine, particularly in patients undergoing intense immunosuppression (IS). Recent observations indicated that patients treated with mTOR inhibitor, e.g. sirolimus, may exhibit favorable outcomes of CMV infection following transplantation. This study was designed to elucidate this effect through investigation of the role of mTORC1 signaling in CMV-specific cytotoxic CD8+ T cells (CTLs). CD8+ T cells were stimulated with artificial antigen-presenting cells (aAPCs) loaded with CMVpp65 peptide.

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The effect of sirolimus on the proliferative capacity, phenotype and functionality of CTLs was determined. Furthermore, we applied next-generation sequencing (NGS) to monitor dynamics of TCR repertoires under the influence of sirolimus as well as detection of signaling pathways and expression of target and effector molecules. Despite the inhibited expansion, sirolimus induced strong T-cell activation, had no effect on the effector memory phenotype and significantly increased antigen-specific effector T-cell response. Key elements of T-cell activation and function such as (1) dynamics of TCR repertoires, (2) phosphorylation of kinases and proteins, and (3) expression of miRNAs and genes were differently affected under sirolimus treatment, indicating their influence in the improved functionality. In contrast to expectations, we showed improved functional qualities of CMV-CTLs exposed to sirolimus. Modulating the environmental cues during CTL formation by IL-2R driven STAT-5 signaling under mTORC1 inhibition allows the fine-tuning of CTL programming to promote antiviral T-cell response with stable dynamics of TCR repertoires. This study provides help for further individualization of IS therapy, indicating a potential benefit of sirolimus in patients with elevated risk of CMV infection.

O-3

INFLUENCE OF ANTIBODIES AGAINST HLA-DQA/DP TO CHRONIC LUNG ALLOGRAFT DYSFUNCTION

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In recent studies de novo donor-specific HLA antibodies (DSA) particularly against HLA-DQB were suspected to contribute to chronic lung allograft dysfunction (CLAD). However, the role of anti-HLA-DQA/DP is completely unknown. The presence of HLA antibodies was examined prospectively in 224 lung patients transplanted between 2013 - 2017. HLA antibodies were investigated by Single Antigen Bead assay prior and one, three, six, 12, 18, 24 and 36 months after transplantation. We compared patient survival and the development of bronchiolitis obliterans syndrome (BOS) in 118 patients with and without anti-HLA DQA/DP, who had at least a follow up time of 24 months. In 60 out of 118 (50.8%) patients, HLA antibodies were not detectable. Anti-HLA class I (A, B, C) and/or II (DRB, DQB) were found in 28 (23.7%) patients, and 30 (25.4%) patients additionally had anti-HLA-DQA/DP. During the first two years after transplantation, 46 (39%) patients developed de novo DSA. In 21 out of 46 (45.7%) patients DSA were persistently detectable. In 25 (52.2%) patients, DSA disappeared and were classified as transient. There was no significant difference between the two-year survival of patients without and with HLA antibodies but patients with additional anti-HLA-DQA/DP had the poorest survival (82.5%, 85.1%, 69.7%, p=0.269). Patients with additional anti-HLA-DQA/DP also showed a significantly higher risk to developing BOS (16.7%, 26.9%, 42.3%, p=0.047). A significantly higher incidence of BOS was also noticed in the DSA group compared to the non-DSA group (36.6%, 18.5%, p=0.037). The highest mortality two years after transplantation was observed in patients with persistent DSA in comparison to patients with transient DSA (56.4%, 91.5%, p=0.005). The additional presence of anti-HLA-DQA/DP increases the risk of developing CLAD. Patient survival was poorer if the HLA-antibodies persisted and were donor-specific. Therefore, anti-HLA-DQA/DP should be taken into consideration during regular HLA-antibody monitoring after lung transplantation.

