Hematopoietic Stem Cell Transplantation Research Literatures

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Abstract: Stem cells are derived from embryonic and non-embryonic tissues. Most stem cell studies are for animal stem cells and plants have also stem cell. Stem cells were discovered in 1981 from early mouse embryos. Stem cells have the potential to develop into all different cell types in the living body. Stem cell is a body repair system. When a stem cell divides it can be still a stem cell or become adult cell, such as a brain cell. Stem cells are unspecialized cells and can renew themselves by cell division, and stem cells can also differentiate to adult cells with special functions. Stem cells replace the old cells and repair the damaged tissues. Embryonic stem cells can become all cell types of the body because they are pluripotent. Adult stem cells are thought to be limited to differentiating into different cell types of their tissue of origin. This article introduces recent research reports as references in the related studies.

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Key words: stem cell; life; research; literature

Introduction

The stem cell is the origin of an organism's life that has the potential to develop into many different types of cells in life bodies. In many tissues stem cells serve as a sort of internal repair system, dividing essentially without limit to replenish other cells as long as the person or animal is still alive. When a stem cell divides, each new cell has the potential either to remain a stem cell or become another type of cell with a more specialized function, such as a red blood cell or a brain cell. This article introduces recent research reports as references in the related studies.

The following introduces recent reports as references in the related studies.

Ambruzova, Z., et al. (2009). "Possible impact of MADCAM1 gene single nucleotide polymorphisms to the outcome of allogeneic hematopoietic stem cell transplantation." Hum Immunol **70**(6): 457-460.

Mucosal addressin cell adhesion molecule-1 (MAdCAM-1) contributes to the recruitment of donor T cells into the mucosal tissues of the recipient after allogeneic hematopoietic stem cell transplantation (aHSCT). The aim of our study was to determine whether selected single nucleotide polymorphisms (SNPs) of the MADCAM1 gene are associated with development of serious complications after aHSCT. Three MADCAM1 gene single nucleotide polymorphisms (rs758502 C/T, rs2302217 A/G, rs3745925 G/T) were genotyped by polymerase chain reaction with sequence-specific primers in 87 Czech, HLA-identical donor-recipient aHSCT pairs. MADCAM1 rs2302217 AA homozygous recipients developed chronic GVHD more frequently than patients with other genotypes (65% vs. 34%; p = 0.025). Furthermore, multivariate analysis revealed the MADCAM1 rs2302217 AA genotype in recipient being also an independent factor associated with development of acute GVHD (p = 0.036) and decreased overall survival (p = 0.001). These data suggest that MADCAM1 gene polymorphisms may be associated with the risk of chronic GVHD and may, also, affect mortality related to aHSCT.

Ansari, M., et al. (2016). "Influence of glutathione S-transferase gene polymorphisms on busulfan pharmacokinetics and outcome of hematopoietic stem-cell transplantation in thalassemia pediatric patients." Bone Marrow Transplant **51**(3): 377-383.

Hematopoietic stem-cell transplantation (HSCT) is currently the only curative therapeutic option for the treatment of thalassemia. In spite of the high cure rate, HSCT can lead to life-threatening adverse events in some patients. Busulfan (Bu) is a key component of the conditioning regimen prior to HSCT. Inter-individual differences in Bu pharmacokinetics (PK) are hypothesized to influence Bu efficacy and toxicity. Since Bu is mainly metabolized by glutathione S-transferase (GST), we investigated the relationship of GSTA1 and GSTM1 genotypes with first-dose PK and HSCT outcomes in 44 children with thalassemia intermedia and thalassemia major. All children received a myeloablative conditioning regimen with IV Bu. Association analysis revealed a relationship between GSTA169C>T (or haplotype *A/*B) and first Bu dose PK that was dependent on sex and Pesaro risk classification (PRC). Among female patients and

patients with PRC I-II, homozygous individuals for the GSTA1T-69 allele defining haplotype *B, had higher Bu exposure and lower clearance (P0.01). Association with HSCT outcomes showed that patients with the GSTM1 null genotypes had higher occurrence of regimen-related toxicity (P=0.01). These results suggest that GST genotypes could be useful to tailor the first Bu dose accordingly to improve HSCT outcome.

Bao, X., et al. (2016). "Donor Killer Immunoglobulin-Like Receptor Profile Bx1 Imparts a Negative Effect and Centromeric B-Specific Gene Motifs Render a Positive Effect on Standard-Risk Acute Myeloid Leukemia/Myelodysplastic Syndrome Patient Survival after Unrelated Donor Hematopoietic Stem Cell Transplantation." Biol Blood Marrow Transplant **22**(2): 232-239.

Donor killer immunoglobulin-like receptor (KIR) group B profiles (Bx) and homozygous of centromeric motif B (Cen-B/B) are the most preferable KIR gene content motifs for hematopoietic stem cell transplantation (HSCT). The risk of transplant from Bx1 donors and the benefit of the presence of Cen-B (regardless of number) were observed for standard-risk acute myeloid leukemia/myelodysplastic syndrome (AML/MDS) patients in this 4-year retrospective study. A total of 210 Chinese patients who underwent unrelated donor HSCT were investigated. Donor KIR profile Bx was associated with significantly improved overall survival (OS; P =.026) and relapse-free survival (RFS; P =.021) and reduced nonrelapse mortality (NRM; P =.017) in AML/MDS patients. A significantly lower survival rate was observed for transplants from Bx1 donors compared with Bx2, Bx3, and Bx4 donors for patients in first complete remission (n = 82; OS: P =.024; RFS: P =.021). Transplant from donors with Cen-B resulted in improved OS (HR =.256; 95% CI,.084 to.774; P =.016) and RFS (HR =.252; 95% CI,.084 to.758; P =.014) in AML/MDS patients at standard risk. However, this particular effect did not increase with a higher number of Cen-B motifs (cB/B versus cA/B; OS: P =.755; RFS: P =.768). No effect was observed on high-risk AML/MDS, acute lymphoblastic leukemia/non-Hodgkin lymphoma, and chronic myelogenous leukemia patients. Avoiding the selection of HSCT donors of KIR profile Bx1 is strongly advisable for standard-risk AML/MDS patients. The presence of the Cen-B motif rather than its number was more important in donor selection for the Chinese population.

