One patient was newly infected with HCV without actual consequences concerning liver function but received a well functioning graft of a 25 year old donor (s. patient and methods). Two patients with underlying HCV infection showed deterioration of liver function one without correlation to transplantation one with genotype crossing. Of the 20 recipients without HCV infection transplanted with kidneys of anti HCV positive donors with negative HCV PCR none got infected. Mean waiting time of the study group was 12 months shorter than mean waiting time in the Eurotransplant region.

**Conclusion:** Acceptance of kidneys of anti HCV positive donors increases the donor pool and is safe for recipients with chronic HCV infections. Also recipients without HCV infection can be transplanted safely if PCR is negative. In rare cases especially in old recipients HCV infection can be accepted as consequence of transplantation.

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**809 SYK, A NOVEL THERAPEUTIC TARGET FOR PTLD, DRIVES EBSTEIN BARR VIRUS (EBV)+ B CELL LYMPHOMA GROWTH AND SURVIVAL THROUGH ACTIVATION OF THE PI3K/AKT PATHWAY**

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Post-transplant lymphoproliferative disorder (PTLD)-associated Epstein Barr Virus (EBV)+ B cell lymphomas are a serious, and often fatal, complication of solid organ and bone marrow transplantation. Currently, therapeutic options are limited and, apart from reducing immunosuppression, there is no consensus regarding treatment strategies. EBV+B cell lymphomas associated with PTLD express several viral genes including latent membrane protein 2A (LMP2A). LMP2A mimics a constitutively active B cell receptor (BCR) and provides survival signals to latently infected host cells in part by the recruitment of the Syk tyrosine kinase and activation of downstream signalling pathways, such as the PI3K/Akt pathway. We hypothesized that inhibition of Syk activity would diminish growth of EBV+B cell lymphoma lines derived from patients with PTLD. Treatment with R406, an orally available inhibitor of Syk, decreased proliferation and induced apoptosis of these lymphomas, as assayed by 3H-thymidine incorporation and Annexin-V/PI staining, respectively. Western Blot analysis for total Syk and autophosphorylated Syk (Y525/526) revealed that Syk is constitutively activated in these PTLD lines. Additionally, treatment with R406 led to a decrease in Syk autophosphorylation at Y525/526, suggesting that Syk is the target of the small molecule inhibitor R406. Syk is the tyrosine kinase exclusively responsible for the phosphorylation of B cell linker protein (BLNK, SLP-65), an important component of the BCR signalling pathway. Treatment of Ramos cells, a human Burkitt’s lymphoma line, with R406 led to a decrease in the phosphorylation of BLNK upon BCR ligation as determined by intracellular staining. Together, these data verify that Syk is an important component of the BCR signalling pathway. Treatment of Ramos cells, a human Burkitt’s lymphoma line, with R406 led to a decrease in the phosphorylation of BLNK upon BCR ligation as determined by intracellular staining. Together, these data verify that Syk is the target of R406. The PI3K/ Akt pathway plays a crucial role in cell growth and survival; in EBV+B cell lymphomas, activation of the PI3K/Akt pathway leads to, among other things, secretion of the autocrine growth factor IL-10. Akt is constitutively activated in EBV+B cell lymphoma lines; however, treatment with R406 or Syk siRNA resulted in a significant decrease in levels of phosphorylated Akt, as determined by Western Blot. Additionally, treatment of PTLD lines with R406 or Syk siRNA led to decreased autocrine IL-10 production. These data indicate that Syk mediates its effects on EBV+B cell lymphoma growth and survival in part through activation of the PI3K/Akt pathway. Thus, these results provide mechanistic insight into how targeting Syk with inhibitors such as R406 may serve as an effective therapeutic strategy for the treatment of EBV-associated PTLD.

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**811 SILENCING OF HLA CLASS I EXPRESSION BY LENTIVIRUS VECTOR-MEDIATED RNA INTERFERENCE RESULTS IN RESISTANCE OF PRIMARY HUMAN CELLS TO ALLOREACTIVE T CELL-MEDIATED CYTOTOXICITY**

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**Background:** Recent advances in vector technology now allow the possibility of genetically engineering graft tissues to express reduced levels of HLA. Reducing HLA expression could help to overcome the limitations imposed by polymorphisms which restrict availability of suitable donors, complicate logistics of procuring and delivering matched tissues and organs, and necessitate life-long immunosuppression. We have previously shown that lentiviral vectors can be used to deliver short hairpin RNAs (shRNA) that knock down HLA expression in a class- or allele-specific manner in established human cell lines, and thereby allow evasion from immune recognition by alloreactive T cells. Here we have further characterized and quantitated the effects of dose-dependent gene transfer and shRNA-mediated knockdown of HLA in human cell lines and primary cells on their ability to be recognized by alloreactive cytotoxic T lymphocytes (alloCTL).