O-4

TOWARDS DEFINING THE IMMUNOGENICITY OF HLA EPITOPES: IMPACT OF EPLETS ON ANTIBODY FORMATION DURING PREGNANCY

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Pregnancy serves as model to study antibody responses to HLA mismatches. Such child-specific antibodies (CSA) are induced by non-self structures of HLA molecules of the unborn child. Comparison of high resolution HLA typing from mother and child reveals the Eplet load on mismatched HLA from paternal alleles. Eplets associated with an increased frequency of CSA may indicate HLA epitopes of higher immunogenicity, potentially relevant as well in the transplant setting. The aim of this study was therefore to determine the impact of Eplet load on the extent and type of CSA formation and to assign the relative immunogenicity of different HLA class I Eplets in the pregnancy setting. 155 mothers (devoid of any pre-sensitizing events) and their first newborn children were examined. Immediately after delivery, all sera from the mothers were tested for IgM- and IgG-type HLA class I antibodies and evaluated for Eplet associated reactivity patterns. 397 (85%) of the 465 paternal HLA class I alleles were mismatched. CSA were detected
The developing immune system of childhood promotes better graft acceptance and longevity but also higher risk for infections and post-transplant (Tx) lymphoproliferative disorders. This may be due to lack of memory B-cells or higher proportions of immune modulatory B-cells (CD24hCD38h phenotypes by multicolor flow cytometry. Participants were separated into infant (0-2 years), child (2-10y) and teenager (10-17y) age groups. Pre-Tx infants had higher B-cell proportions than children and teenager (p<0.01), but fewer CD27+ memory B cells of IgM+ (p<0.05) and switched IgM- (p<0.01) phenotype. Memory B-cells increased with age throughout childhood (p<0.05). Memory B-cell development was not affected by Tx but showed similar age-related increase similar to cross sectional values of pre-Tx children despite immune suppression. TrB were higher in infants (p<0.05) and declined to adult values thereafter (p<0.05). Pre-Tx, liver recipients had more B-cells, TrB and CD21+ B-cells (p<0.05) and kidney less CD4+ and more CD8+ T-cells (p<0.05) compared to other organ groups, while post-Tx all organ groups were similar, showing lower CD4+ and higher CD8+ T-cells than pre-Tx. Breg proportion was not associated with age, Tx stage or organ type. While Tx and immune suppression significantly alter T-cell proportions in childhood, B-cells show a similar maturation profile with age as in a non-manipulated immune system. Lack of memory and increased proportions of immature B-cells likely contribute to the better graft acceptance in infants. The role of the individual immune profile and organ related differences on the clinical course are being prospectively assessed in long term follow up.

THE IMPACT OF CHILDHOOD SOLID ORGAN TRANSPLANTATION ON B-CELL MATURATION

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The ability of TCRs to simultaneously scan self-HLA molecules and bound fragments of non-self-origin is a unique event in receptor-ligand interactions. This highly specific immune function is impaired following HSCT for a timespan of several months needed for the maturation of T cells. The progression of HCMV disease in immunocompromised transplant patients induces life-threatening situations. Therefore, the need for a new immune system that delivers vital and potent CD8+ T cells carrying TCRs that recognize even one HCMV peptide/HLA molecule and clear the viral infection long term is clear. The transcription and translation of HCMV proteins in the lytic cycle is a precisely regulated cascade of processes, therefore it is a highly sensitive challenge to adjust the exact time point of HCMV-peptide recruitment over self-peptides. We previously detected the infrequency of viral peptide presentation during infection where the presentation of self-peptides prevalently occurred. We utilized soluble HLA technology in HCMV-infected fibroblasts and sequenced naturally sHLA-A*24:02 presented HCMV-derived peptides. We detected 28 HCMV specific peptides that ranged between nine and 16 AA in...
length and are derived from proteins of different time stages of infection from immediate-early, early and late proteins. Following synthetization these peptides have been used as stimulators for PBMCs from A*24:02 positive or negative donors with or without previous HCMV infection. CD8+ T-cells have been tested for sensitivity and specificity using recombinant A*24:02 expressing T2 cells as target cells bound to the respective peptide. All peptides demonstrated immunogenicity and specificity for HLA-A*24:02. One peptide of 14 AAs length derived from the IE2 antigen induced the strongest T-cell responses; this peptide can be detected with a low ranking score in general peptide prediction databases. These results highlight the need for elaborate and HLA-allele specific peptide selection.

O-7

ADOPTIVE TRANSFER OF CELLULAR IMMUNITY AGAINST CYTOMEGALOVIRUS BY VIRUS-SPECIFIC LYMPHOCYTES FROM A THIRD PARTY FAMILY DONOR