Berro, M., et al. (2010). "Association of functional polymorphisms of the transforming growth factor B1 gene with survival and graft-versus-host disease after unrelated donor hematopoietic stem cell transplantation." Haematologica **95**(2): 276-283.

BACKGROUND: Many genetic factors play major roles in the outcome of hematopoietic stem cell transplants from unrelated donors. Transforming growth factor beta1 is a member of a highly pleiotrophic family of growth factors involved in the regulation of numerous immunomodulatory processes. DESIGN AND METHODS: We investigated the impact of single nucleotide polymorphisms at codons 10 and 25 of TGFB1, the gene encoding for transforming growth factor beta1, on outcomes in 427 mye-loablative-conditioned transplanted patients. In addition, transforming growth factor beta1 plasma levels were measured in 263 patients and 327 donors. RESULTS: Patients homozygous for the single nucleotide polymorphism at codon 10 had increased non-relapse mortality (at 3 years: 46.8% versus 29.4%, P=0.014) and reduced overall survival (at 5 years 29.3% versus 42.2%, P=0.013); the differences remained statistically significant in multivariate analysis. Donor genotype alone had no impact, although multiple single nucleotide polymorphisms within the pair were significantly associated with higher non-relapse mortality (at 3 years: 44% versus 29%, P=0.021) and decreased overall survival (at 5 years: 33.8% versus 41.9%, P=0.033). In the 10/10 HLA matched transplants (n=280), recipients of non-wild type grafts tended to have a higher incidence of acute graft-versus-host disease grades II-IV (P=0.052). In multivariate analysis, when analyzed with patients' genotype, the incidences of both overall and grades II-IV acute graft-versus-host disease were increased (P=0.025 and P=0.009, respectively) in non-wild-type pairs. CONCLUSIONS: We conclude that increasing numbers of single nucleotide polymorphisms in codon 10 of TGFB1 in patients and donors are associated with a worse outcome following hematopoietic stem cell transplantation from unrelated donors.

Bock, F., et al. (2016). "Outcome of Patients with Multiple Myeloma and CKS1B Gene Amplification after Autologous Hematopoietic Stem Cell Transplantation." Biol Blood Marrow Transplant **22**(12): 2159-2164.

The gain/amplification of the CKS1B gene on chromosome 1q21 region is associated with a poor outcome in patients with multiple myeloma (MM). However, there are limited data on the outcome of patients with CKS1B amplification after a single high-dose chemotherapy and autologous hematopoietic stem cell transplantation (auto-HCT). We retrospectively evaluated the outcome of patients with CKS1B amplification who received an auto-HCT between June 2012 and July 2014 at our institution. We identified 58 patients with MM and CKS1B gene

amplification detected by fluorescent in situ hybridization (FISH). We compared their outcomes with a propensity score-matched control group of 58 patients without CKS1B amplification who were treated at approximately the same time. The primary objective was to compare the progression-free (PFS) and overall survival (OS) between the CKS1B and the control groups. Stratified log-rank test with the matched pairs as strata and double robust estimation under the Cox model were used to assess the effect of CKS1B gene amplification on PFS or OS in the matched cohort. Patients in the CKS1B and control groups were well matched for age, gender, disease status, year of auto-HCT, response to pretransplantation therapy, and baseline hemoglobin level. In both groups, 57% patients were in first remission and 43% had relapsed disease at auto-HCT. Twenty-seven (47%) patients with CKS1B amplification had concurrent monosomy 13 or 13q deletion; 6 (10%) by conventional cytogenetics only, 16 (28%) by FISH only, and 5 (9%) by both. Median follow-up after auto-HCT was 25.4 months. The median PFS of the CKS1B and the control groups were 15.0 months and 33.0 months (P =.002), respectively. The median OS have not been reached yet. The 2-year OS rates in the CKS1B and the control groups were 62% and 91% (P =.02), respectively. In conclusion, Patients with CKS1B amplification are more likely to have additional high-risk cytogenetic abnormalities and a shorter PFS and OS after an auto-HCT.

Burek Kamenaric, M., et al. (2017). "The impact of KIR2DS4 gene on clinical outcome after hematopoietic stem cell transplantation." Hum Immunol **78**(2): 95-102.

Killer cell immunoglobulin-like receptors (KIR) are a family of inhibitory/activating receptors expressed on NK cells. Interactions of KIR receptors with KIR ligands have been shown to modify hematopoietic stem cell transplantation (HSCT) outcome. The aim of this research was to determine the KIR2DS4 allele variants distribution among 111 patients with different hematological malignancy who underwent HSCT and their donors, and to evaluate KIR2DS4 alleles' impact on HSCT outcome. The KIR gene frequency analysis showed a significantly higher incidence of full-length KIR2DS4 alleles among patients. The impact of KIR2DS4 alleles on transplantation outcomes revealed that donors' full-length KIR2DS4 alleles is associated with lower overall survival rates, higher risk of GVHD and higher relapse incidence. The expression of full-length KIR2DS4 allele variants may contribute to a worse clinical outcome after HSCT. KIR typing for KIR2DS4 could be used as an additional criterion for selecting suitable donors in cases when more than one HLA identical donor is identified for a specific patient.

Burt, R. K., et al. (2000). "Gene-marked autologous hematopoietic stem cell transplantation of autoimmune disease." J Clin Immunol **20**(1): 1-9.

In phase I (safety) trials, we have demonstrated the feasibility of autologous hematopoietic stem cell transplantation (HSCT) for patients with autoimmune diseases. Although this review comments on results of our phase I trials, the focus is on phase II (efficacy) trials using gene-marked autologous stem cells.

Cartier, N. and P. Aubourg (2010). "Hematopoietic stem cell transplantation and hematopoietic stem cell gene therapy in X-linked adrenoleukodystrophy." Brain Pathol **20**(4): 857-862.