**Materials & Methods:** Lentiviral vectors for shRNA targeting Class I-specific and HLA-A0201 allele-specific sequences were employed to transduce 293T human embryonic kidney cells and CD34+ human primary hematopoietic progenitor cells. Transduced target cells were analyzed for sensitivity to cytolyis by incubation with HLA-A2-activated alloCTL at an E:T ratio of 10:1.
Interferon-gamma production from the alloCTLs was measured by ELISA, and viability of target cells was determined by MTS assay, annexin V staining, and complement-mediated cytotoxicity assay.

Results: With higher multiplicities of infection (MOI=10-30), cell surface expression of HLA-A2 and HLA-ABC was reduced in a dose-dependent manner by 50% or >80%, respectively, compared to cells transduced with control vector. Additionally, relative to target cells transduced with control vector, target cells transduced with HLA-ABC shRNA and HLA-A0201 shRNA vectors induced 2.5- and 4.5-fold less interferon production from alloCTL, respectively, and exhibited >2-fold enhanced resistance to alloCTL-mediated killing (p<0.05). However, there were no significant differences in survival of shRNA vector-transduced target cells compared to control vector-transduced cells after incubation with non-HLA-restricted LAK cells derived from the same donor.

Conclusions: Reducing expression of HLA in a class- or allele-specific manner may be a relevant tool for regulation of immune responses, even when these are presented by non-professional antigen-presenting cells. We investigated whether human plasmacytoid dendritic cells (pDC) can induce hyporesponsiveness and suppressive capacity in allogeneic T-cells containing memory T-cells, pancreatic islet cells, and keratinocytes.

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812 HUMAN PLASMACYTOID DENDRITIC CELLS INDUCE PROFOUND HYPORESPONSIVENESS AND SUPPRESSIVE CAPACITY IN ALLOGENIC T-CELLS

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BACKGROUND: A major barrier for induction of liver transplant tolerance in humans is their large repertoire of allo-reactive memory T-cells, which rapidly respond to allo-antigens, even when these are presented by non-professional antigen-presenting cells. We investigated whether human plasmacytoid dendritic cells (pDC) can induce hyporesponsiveness and suppressive capacity in allogeneic T-cells containing memory T-cells.

METHODS: Purified PDC from human blood, were stimulated with Toll-Like Receptor (TLR)-7 agonist lexoxiridine (LOX) or TLR-9 ligand CpG A. After 20 hours, allogeneic T-cells containing CD45RA- naïve and CD45RO+ memory cells, were added. T-cell proliferation, cytokine production, CD25 and Foxp3 expression were determined after 7 days. Hyporesponsiveness was assessed in re-stimulations with LPS-matured monocyte-derived DC (MoDC), and suppressive capacity was determined by adding graded numbers of PDC-primed T-cells to responder T-cells stimulated by mature MoDC, either derived from the same donor as PDC, or from a third party.

RESULTS: Toll-like receptor (TLR)-stimulated PDC primed allogeneic T-cells to produce IL10 (CpG-PDC: 668±260 pg/ml; LOX-PDC: 715±369 pg/ml). After stimulation with CpG-PDC or LOX-PDC 13±2% and 14±2%, respectively, of allogeneic CD4 T-cells acquired Foxp3 and CD25 expression. CFSE-dilution showed that TLR-stimulated PDC induced proliferation of CD4+Foxp3+ T-cells. No Foxp3+ T-cells were generated when CD25- T-cells were depleted from allogeneic T-cells prior to their stimulation with PDC, showing that their enrichment was due to expansion from pre-existing Treg. T-cells primed by TLR-stimulated PDC were hyporesponsive upon restimulation with mature MoDC derived from the same donor (90% decrease in proliferation compared with fresh T-cells), and suppressed responder T cells stimulated by mature MoDC in a dose-dependent and donor-specific fashion (55% inhibition of proliferation at suppressor:responder ratio of 1:2). Suppression was abrogated by anti-IL10 receptor antibody.

CONCLUSION: TLR-stimulated human PDC-induced profound hyporesponsiveness and suppressive capacity in allogeneic T-cells, including memory T-cells. Cellular immunotherapy with PDC from donor blood may be considered as a promising approach to silence the allo-reactive repertoire of liver transplant recipients.