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After hematopoietic stem cell transplantation, reactivation of cytomegalovirus (CMV) is associated with severe complications. We here describe the course of an AML patient with CMV reactivation who did not respond adequately to antiviral therapy and immunoglobulins. She was treated with CMV-specific T cells (virus-specific T cells, VSTs) from her HLA-haploidentical sister; because the unrelated donor was CMV IgG negative. Manufacturing of CMV-specific T cells was carried out at Hannover Medical School with the GMP PepTivator HCMVpp65 for antigenic restimulation using the CliniMACS Cytokine Capture System (Miltenyi Biotec, Bergisch Gladbach, Germany). The final T cell product had a viability of 79.9% with a purity of 41.8% CMV-specific IFN-gamma+ T cells. Viral load and cellular CMV immunity were closely monitored for almost three years. The origin of the CMV-specific cells that provided long-term control of the virus was analyzed by highly sensitive hematopoietic chimerism testing (KMRtype and KMRtrack Chimerism Monitoring Reagents, GenDx, Utrecht, Netherlands). After infusion of VSTs CMV viral load decreased until day 137 after transplantation (day 41 after VSTs) and reached a minimum of 63 copies (98 IU)/ml. Thereafter, it increased again and remained detectable until day 551. From day 566 onwards, CMV viral load was below the detection limit. After VSTs, CMV pp65-specific spots increased until day 713 (367 spots increment). However, a minimum at day 110 and day 144 could be observed. CMV IE1-specific spots reached a maximum at day 123 (19.5 spots increment) and then started to decrease. In summary, viral control by VSTs for at least 41 days could be assumed. After VSTs only moderate side-effects (GvHD grade 2) occurred. At day 713 after transplantation, hematopoietic chimerism testing indicated a 100% donor chimerism in CMV pp65-specific cells. Most likely, long-term CMV-specific immunity was established from the unrelated donor-derived immune system.

O-8

VIRUS-SPECIFIC T CELLS FROM STEM CELL, FAMILY AND THIRD PARTY T-CELL DONORS: PATIENT MONITORING, DONOR SELECTION AND GMP-COMPATIBLE MANUFACTURING

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Intensive immunosuppressive therapy puts patients before and after hematopoietic stem cell (HSCT) or solid organ transplantation (SOT) at risk of opportunistic infections. Among those, infection with and reactivation of cytomegalovirus (CMV), Epstein-Barr virus (EBV), human herpesvirus 6 (HHV6), adenovirus (ADV) and polyoma virus BK (BKV) are frequent and associated with significant morbidity and mortality. Shortcomings of conventional therapies have increased the interest in antiviral T-cell transfer. Efficacy and clinical outcome can be improved by rapid recruitment of a suitable T-cell donor and an established method for fast manufacturing of antiviral T cells. A registry (alloCELL) for unrelated T-cell donors was established, which currently records >200 HLA-typed donors extensively screened for their antiviral T-cell repertoire. The alloCELL lab further established comprehensive protocols to consider clinical requirements of patients at high risk or with failed conventional therapy. Manufacturing license was obtained...
O-9

GENERATION OF CD19-SPECIFIC CHIMERIC ANTIGEN RECEPTOR NATURAL KILLER CELLS FOR THE TREATMENT OF ACUTE LYMPHOBLASTIC LEUKEMIA

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Relapsed and refractory acute lymphoblastic leukemia (ALL) is difficult to treat and relies on therapies such as allogenic hematopoietic stem cell transplantation. A new therapeutic approach with autologous chimeric antigen receptor-modified (CAR) T cells with specificity for CD19 showed potent antitumor efficacy. Similar to these CD19-CAR-T cells, CD19-CAR natural killer (NK) cells pre-stimulated with feeder cells mediate efficient anti-tumor effects against patient derived ALL cells. Compared to T cells, CAR-transduced NK cells exhibit several advantages, such as safety in clinical use and multiple mechanisms by which they recognize cancer cells besides the CAR-specific antigen. Therefore, NK cells possess the potential to kill heterologous leukemia cells which have lost CD19, a persisting problem in the therapy with CD19-CAR-T cells. Mostly, lentiviral vectors are used to genetically engineer primary CAR-NK cells, but transduction efficiency is low and needs improvement. Additionally, protocols for generation of CD19-CAR-NK cells often include pre-stimulation by feeder cells, bearing potential risks in clinical application. Therefore, we focused on the optimization of generating CD19-CAR-NK cells by viral transduction under feeder-cell free conditions. NK cells were isolated from peripheral blood mononuclear cells using CD56 selection or CD3/CD19 depletion kits and stimulated by interleukin 15. After a short expansion phase NK cells were transduced with a lentiviral vector encoding for a CD19-CAR. Different substances which enhance transduction efficiency of lymphocytes were compared in order to further optimize gene modification. The resulting CD19-CAR NK cells further proved their high anti-leukemic potential. Finally, we developed a NK cell specific transduction protocol to successfully generate highly cytotoxic CD19-CAR-NK cells under feeder-cell free conditions.