Allogeneic hematopoietic stem cell transplantation (HSCT) is the only therapeutic approach that can arrest cerebral demyelination of X-linked adrenoleukodystrophy (ALD) in boys and results in long-term in a good quality of life, provided the procedure is performed at an early stage of disease. Similar benefits of allogeneic HSCT have been demonstrated in adults with cerebral ALD. However, it is not yet known whether allogeneic HSCT can prevent or rescue adrenomyeloneuropathy. Allogeneic HSCT remains associated with significant morbidity and mortality risks, particularly in adults, and not all ALD patients have donors despite the availability of cord blood. The absence of biological markers that can predict the evolutivity of cerebral disease is a major limitation to propose in due time allogeneic HSCT to ALD patients. Recently, HSC gene therapy using lentiviral vector was shown to have comparable efficacy than allogeneic HSCT in two boys with cerebral ALD who had no Human-leukocyte-antigen (HLA)-matched donor. If these results are confirmed in an extended series of patients, HSC gene therapy may become the first therapeutic option for all ALD male patients who develop cerebral demyelination.

Cho, H. J., et al. (2012). "Impact of vitamin D receptor gene polymorphisms on clinical outcomes of HLA-matched sibling hematopoietic stem cell transplantation." Clin Transplant **26**(3): 476-483.

We hypothesized that polymorphisms of the vitamin D receptor (VDR) gene might affect clinical outcomes of allogeneic hematopoietic stem cell transplantation (HSCT). Three VDR gene polymorphisms (BsmI G>A, ApaI G>T, and TaqI T>C) were genotyped in 147 patients who underwent HLA-matched sibling allogeneic HSCT. Frequencies of infection, graft-vs.-host disease (GVHD), overall survival (OS), and disease-free survival (DFS) were compared according to genotypes and haplotypes.

Infection and acute GVHD had trends to be less frequent in patients with ApaI TT genotype than non-TT genotypes (p = 0.061 and p = 0.059, respectively). For TaqI genotypes, there were no statistical differences in frequency of infection and acute GVHD (p = 0.84 and p = 0.30, respectively), but TC genotype was associated with longer OS and DFS than TT genotype (p = 0.022 and p = 0.038, respectively). In the ApaI-TaqI haplotype analysis, patients with TC haplotype had significantly longer OS and DFS than those without TC haplotype (p = 0.022 and p = 0.038, respectively). In multivariable analysis, TaqI genotype and ApaI-TaqI haplotype of recipients were independent prognostic factors for both OS and DFS. This study suggests that the genotype and haplotype of VDR in recipient might be associated with clinical outcome of sibling HLA-matched HSCT.

Cho, I. H., et al. (2015). "Association between interleukin-10 promoter gene polymorphisms and acute graft-versus-host disease after hematopoietic stem cell transplantation: a systematic review and meta-analysis." Hematology **20**(3): 121-128.

OBJECTIVES: Interleukin-10 (IL-10) is an important immunomodulatory cytokine. The association between IL-10 promoter gene polymorphisms and acute graft-versus-host disease (aGVHD) risk is established; however, results of these studies remain inconclusive. We performed a meta-analysis to clarify the effects of IL-10 promoter gene polymorphisms on aGVHD risk. METHODS: The authors searched MEDLINE, EMBASE, and Cochrane Library databases. Two independent authors extracted data, and the effects were estimated from an odds ratio (OR) with 95% confidence intervals (CIs). Subgroup and sensitivity analyses identified sources of heterogeneity. RESULTS: Finally, a total of 11 studies encompassing 3588 recipients and 3221 donors were included to study IL-10 -1082 G > A, -819 C > T, and -592 C > A polymorphisms. IL-10 -819 CC genotype was associated with an increased aGVHD risk (grade I-IV: OR, 2.722 (95% CI, 1.360-5.450); grade II-IV: OR, 2.265 (95% CI, 1.015-5.053)). Furthermore, patients who received grafts from donors with an IL-10 -819 CC genotype experienced more frequent grade I-IV aGVHD (OR, 2.306 (95% CI, 1.168-4.551)). Recipients with IL-10 -592 CC genotypes were at increased risk for grade II-IV aGVHD (OR, 1.999 (95% CI, 1.230-3.250)). Together, this meta-analysis found that IL-10 -819 CC and -592 CC polymorphisms increased aGVHD risk. DISCUSSION AND CONCLUSION: This meta-analysis found the evidence that the IL-10 -819 CC and -592 CC genotypes in both recipients and donors increased the risk of aGVHD in allogeneic hematopoietic stem cell transplantation (HSCT) patients. These results contribute towards improving patient outcome through insight and rationale for individualized treatment strategies considering genetic determinants.

Dickinson, A. M., et al. (2001). "GvHD risk assessment in hematopoietic stem cell transplantation: role of cytokine gene polymorphisms and an in vitro human skin explant model." Hum Immunol **62**(11): 1266-1276.

This present review concentrates on the recent results investigating the role of certain cytokine gene polymorphisms, including tumor necrosis factor alpha, interferon gamma, interleukin-6 (IL-6), IL-10, and IL-1 receptor antagonist, in allogeneic stem cell transplantation. The review discusses their potential role in predicting outcome and the development of a genetic risk index for graft-versus-host disease in human leukocyte antigen matched sibling transplants. By the comparative use of an in vitro human skin explant model, initial results suggest that certain polymorphisms may be associated with more severe disease.

Dou, L. P., et al. (2007). "[Distribution of immunoglobulin like receptor gene in Han population in China and the impact thereof on the HLA-identical sibling hematopoietic stem cell transplantation]." Zhonghua Yi Xue Za Zhi **87**(44): 3111-3114.

OBJECTIVE: To investigate the distribution of killer immunoglobulin like receptor (KIR) gene in the Han population in north China and the impact of donor KIR and patient HLA genotypes on the outcome of HLA-identical sibling hematopoietic stem cell transplantation patients with hematological malignancy. METHODS: Polymerase chain reaction with sequence-specific primers (PCR-SSP) was used to detect the KIR distribution of 150 healthy people and the KIR genotype of donor and HLA genotype of allogeneic stem cell transplantation recipients of 74 donor-recipient pairs, and a retrospective study was carried out to analyze the outcomes of 45 patients with various hematological malignancies who received non T-cell-depleted transplant from HLA-identical sibling donors, all the subjects being of Han nationality in north China. RESULTS: The gene frequencies of KIR2DL1, KIR2DL2, KIR2DL3, and KIR3DL1 were 100%, 20%, 100%, and 95% respectively. 96% of the allogeneic donors carried one of the 3 class I ligands of inhibitory KIR. 36 of the 45 (80%) donor-recipient HLA-identical sibling transplant pairs lacked recipient HLA ligand for donor KIR, among which 35 lacked recipient HLA ligand for donor KIR2DL1, 1 pair lacked that for KIR2DL2/2DL3, and 31 pairs lacked that for KIR3DL1. Cumulative incidence analysis of graft versus host disease (GVHD) in the patients undergoing HLA-identical sibling hematopoietic stem

cell transplantation demonstrated that the incidence of severe acute GVHD (aGVHD) in the patients lacking HLA ligand for donor-inhibitory KIR2DL1 was 31%, significantly lower than that of the patients with HLA ligand for donor-inhibitory KIR2DL1 (70%, P = 0.029), and the incidence of severe aGVHD in the acute myeloid leukemia patients lacking HLA ligand for donor-inhibitory KIR and KIR2DL1 was 18%, significantly lower than that of the KIR compatible patients (75%, P = 0.03). CONCLUSION: Almost all Chinese of Han nationality in north China carry having one of the 3 known class I ligands of inhibitory KIR. KIR and KIR2DL1 mismatch is associated with lower aGVHD after HLA-identical sibling hematopoietic stem cell transplantation.

Dou, L. P., et al. (2008). "The diversity of KIR gene in Chinese Northern Han population and the impact of donor KIR and patient HLA genotypes on outcome following HLA-identical sibling allogeneic hematopoietic stem cell transplantation for hematological malignancy in Chinese people." Int J Hematol **87**(4): 422-433.

Killer cell immunoglobulin-like receptors (KIRs) are members of a group of molecules that specifically recognize HLA class I ligands and are found on subsets of human lymphopoetic cells. The number of KIR loci can vary between individuals, resulting in a heterogeneous array of possible KIR genes. The range of observed profiles has been explained by the occurrence of two haplotype families termed A and B, which can be distinguished on the basis of certain KIR sequences. Immunogenetic analysis of different ethnic populations shows significant differences in terms of the distribution for group A and group B haplotypes. Recently, attention has been focused on the role of killer cell immunoglobulin-like receptor (KIR)-ligand incompatibility in the graft-versus-host direction between donor and recipient in allogeneic hematopoietic stem-cell transplantation (ASCT). The goal of this study was to study the frequency of specific KIR genes in Chinese Northern Han population and evaluate the role of KIR-ligand mismatch in Chinese HLA-identical sibling hematopoietic stem cell transplantation patients with hematological malignancy. Here genomic DNA from 150 Northern Chinese Han individuals was typed for the presence or absence of KIR genes. Seventy-four allogeneic stem cell transplantation donor/recipient pairs were typed for HLA-A, B, C and KIR. Sixteen KIR genes were observed in the population, and framework genes 3DL3, 3DP1, 2DL4, and 3DL2 were present in all individuals. Twenty-two different genotypes were found. Group A haplotypes outnumbered group B haplotypes in frequency by approximately 3:1, with individuals having two group A haplotypes accounting for 51.9% (78/150). We observed that 57 out of 74 (77.3%) donor-recipient pairs could be characterized by lack of recipient HLA ligand for donor KIR. We observed that 36 out of 45 (80%) donor-recipient HLA-identical sibling transplant pairs could be characterized by lack of recipient HLA ligand for donor KIR. Cumulative incidence analysis of aGVHD in patients undergoing HLA-identical sibling hematopoietic stem cell transplantation in this study demonstrated a decreased incidence of severe aGVHD in patients lacking HLA ligand for donor-inhibitory KIR2DL1 (31.4 vs. 70%, P = 0.029). And also in AML (acute myeloid leukemia) patients lacking HLA ligand for donor-inhibitory KIR and KIR2DL1 (17.6 vs. 75%, P = 0.03). Our data demonstrated that the Chinese Han population is distinct in KIR gene frequencies and putative KIR haplotypes in comparison to some other populations. Almost all allogeneic donors could be characterized as having an inhibitory KIR for each of the three known class I ligands. KIR and KIR2DL1 mismatch is associated with lower aGVHD in Chinese after HLA-identical sibling hematopoietic stem cell transplantation.

Dreyer, Z. E., et al. (2011). "Analysis of the role of hematopoietic stem-cell transplantation in infants with acute lymphoblastic leukemia in first remission and MLL gene rearrangements: a report from the Children's Oncology Group." J Clin Oncol **29**(2): 214-222.

PURPOSE: Although the majority of children with acute lymphoblastic leukemia (ALL) are cured with current therapy, the event-free survival (EFS) of infants with ALL, particularly those with mixed lineage leukemia (MLL) gene rearrangements, is only 30% to 40%. Relapse has been the major source of treatment failure for these patients. The parallel Children's Cancer Group (CCG) 1953 and Pediatric Oncology Group (POG) 9407 studies were designed to test the hypothesis that more intensive therapy, including dose intensification of chemotherapy, and hematopoietic stem-cell transplantation (HSCT) would improve the outcome for this group of patients. PATIENTS AND METHODS: One hundred eighty-nine infants (CCG 1953, n = 115; POG 9407, n = 74) were enrolled between October 1996 and August 2000. For infants with the MLL gene rearrangement and an appropriate donor, HSCT was the preferred treatment on CCG 1953 and investigator option on POG 9407 after completion of the second phase of therapy. Fifty-three infants underwent HSCT. RESULTS: The 5-year EFS rate was 48.8% (95% CI, 33.9% to 63.7%) in patients who received HSCT and 48.7% (95% CI, 33.8% to 63.6%) in patients treated with chemotherapy alone (P =.60). Transplantation outcomes were not affected

by the preparatory regimen or donor source. CONCLUSION: Our data suggest that routine use of HSCT for infants with MLL-rearranged ALL is not indicated. However, limited by small numbers, this study should not be considered the definitive answer to this question.

Fujii, H., et al. (2018). "Application of next-generation sequencing to detect acyclovir-resistant herpes simplex virus type 1 variants at low frequency in thymidine kinase gene of the isolates recovered from patients with hematopoietic stem cell transplantation." J Virol Methods **251**: 123-128.