O-10

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

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HLA and minor antigen mismatches are the main causes of allograft rejection and graft failure. Previously, we have shown in mice and rats that MHC silenced cells and tissues are protected against immune rejection. We also demonstrated in porcine lungs that SLA expression can be turned off in a complex vascularized organ. In this study we evaluated the effect of MHC I silencing prior to allogeneic lung transplantation (Tx) in an established porcine Tx model by monitoring the cytokine response during the first 12 weeks after Tx with immunosuppression given only in the first 4 weeks. SLA I was permanently silenced during normothermic ex vivo perfusion with lentiviral vectors encoding short hairpin RNAs targeting beta2-microglobulin. A lentivirally transduced non-specific shRNA was used in the control lung. NanoLuc was used as a reporter gene in both groups. In each transplant experiment both donor lungs were genetically engineered with one lung being transplanted and the other lung used for quality control. Levels of beta2-microglobulin mRNA and SLA were quantified by RT-PCR and flow cytometry. SLA downregulation of the endothelial cells was designed to reach a level of 70%. Cytokines were monitored every second day after Tx and weekly after the post-operative day (POD) 7 using multiplex technology. Already 1h after Tx the serum levels of IL-1beta,
anti-HLA IGA ANTIBODIES AND KIDNEY GRAFT SURVIVAL IN TRANSPLANTED PATIENTS

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IgA isotype antibodies (Abs) recognize their target antigens in a very specific manner with high affinity as well as high avidity. Little is known about the influence of these non-complement fixing IgA isotype on organ graft survival. Previously, we reported a high prevalence of IgA Abs in serum of kidney re-transplant (Tx) patients leading us to investigate the donor specificity and the effect of IgA isotype on graft survival. Sera from 276 kidney re-Tx candidates within the waitlist of Erlangen-Nuremberg were selected. Importantly, the time to first dialysis after Tx (TtD), measured in months (mo), was used as a precise endpoint marker for loss of graft function. IgG and IgA Abs were investigated using Luminex generic and specific assays. Of the 276 sera, 89 tested positive for IgA and 243 for IgG Abs. Interestingly, the presence of IgA was highly linked to the presence of IgG (p<0.0001). As expected, stratification by IgA and IgG status revealed that grafts silenced for MHC I expression are immunologically invisible and may successfully combat the burden of rejection and immunosuppression.

O-12

HUMAN LEUKOCYTE ANTIGENS (HLA) AND PHARMACOGENETICS

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Pharmacogenomics is the science of how genes affect the way people respond to drugs. How genes affect the way our body processes drugs (pharmacokinetics), the interaction of drugs with receptors (pharmacodynamics) and the treatment efficacy and adverse side effects. Pharmacogenetics is a subset of pharmacogenomics. The study of how inherited variation affects drug response and metabolism. The interactions of drugs with HLA molecules in vivo and in vitro, is a small, but vital part of pharmacogenetics. Today the most important known adverse HLA-drug interactions are: HLA-B*57:01 with Abacavir and Flucloxacillin, HLA-B*15:02 and HLA-A*31:01 with Carbamazepine and Phenytoin and HLA-B*58:01 and HLA-A*33:03 with Allopurinol. These HLA-drug interactions can cause different adverse drug reactions like hypersensitivity syndrome, DRESS (drug reaction (or rash) with eosinophilia and systemic symptoms), SJS (Stevens-Johnson syndrome), TEN (toxic epidermal necrolysis) and DILI (drug-induced liver injury). In the future the diagnostics of these HLA-drug interactions will become more and more important to prevent HLA-triggered adverse drug reactions.

O-13

WHOLE GENE HLA SEQUENCING OF NON-RELATED DONOR-RECIPIENT PAIRS: A RETROSPECTIVE ANALYSIS

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Serious risk of acute graft-versus-host disease (GvHD) and increased mortality in unrelated hematopoietic stem cell transplantation (HSCT) is associated with HLA disparity between donor and recipient. According to EFI standards,
HLA matching is sufficient for exon 2 and 3 of class I and exon 2 of class II, however, little is known about the impact of mismatches beyond the antigen-binding sites. Therefore, we retrospectively analyzed 89 patient-donor pairs for HLA protein disparities outside exon 2 and 3 of the HLA-A, -B, -C, -G, -E, -F, -DRB1, -DQA1, -DQB1, -DPAl and -DPB1 genes. Of these pairs, 72% had been matched for HLA-A, -B, -C (exon 2 and 3) and -DQB1 and -DRB1 (exon 2). Whole genome sequencing revealed that 4.5% of the pairs had protein sequence disparities, surprisingly affecting only exon 4 of HLA class I genes. HLA-DQB1 and -DRB1 did not show any differences at the protein level, whereas HLA-DPA1 and -DPB1 accounted for most of the differences between donor and recipient. A total of 83% of the 89 pairs were mismatched for HLA-DP at the 2-field level: 40% with either a DPA1 or DPB1 locus difference and 43% were mismatched for both loci. Allelic-resolution genotype matching for all loci (22/22 match) was only observed in 10%; 26% had single or double mismatches for HLA-DPA1 or -DPB1. Regarding disparities in non-classical HLA loci, 57% were mismatched in HLA-E, -F or -G, where HLA-E was the most common mismatched locus (24.5%). HLA-G was additionally mismatched in 12% of the pairs. Only 20% of the donor-recipient pairs had a single locus mismatch in the analyzed HLA loci, whilst another 20% showed mismatches in two different HLA loci. In summary, a high number of allele level variations were detected in the classical as well as non-classical HLA loci by sequencing the complete genes. In the next step, clinical data from the patient-donor pairs will be related to the observed HLA sequence variations to determine the clinical relevance on the outcome of transplantation.

O-14

IMPACT OF NK CELLS ON THE BALANCE OF GVL AND GVHD

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Allogeneic hematopoietic stem cell transplantation (HSCT) often remains the only curative treatment for hematological disorders. However, its success is frequently limited by acute and chronic graft-versus-host disease (GVHD) causing significant morbidity and mortality. Clinical studies exploiting the impact of natural killer (NK) cells in allo-HSCT have provided promising results due to the capability of NK cells to induce a graft-versus-leukemia effect (GVL) without causing GVHD. However, it is known that NK cells are a heterogeneous population and can be divided into functionally distinct NK cell subpopulations. Murine NK cells can be separated along their expression of CD117, CD27 and CD11b, whereas human NK cells can be divided into immature NK cells (CD56highCD16dim) and mature cytotoxic NK cells (CD56dimCD16high). In our studies, we focus on the functional relevance of distinct NK cell subsets on the balance of GVL and GVHD. Our data outline that GVHD is associated with a severe defect in NK cell reconstitution. First, in a fully MHC mismatched HSCT mouse model, cytotoxic CD11b+ NK cells, migrating to the peripheral GVHD target organs and providing the most efficient suppression of allogeneic T cell proliferation, protect against GVHD, but maintain GVL. Furthermore, in the longitudinal analysis of immune reconstitution in patients after allo-HSCT, we observed a significant correlation between the incidence and severity of acute GVHD and the regeneration of NK cell subsets. In summary, our data suggests a negative impact of acute GVHD on early NK cell immune reconstitution, maturation and NK subset distribution. Finally, monitoring of early NK cell reconstitution after HSCT may help to identify patients at risk for the development of severe aGVHD.

O-15

FLOW-CYTOMETRIC ANALYSIS OF HLA DISPARITIES AS A HIGHLY EFFICIENT TOOL TO DETECT CHIMERISM IN ANTIGEN-SPECIFIC T CELLS AFTER NON-IDENTICAL TRANSPLANTATION AND ADOPTIVE T-CELL TRANSFER

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Chimerism analysis after HLA-mismatched stem cell transplantation (SCT) represents an essential diagnostic tool in patient monitoring. In particular, flow-cytometric analysis using monoclonal anti-HLA antibodies (mAbs) offers an attractive approach to detect the chimerism status in single cell subsets in transplanted patients. We established a flow-cytometric protocol providing a stable and feasible platform for monitoring chimerism in antigen-specific T cells. For validation, CMVpp65 was selected as target and anti-HLA (IgM) A1/11/26+ and A2 mAbs were used. Donors were selected from the alloCELL registry (www.alloCELL.com) according to their HLA type, CMV serology and antiviral T-cell frequencies. PBMCs from HLA-A01+/02- (donor 1, D1) and A01-/02+ (donor 2, D2) donors were briefly restimulated with CMVpp65 peptide pool and mixed 1:1 and
INVESTIGATING THE IMPACT OF NON-SHARED HLA-C ALLOTYPE EXPRESSION LEVELS ON HLA-C MISMATCHED UNRELATED HSCT USING TWO DIFFERENT HLA-C EXPRESSION PROXY MODELS