Ion Torrent next-generation sequencing (NGS) technology was applied to study the mode of emergence of acyclovir (ACV)-resistant (ACVr) herpes simplex virus type 1 (HSV-1) in patients with hematopoietic stem cell transplantation (HSCT) by quantitatively detecting mutations in the viral thymidine kinase (vTK) gene in the HSV-1 isolates recovered from HSCT patients. All of the mutations detected with the Sanger sequencing method in the vTK genes of HSV-1 isolates were also detected with the NGS assay. Furthermore, different mutations, which conferred ACV resistance and were not detected with the Sanger sequencing method, were also detected in a quantitative manner by using the NGS assay. The approach described here is applicable to studying the emergence process of vTK gene mutation-associated ACVr HSV-1 more in detail than the Sanger method. The NGS assay makes it possible to make a diagnosis of vTK gene mutation-associated ACVr HSV-1 infections at the early stage, which the ratio of ACVr HSV-1 is much lower than that of ACV-sensitive (ACVs) HSV-1.

Gagne, K., et al. (2009). "Donor KIR3DL1/3DS1 gene and recipient Bw4 KIR ligand as prognostic markers for outcome in unrelated hematopoietic stem cell transplantation." Biol Blood Marrow Transplant **15**(11): 1366-1375.

Given their antileukemic activity, natural killer (NK) cells can alter the outcome of hematopoietic stem cell transplantation (HSCT). The physiologic functions of NK cells are regulated by the interaction of killer immunoglobulin-like receptors (KIR) with specific HLA class I ligands. In the literature, different models based on HLA class I and/or KIR donor (D)/recipient (R) gene disparities are considered as predictors of NK cell alloreactivity. In this retrospective and multicentric French study, we analyzed the clinical impact of the different NK-alloreactivity models in 264 patients who underwent T repleted unrelated HSCT. First, we did not observe that the "KIR ligand-ligand" model had a significant clinical impact on unrelated HSCT outcome, whereas the "missing KIR ligand" model had a significant but limited effect on unrelated HSCT, because only the absence of C1 ligand in patients with myelogenous diseases was associated with a decreased overall survival (OS) (hazard ratio=2.17, P=.005). The "KIR receptor-receptor" and the "KIR receptor-ligand" models seemed the most capable of predicting NK alloreactivity because they had a significant impact on acute graft-versus-host disease (aGVHD) occurrence, OS, and relapse incidence in D/R unrelated pairs. In particular, KIR3DL1 gene mismatches in the GVH direction (D (+)R (-)) and the D KIR3DL1(+)/3DS1(+) and R Bw4(-) combination were respectively correlated with the lowest OS in HLA identical pairs (HR=1.99, P =.02) and the highest incidence of relapse in HLA nonidentical D/R unrelated pairs (HR=4.72, P =.03). Overall, our results suggest a detrimental effect of KIR3DL1(+)/3DS1(+) donor NK cells transplanted into HLA-Bw4(-) patients in the absence of an educational process via KIR3DL1/HLA-Bw4 interactions.

Goussetis, E., et al. (2011). "Cytokine gene polymorphisms and graft-versus-host disease in children after matched sibling hematopoietic stem cell transplantation: a single-center experience." Cell Mol Immunol **8**(3): 276-280.

Various polymorphisms in cytokine genes have recently been investigated as candidate risk factors in allogeneic hematopoetic stem cell transplantation (allo-HSCT). We retrospectively analyzed specific polymorphisms in genes for interleukin (IL)-10, IL-6, tumor-necrosis factor alpha (TNF-alpha) and interferon gamma (IFN-gamma) in a pediatric cohort of 57 histocompatibility leucocyte antigen (HLA)-identical sibling myeloablative transplants. Both recipient and donor genotypes were tested for association with graft-versus-host disease (GVHD) by statistical methods including Cox regression analysis. We found a significant association between the IL-10 promoter haplotype polymorphisms at positions -1082, -819 and -592 with the occurrence of severe (grades III-IV) acute GVHD (aGVHD). Recipients with the haplotype GCC had a statistically significant decreased risk of severe aGVHD (hazard risk (HR)=0.20, 95% confidence interval (CI): 0.06-0.67) in comparison with patients with other IL-10 haplotypes (P=0.008). Transplant-related mortality at 1 year was significantly lower in recipients with this haplotype (HR=0.17, 95% CI: 0.012-0.320) versus other IL-10 haplotypes (P=0.03), whereas overall survival was not influenced by IL-10 haplotype polymorphisms. In multivariate analysis, the presence of the IL-10 GCC haplotype was found as the only variable associated with a statistically significant decreased hazard of severe aGVHD development

(P=0.02, HR=0.21, 95% CI: 0.05-0.78). These results suggest that pediatric patients possessing the IL-10 GCC haplotype may be protected from the occurrence of severe aGVHD in the setting of matched sibling HSCT.

Gruhn, B., et al. (2009). "Polymorphism of interleukin-23 receptor gene but not of NOD2/CARD15 is associated with graft-versus-host disease after hematopoietic stem cell transplantation in children." Biol Blood Marrow Transplant **15**(12): 1571-1577.

Graft-versus-host disease (GVHD) is a major cause of morbidity and mortality after hematopoietic stem cell transplantation (HSCT). The selection of a suitable donor is the most critical issue in preventing severe GVHD. Recent data suggest that the risk of GVHD does not only depend on human leukocyte antigens (HLA) but also on polymorphisms of genes that influence immune responses. We analyzed the 1142 G>A single-nucleotide polymorphism (SNP) in the interleukin-23 receptor gene (IL23R) and 3 SNPs in the NOD2/CARD15 gene in a cohort of 231 children who underwent allogeneic stem cell transplantation and/or their respective donors. No association was observed between any of the NOD2/CARD15 polymorphisms and GVHD in either donor or recipient. Likewise, the IL23R polymorphism in the recipient was not significantly associated with GVHD. We found a significantly reduced incidence of acute GVHD (aGVHD) grade II-IV in patients who were transplanted from a donor with the IL23R polymorphism (5.0% versus 33.3%; P=.009). There was no case of aGVHD grade III-IV if this polymorphism occurred in the donor. These findings could be particularly relevant for children with inborn metabolic or immunologic disorders who do not benefit from a graft-versus-tumor effect, and therefore, selection of a donor with the IL23R polymorphism might be beneficial.