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Petersdorf et al. reported in 2014 an association between patient non-shared (PNS) high expressed HLA-C (C) allootypes and inferior HSCT outcome using an imputed HLA-C-expression model (Apps et al. 2013). This study aims at: a) examining the validity of Apps et al. model in Caucasians by using the same methodology in a sample of 400 healthy German blood donors. b) investigating the effect of PNS HLA-C expression levels on outcome by applying HLA-C expression imputed data in an HLA-C-mismatched HSCT setting. Buffy coats from 400 healthy German blood donors were tested by flow cytometry as previously described (Apps et al. 2013) in order to determine HLA-C expression on lymphocytes. With reference to the mean fluorescence intensity (MFI) values measured, HLA-C antigens were categorized as high- or low- expressed. In a cohort of 324 single HLA-C-mismatched transplant pairs both HLA-C expression models were used in order to investigate the impact of high- and low-expressed PNS-C on HSCT outcome. Overall survival (OS), disease free survival (DFS), relapse incidence (RI) and non-relapse mortality (NRM) were set as outcome endpoints, while statistical significance was set at p<0.05. With the exception of HLA-C*02 and *15, the two expression models were fully concordant as to the definition of low- and high-expressed HLA-C antigens. Analysis of PNS-C expression effect on HSCT outcome revealed an unexpected correlation between high expression and better OS (49% vs 33%, p=0.04; HR=0.43, p=0.002) due to lower NRM (37% vs 47%, p=0.02; HR=0.29, p<0.001). Although similar trends were observed, statistical significance was only met with the Apps et al. model. Death cause analysis revealed no differences for GvHD-associated death, while infection related mortality was only slightly higher in the low PNS-C Group of patients (14% vs 10%). Interestingly, donor as well as cumulative donor and patient non-shared HLA-C expression levels had no significant effect on any outcome endpoint. To conclude a) HLA-C antigens exhibit similar expression patterns regardless of race. b) The lower OS observed in lower expressed PNS HLA-C mismatches was due to higher NRM, yet, through an undefined mechanism. Albeit noteworthy, these findings must be confirmed by larger independent cohorts, before definitive conclusions can be drawn.

IN SILICO PREDICTION OF NON-PERMISSIVE HLA-DBP1 MISMATCHES IN UNRELATED HEMATOPOIETIC CELL TRANSPLANTATION BY FUNCTIONAL DISTANCE

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Expression of the non-classical human leukocyte antigen-G (HLA-G) promotes cancer invasiveness and metastatic progression. HLA-G can exist as cell surface molecule or in soluble forms (sHLA-G) including secreted or shed molecules or released molecules via extracellular vesicles (EVs). HLA-G expression is associated with single nucleotide polymorphisms (SNPs) in the HLA-G 3' untranslated region (UTR) on particular haplotypes. In this study, we addressed the question how HLA-G 3' UTR SNP containing haplotypes and sHLA-G impact the clinical parameters and disease outcome in epithelial ovarian cancer (EOC). For this, we (i) sequenced the HLA-G 3' UTR in histologically confirmed EOC patients encompassing nine SNPs located between +2960 and +3227, (ii) defined the 3' UTR SNP containing haplotypes, (iii) inferred the total amount of sHLA-G (sHLA-Gtot) and vesicular sHLA-G (sHLA-GEV) and (iv) analyzed the relationship of sHLA-G and 3' UTR SNP containing haplotypes to EOC. Levels of sHLA-Gtot and sHLA-GEV were significantly increased in serous EOC patients compared to healthy donors (p<0.0001). In EOC patients, elevated levels were associated with advanced disease stage (p<0.0001) mirroring the tumor burden. Strikingly, release of sHLA-GEV was promoted in EOC (p=0.0003) and strongly associated (p<0.01) with the presence of circulating tumor cells (CTC). Although sHLA-Gtot and sHLA-GEV levels were independent of HLA-G 3' UTR SNP containing haplotypes, the homozygous UTR-2 genotype was associated with the presence of CTC prior to therapy (p=0.026). Patients carrying UTR-7 had a significantly improved overall survival (p=0.041) compared to UTR-7 negative ones. This study gives evidence that EOC burden is associated with an enhanced expression of sHLA-G and that EOC favors the release of vesicular sHLA-G. It seems that regulatory elements targeting SNPs specific for UTR-2 or UTR-7 haplotypes are operative in the course of disease of EOC.

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THE NATURE OF A LEUKEMIC MALIGNANCY DETERMINES THE HLA-G-RESTRICTED PEPTIDE RECRUITMENT

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In silico prediction of high-risk non-permissive donor-recipient HLA mismatches after unrelated donor (UD) hematopoietic cell transplantation (HCT) is an attractive yet elusive objective. Non-permissive T-cell epitope (TCE) group mismatches have been defined by alloreactive T-cell cross-reactivity patterns for 72/942 (7.6%) HLA-DPB1 (DPB1) alleles identified to date (TCE-X), including 52/80 (65%) DPB1 alleles reported as common or well documented (CWD) in the EFL and ASHI catalogues. A numerical functional distance (FD) scoring system for in silico prediction of TCE groups based on the median impact of key amino acid polymorphism in exon 2 was developed for all DPB1 alleles (TCE-FD), including 28/80 (35%) CWD alleles not assignable by TCE-X. Here, we compared clinical outcome associations of non-permissive DPB1 mismatches defined by TCE-X or TCE-FD in 2730 patients who received an 8/8 HLA-matched UD-HCT for AML, ALL, MDS or CML between 1999 and 2011. Concordance between the two models was 92.3%, with most differences arising from DPB1*06:01 and DPB1*19:01 differently assigned by TCE-X and TCE-FD. Only 9/28 (32.1%) of CWD alleles not assignable by TCE-X were found in this cohort, occurring in 33/2730 (1.2%) of pairs. In both TCE-X and TCE-FD, non-permissive mismatches were associated with reduced overall survival (HR 1.15, p<.006 and HR 1.12, p<.03), increased transplant-related mortality (HR 1.31, p<.001 and HR 1.26, p<.001), as well as acute (HR 1.16, p<.02 and HR 1.22, p<.001) and chronic graft-versus-host disease (HR 1.20, p<.003 and HR 1.22, p<.001). Our finding that in silico prediction of non-permissive DPB1 mismatches based on experimentally-elaborated FD scores is feasible for any DPB1 allele with known exon 2 sequence and improves prediction of major transplant outcomes including acute and chronic graft-versus-host disease, has important implications for UD searches. TCE-FD is likely to become increasingly useful as new alleles are detected by refined tissue typing techniques, and with the extension of unrelated HCT to new ethnic groups. Our proof-of-principle observation opens new potential avenues for developing risk prediction models across the HLA system.
Tumor immune evasion mechanisms implement the down-regulation of HLA-Ia and the up-regulation of HLA-Ib, particularly HLA-G. While the fate of cells presenting pHLA-Ia molecules is dictated by their polymorphism and thus ability to present a broad range of healthy or pathogenic ligands, HLA-G presenting cells are vice versa protected against immune recognition and thought to present a narrow variety of peptides based on their non-polymorphic nature. To antagonize NK cell mediated lysis due to the lack of HLA-Ia, acute leukemia, multiple myeloma, non-Hodgkin and Hodgkin’s Lymphoma cells induce over-expression of HLA-G. We recently demonstrated that allelic HLA-G variants differ substantially in their peptide repertoires and features, despite their exiguously appearing polymorphism. This unexpected immune function might translate to differential NK cell inhibition between immune privileged organs and tumor cells. To understand if the availability of peptides within a cell influences the selection of tumor- over self-peptides, we engineered sHLA-G*01:01 expressing K562 and HDLM-2 cells for mass spectrometric peptide analysis. While the source of K562 and HDLM-2 derived peptides is of identical origin, e.g. nuclear or cytosolic origin, the features of the bound peptides differ considerably. In particular, p1 can be discriminated by being a K for K562 derived peptides but an R for HDLM-2 derived peptides. No obvious p2 anchor could be determined, yet all peptides are anchored by P at p3 and L at p§Ù. Structural investigations of HLA-G*01:01 bound to peptides with K or R at p1 indicate no difference in peptide topology or pocket A binding. Protein searches in the Proteinatlas revealed a similar availability of proteins in both cell lines. We found the association of HLA-G*01:01 with proteins of the peptide loading complex in both cell types being identical. These results suggest that the differential selection of peptides can be attributed to an exquisite immune function of HLA-G.

**MONITORING OF HUMAN LEUKOCYTE ANTIGEN ANTIBODIES IN KIDNEY TRANSPLANTATION. RESULTS OF A LONG-TERM FOLLOW-UP STUDY AT THE UNIVERSITY TRANSPLANT CENTER IN FRANKFURT.**

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Human leukocyte antigens are a risk factor for transplantation and donor specific antibodies (DSA) are a contra-indication for transplantation. Methods for the detection of these antibodies have been developed that allow for the definition of allele specificities leading to an increased range of poly-immunized patients with high PRA values. This study included a total of 197 patients who received deceased or living donor kidneys. The pre-transplant HLA-Ab status was monitored quarterly. Routine monitoring for HLA class I and HLA class II Ab was performed by ELISA and Micro-Bead Assay Single Antibody test systems (One Lambda; Thermo Fischer) for screening and the for HLA-Ab differentiation. In addition, twice a year, CDC was performed for all patients on the kidney transplant waiting list and quarterly for all immunized patients using an in-house panel with unseparated T/B cells from at least 60 donors or the Lambda Cell Tray 1W60 (One Lambda). Based on HLA-Ab monitoring, unacceptable HLA-A and HLA-B antigens were excluded for donor selection based on the Eurotransplant allocation system (ENIS). An increased risk for death censored graft loss was found in patients with pre-transplant SAB-HLA-Ab (p=0.008). The risk for increased graft loss was also significant in patients with pre-transplant SAB-HLA-Ab but without SAB-detected donor-specific Ab (SAB-DSA) (p=0.012). ELISA was not sufficient to identify pretransplant immunized patients with an increased risk for graft loss. In immunized patients, graft loss was predominantly present in patients who received transplants with a mismatch on the HLA-DR locus. Analysis of these patients based on complement fixing antibodies based on the C1q SAB assay confirmed the detrimental effect of DSA. However, they did not show an additional prospective definition of patients at risk for graft failure. Our results show an increased risk for long-term graft loss in patients with pretransplant SAB-HLA, even in the absence of DSA. SAB-HLA-Ab-positive patients being negative in ELISA or CDC assay, might profit from a well HLA-DR matched graft and intensified immunosuppression. The clinical relevance of C1q Ab pre-transplantation may be limited of individual cases, despite the relevance of these antibodies post-transplantation.