Hacke, K., et al. (2009). "Suppression of HLA expression by lentivirus-mediated gene transfer of siRNA cassettes and in vivo chemoselection to enhance hematopoietic stem cell transplantation." Immunol Res **44**(1-3): 112-126.

Current approaches for hematopoietic stem cell (HSC) and organ transplantation are limited by donor and host-mediated immune responses to allo-antigens. Application of these therapies is limited by the toxicity of preparative and post-transplant immunosuppressive regimens and a shortage of appropriate HLA-matched donors. We have been exploring two complementary approaches for genetically modifying donor cells that achieve long-term suppression of cellular proteins that elicit host immune responses to mismatched donor antigens, and provide a selective advantage to genetically engineered donor cells after transplantation. The first approach is based on recent advances that make feasible targeted down-regulation of HLA expression. Suppression of HLA expression could help to overcome limitations imposed by extensive HLA polymorphisms that restrict the availability of suitable donors. Accordingly, we have recently investigated whether knockdown of HLA by RNA interference (RNAi) enables allogeneic cells to evade immune recognition. For efficient and stable delivery of short hairpin-type RNAi constructs (shRNA), we employed lentivirus-based gene transfer vectors that integrate into genomic DNA, thereby permanently modifying transduced donor cells. Lentivirus-mediated delivery of shRNA targeting pan-Class I and allele-specific HLA achieved efficient and dose-dependent reduction in surface expression of HLA in human cells, and enhanced resistance to allo-reactive T lymphocyte-mediated cytotoxicity, while avoiding non-MHC restricted killing. Complementary strategies for genetic engineering of HSC that would provide a selective advantage for transplanted donor cells and enable successful engraftment with less toxic preparative and immunosuppressive regimens would increase the numbers of individuals to whom HLA suppression therapy could be offered. Our second strategy is to provide a mechanism for in vivo selection of genetically modified HSC and other donor cells. We have uniquely combined transplantation during the neonatal period, when tolerance may be more readily achieved, with a positive selection strategy for in vivo amplification of drug-resistant donor HSC. This model system enables the evaluation of mechanisms of tolerance induction to neo-antigens, and allogeneic stem cells during immune ontogeny. HSC are transduced ex vivo by lentivirus-mediated gene transfer of P140K-O (6)-methylguanine-methyltransferase (MGMT (P140K)). The MGMT (P140K) DNA repair enzyme confers resistance to benzylguanine, an inhibitor of endogenous MGMT, and to chloroethylating agents such as BCNU. In vivo chemoselection enables enrichment of donor cells at the stem cell level. Using complementary approaches of in vivo chemoselection and RNAi-induced silencing of HLA expression may enable the generation of histocompatibility-enhanced, and eventually, perhaps "universally" compatible cellular grafts.

Hara, H., et al. (2009). "Intratumoral interferon-alpha gene transfer enhances tumor immunity after allogeneic hematopoietic stem cell transplantation." Cancer Immunol Immunother **58**(7): 1007-1021.

One of the major challenges in the treatment of solid cancers by allogenic hematopoietic stem cell transfer (alloHSCT) is the specific enhancement of

antitumor immunity. Interferon (IFN) is a cytokine with pleiotropic biological functions including an immunomoduration, and our preclinical studies have shown that an intratumoral IFN-alpha gene transfer induced strong local tumor control and systemic tumor-specific immunity. In the present study, we examined whether the IFN-alpha gene transfer could enhance recognition of tumor-associated antigens by donor T cells and augment the antitumor activity of alloHSCT. First, when a mouse IFN-alpha adenovirus vector (Ad-mIFN) was injected into subcutaneous xenografts of syngeneic renal and colon cancer cells, tumor growth was significantly suppressed in a dose-dependent manner. A significant tumor cell death and infiltration of immune cells was recognized in the Ad-mIFN-injected tumors, and the dendritic cells isolated from the tumors showed a strong Th1-oriented response. The antitumor effect of Ad-mIFN was then examined in a murine model of minor histocompatibility antigen-mismatched alloHSCT. The intratumoral IFN-alpha gene transfer caused significant tumor suppression in the alloHSCT recipients, and this suppression was evident not only in the gene-transduced tumors but also in simultaneously inoculated distant tumors which did not receive the vector injection. A cytotoxicity assay showed specific tumor cell lysis by donor T cells responding to IFN-alpha. Graft-versus-host disease was not exacerbated serologically or clinically in the mice treated with IFN-alpha. This combination strategy deserves evaluation in future clinical trials for human solid cancers.

Hashimoto, H., et al. (2015). "Infusion of donor lymphocytes expressing the herpes simplex virus thymidine kinase suicide gene for recurrent hematologic malignancies after allogeneic hematopoietic stem cell transplantation." Int J Hematol **102**(1): 101-110.

The infusion of donor lymphocytes expressing the herpes simplex virus thymidine kinase suicide gene (TK-cells) is a promising strategy for the treatment of hematologic malignancies relapsing after allogeneic hematopoietic stem cell transplantation. Here we report the results of a phase I clinical trial designed to examine the feasibility, safety, and efficacy of donor lymphocyte infusion (DLI) of TK-cells. Three patients (two with malignant lymphomas, one with acute myeloid leukemia) were enrolled in the trial and received a single DLI of 1 x 10(7) or 5 x 10(7) TK-cells/kg. No local or systemic toxicity related to the gene-transfer procedure was observed. Two patients achieved stable disease. No patient had severe graft-versus-host disease requiring systemic steroid and/or ganciclovir administration. TK-cells were detected in the peripheral blood of all three patients by PCR, but did not persist longer than 28 days. Analysis of cytotoxic T lymphocyte activity detected no immune response against TK-cells by the recipient's own T cells. Flow cytometric analysis showed low proliferative activity and cytotoxic function of TK-cells. In conclusion, DLI of TK-cells was safely performed in all three patients. Our analysis suggests the probable cause of rapid disappearance of TK-cells to be insufficient in vivo expansion of TK-cells in these patients.