**COMPUTATIONAL SIMULATIONS DEMONSTRATE THE FEASIBILITY AND BENEFIT OF EPITOPE MATCHING IN DECEASED DONOR KIDNEY ALLOCATION**

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The Eurotransplant (ET) Kidney Allocation System (ETKAS) aims to allocate organs to patients on the waiting
list fairly whilst optimizing HLA matching. ETKAS currently considers the count of HLA-A, -B and -DR mismatches on a broad and split level. Evidently, epitope matching (EM) is biologically and clinically more relevant. Here, we performed ETKAS-based computer simulations to evaluate the potential benefit of EM on allocation efficacy. A virtual population of 400,000 individuals was generated using the NMDP haplotype frequency dataset of 2011. A waiting list of 10,400 patients was constructed and maintained during the simulation, matching the 2015 ET Annual Report characteristics. Within ten simulated years, Markov Chain Monte Carlo simulations allocated 22,600 kidneys. T-cell epitopes were calculated using the pirche.org algorithm. Besides comparing four EM scenarios, the impact of applying EM in all ET countries was compared to applying EM only in Germany. The best-balanced scenario prioritized HLA-A, -B and -DR fully matched donors, replaced the HLA match grade by PIRCHE-II score and exchanged the HLA mismatch probability by an epitope mismatch probability polynomial. This setup showed negligible impact on kidney exchange rates and waiting time, whilst HLA match grades decreased mildly. The number of transplantations with PIRCHE-II scores of 50 or better increases from 54% to 77% in ET when EM is applied in all ET countries and respectively from 63% to 88% in Germany when EM is applied only in Germany. Applying EM only in one participant country showed no impact on the other ET countries’ HLA match grades, waiting time or transplant numbers. The anticipated reduced graft loss rates may not only benefit transplanted patients but also patients on the waiting list. We conclude that epitope-based matching may lead to an improved outcome while keeping equal balances on the waiting list.

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PREDICTION OF ANTIBODY- AND CELL-MEDIATED REJECTION OF KIDNEY TRANSPLANTS BY HLA EPITOPE MATCHING

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Our previous study has demonstrated that epitope matching as performed by both the HLAMatchmaker and the Predicted Indirectly Recognizable HLA Epitope (PIRCHÉ) algorithm is an independent predictor for de novo donor-specific HLA antibodies (DSA). Here, we analyzed the correlation between HLA epitope matching and allograft rejection following kidney transplantation. A total of 1,083 consecutive deceased and living kidney transplants performed between 1995 and 2015 were enrolled to the study cohort. All patients revealed no DSA prior to transplantation as detected by solid-phase immunoassays. HLA epitope mismatches were determined by both the HLAMatchmaker and PIRCHÉ algorithm. Rejections were diagnosed according to current Banff criteria. During follow-up 63 (6%) patients developed antibody-mediated rejection (ABMR). T-cell-mediated rejection (TCMR) was observed for 226 (21%) patients during follow-up. There was a direct correlation between the degree of HLA epitope matching and the incidence of ABMR and TCMR. At ten years of follow-up, patients with an HLAMatchmaker score <5 (n=123), >5 to <18 (n=173), >18 to <36 (n=469) and >36 (n=318) revealed a predicted incidence of ABMR and TCMR of 1% and 8%, 5% and 14%, 8% and 25% as well as 15% and 29%, respectively. Patients with a PIRCHÉ-II score <9 (n=107), >9 to <35 (n=149), >35 to <90 (n=504) and >90 (n=323) had a predicted incidence of ABMR and TCMR of 1% and 7%, 3% and 14%, 8% and 25% as well as 14% and 28%, respectively. These findings suggest a potential predictive value of HLA epitope matching for renal allograft rejection.