Hong, Y., et al. (2017). "[Clinical significance of monitoring ETV6-RUNX1 fusion gene expression in children with acute lymphoblastic leukemia after allogeneic hematopoietic stem cell transplantation]." Zhonghua Xue Ye Xue Za Zhi **38**(8): 680-684.

Objective: To investigate the clinical significance of monitoring ETV6-RUNX1 fusion gene in children with acute lymphoblastic leukemia (ALL) after allogeneic stem cell transplantation (allo-HSCT). Methods: Clinical data of 13 children received allo-HSCT in Peking University Institute of Hematology from May 2009 to March 2016 were retrospectively collected. The ETV6-RUNX1 gene was examined by real-time quantitative polymerase chain reaction (RQ-PCR). The correlation between its expression level and the disease status was analyzed. Results: Of 13 enrolled ALL cases, the ETV6-RUNX1 expression of 7 patients converted to positive after transplant at a median time of 137 days (range, 28-270 days). The expression level of the first positive sample was 0.034% (range, 0.004%-0.061%). The duration from ETV6-RUNX1 positive to hematological relapse was 196 days (range, 28-666 days). Four patients experienced relapse at a median time of 294 days (range, 104-803 days) after allo-HSCT. The ETV6-RUNX1 expression converted to positive prior to MRD. Patients with positive ETV6-RUNX1 gene expression pre-transplantation would be more likely to relapse. Conclusion: Monitoring ETV6-RUNX1 by RQ-PCR could be used to evaluate MRD status after allo-HSCT. Patients with positive ETV6-RUNX1 after transplant had a poor prognosis.

Hu, P., et al. (2016). "Hematopoietic Stem cell transplantation and lentiviral vector-based gene therapy for Krabbe's disease: Present convictions and future prospects." J Neurosci Res **94**(11): 1152-1168.

Currently, presymtomatic hematopoietic stem and progenitor cell transplantation (HSPCT) is the only therapeutic modality that alleviates Krabbe's disease (KD)-induced central nervous system damage. However, all HSPCT-treated patients exhibit severe deterioration in peripheral nervous system function characterized by major motor and expressive language pathologies. We hypothesize that a combination of

several mechanisms contribute to this phenomenon, including 1) nonoptimal conditioning protocols with consequent inefficient engraftment and biodistribution of donor-derived cells and 2) insufficient uptake of donor cell-secreted galactocerebrosidease (GALC) secondary to a naturally low expression level of the cation-independent mannose 6-phosphate-receptor (CI-MPR). We have characterized the effects of a busulfan (Bu) based conditioning regimen on the efficacy of HSPCT in prolonging twi mouse average life span. There was no correlation between the efficiency of bone marrow engraftment of donor cells and twi mouse average life span. HSPCT prolonged the average life span of twi mice, which directly correlated with the aggressiveness of the Bu-mediated conditioning protocols. HSPC transduced with lentiviral vectors carrying the GALC cDNA under control of cell-specific promoters were efficiently engrafted in twi mouse bone marrow. To facilitate HSPCT-mediated correction of GALC deficiency in target cells expressing low levels of CI-MPR, a novel GALC fusion protein including the ApoE1 receptor was developed. Efficient cellular uptake of the novel fusion protein was mediated by a mannose-6-phosphate-independent mechanism. The novel findings described here elucidate some of the cellular mechanisms that impede the cure of KD patients by HSPCT and concomitantly open new directions to enhance the therapeutic efficacy of HSPCT protocols for KD. (c) 2016 The Authors. Journal of Neuroscience Research Published by Wiley Periodicals, Inc.

Humbert, O., et al. (2018). "A Nonhuman Primate Transplantation Model to Evaluate Hematopoietic Stem Cell Gene Editing Strategies for beta-Hemoglobinopathies." Mol Ther Methods Clin Dev **8**: 75-86.

Reactivation of fetal hemoglobin (HbF) is a promising approach for the treatment of beta-hemoglobinopathies and the targeting of genes involved in HbF regulation is under intensive investigation. Here, we established a nonhuman primate (NHP) transplantation model to evaluate hematopoietic stem cell (HSC)-based gene editing strategies aimed at reactivating HbF. We first characterized the transient HbF induction to autologous HSC transplantation in pigtailed macaques, which was comparable in duration and amplitude to that of human patients. After validating function of the HbF repressor BCL11A in NHPs, we transplanted a pigtailed macaque with CD34(+) cells electroporated with TALE nuclease mRNA targeting the BCL11A coding sequence. In vivo gene editing levels were low, but some BCL11A deletions were detected as late as 200 days post-transplantation. HbF production, as determined by F-cell staining and gamma-globin expression, was slightly increased in this animal as compared to transplant controls. We also provided proof-of-concept results for the selection of edited NHP CD34(+) cells in culture following integration of the P140K/MGMT cassette at the BCL11A locus. In summary, the NHP model described here will allow the testing of novel therapeutic approaches for hemoglobinopathies and should facilitate clinical translation.

Isgro, A., et al. (2010). "Progress in hematopoietic stem cell transplantation as allogeneic cellular gene therapy in thalassemia." Ann N Y Acad Sci **1202**: 149-154.

Allogeneic hemopoietic stem cell transplantation (HSCT) represents one of the best cures for thalassemia. Currently, HSCT for thalassemia consists of allogeneic stem cell gene therapy and still awaits autologous genetically modified stem cell transplantation. HSCT for thalassemia has substantially improved over the last two decades, due in large part to improvements in preventive strategies, the effective control of transplant-related complications, and the development of new preparative regimens. A risk classes-based approach to transplantation in thalassemia has led to disease-free survival probability of 87, 85, and 80% in classes 1, 2, and 3 patients, respectively. Adult thalassemia patients, who are higher risk patients for transplant-related toxicity due to an advanced phase of the disease, have a cure rate of 65% with current treatment protocol. Patients who do not have matched family or unrelated donors could benefit from haploidentical mother-to-child transplantation. Overall, the results of this type of transplantation appear encouraging.

Jin, L., et al. (2010). "[Relationship of tumor necrosis factor gene polymorphism and acute graft-versus-host disease after unrelated allogeneic hematopoietic stem cell transplantation]." Zhonghua Nei Ke Za Zhi **49**(4): 320-324.

OBJECTIVE: To explore the relationship between tumor necrosis factor (TNF) gene polymorphisms in donors and recipients and the incidence and severity of acute graft-versus-host diseases (aGVHD) after unrelated allogeneic hematopoietic stem cell transplantation (allo-HSCT). METHODS: Single nucleotide polymorphisms (SNPs) of TNFalpha-238 (G/A), TNFalpha-857 (C/T), TNFalpha-863 (C/A), TNFalpha-1031 (T/C), TNFbeta + 252(A/G) were analyzed by Multiplex SNaPshot analysis in 76 pairs of donors and recipients. RESULTS: Transplantation involving donors with TNFalpha-857 CC genotype resulted in a higher incidence of grade II-IV aGVHD than donors with CT

genotype (91.3% vs 8.7%, P = 0.039). In the 23 patients with grade II-IV aGVHD, no patients had TNFbeta + 252 AA genotype, 19 (82.6%) had GA genotype and 4 (17.4%) had GG genotype. There was a significant difference in the distribution pattern of the TNFbeta + 252 (AA, GA and GG) genotypes in these patients (P = 0.03). There was no significant association of TNFalpha-238 (G/A), TNFalpha-863 (C/A) and TNFalpha-1031(T/C) polymorphisms with the risk of aGVHD. CONCLUSION: These results suggest donor TNFalpha-857 CC genotype is related to a higher incidence of grade II-IV aGVHD, and patients with TNFbeta + 252 AA genotype have protection against the risk of grade II-IV aGVHD.

Kamel, A. M., et al. (2018). "The impact of cytokine gene polymorphisms on the outcome of HLA matched sibling hematopoietic stem cell transplantation." Cytokine **110**: 404-411.

Graft-versus-host disease (GVHD) is the major complication of allogeneic hematopoietic stem cell transplantation (HSCT); cytokines are recognized as important mediators in its pathogenesis. In this study we investigated the role of cytokine gene polymorphisms on HSCT outcome. A total of 106 patient and 98 donors were genotyped by polymerase chain reaction sequence specific primers (PCR-SSP) based assay for tumor necrosis factor-alpha-308 (TNFalpha -308), interleukin (IL)-6-174, IL-10-1082, -819, -592, Interferon-gamma+874 (IFN-gamma+874), and transforming growth factor-beta1 (TGF-beta1) codon10 and 25 polymorphisms. Except one in each category, all patients and donors were TNFalpha -308 high producers and the majority were IL-6-174 high producers (93.3% and 90.8% respectively); a pattern that would alleviate any potential biological impact. Patient's IFN-gamma+874 showed significant association with the development of chronic GVHD. Patients with IFN-gamma +874 high producer showed an 8 folds likelihood to develop chronic GVHD as compared to those with IFN-gamma+874 low producer predicted phenotype (95% CI: 1.59-40.2, p=0.01). Patient's TGFbeta1-codon 10 and 25 high/intermediate producers showed a lower incidence of acute GVHD though it did not achieve statistical significance (p=0.065) on account of the low frequency of this genotype in our patients and donors (11.4 and 8.2% respectively). Other factors contributing to risk of GVHD included older age for both acute and chronic (p=0.01 and 0.02 respectively) with age 24 as the best discriminating cutoff; CD34+ cell dose for chronic GVHD (p=0.045) with a dose of 8x10(6)/kg as the best discriminating cutoff; and conditioning regimen with Flu/Bu associated with the lowest incidence of acute GVHD (p=0.003) and no impact on chronic GVHD. In conclusion the current study further indicates a potential role of some cytokine gene polymorphisms in the development of GVHD. The relative distribution of high and low producer genotypes in different ethnic groups contributes to their biological impact in different populations.

Karabon, L., et al. (2018). "The Influence of Genetic Variations in the CD86 Gene on the Outcome after Allogeneic Hematopoietic Stem Cell Transplantation." J Immunol Res **2018**: 3826989.

CD86 molecule is the ligand for both costimulatory (CD28) and coinhibitory (CTLA-4) molecules, and it regulates immune response after allogeneic hematopoietic stem cell transplantation (alloHSCT). Therefore, we postulate that CD86 gene variations might influence the outcome after alloHSCT. Altogether, 295 adult patients (pts) undergoing related (105 pts) and unrelated (190 pts) donor-matched HSCT were genotyped for the following CD86 gene polymorphisms: rs1129055, rs9831894, and rs2715267. Moreover, the donors' rs1129055 polymorphism was determined. None of the investigated SNPs alone were associated with aGvHD and rate of relapse. However, we showed that rs2715267 SNP influenced overall survival (OS) after alloHSCT. The 24-month OS for the rs271526GG recipients was worse than that for the recipients possessing T allelle (TT or GT genotypes) (p = 0.009). Moreover, analysis of gene-gene interaction between CD86 and CTLA-4 showed that having both the A allele for CD86 rs1129055 and the CTLA-4 CT60GG genotype in recipients increased the risk of aGvHD about 3.5 times. Interestingly, the donors' rs1129055GG genotype and the recipients' CT60GG genotype also increased the risk of aGvHD about 2.7-fold. We postulate that recipients' CD86 gene polymorphisms influence the overall survival after alloHSCT and, together with CTLA-4 polymorphisms, might be considered a risk factor for aGvHD.

Karabon, L., et al. (2015). "A CT60G>A polymorphism in the CTLA-4 gene of the recipient may confer susceptibility to acute graft versus host disease after allogeneic hematopoietic stem cell transplantation." Immunogenetics **67**(5-6): 295-304.

T cell activation plays a crucial role in the development of acute graft versus host disease (aGvHD). Cytotoxic T cell antigen-4 (CTLA-4) is a co-inhibitory molecule that negatively regulates T cell activation, differentiation, and proliferation. Single-nucleotide polymorphisms (SNPs) in CTLA-4 gene may affect its function. Inconsistent observations have been reported regarding the associations of CTLA-4 SNPs with complications after hematopoietic stem cell transplantation (HSCT). Moreover, the majority of the

observations were focused on the donors' SNPs. Recently, a few studies have shown that recipients' genetic variations in the CTLA-4 gene might influence HSCT results. The aim of our study was to determine the influence of the CTLA-4 gene polymorphisms of the donors and the recipients on the outcome of HSCT. Altogether, 312 donor-recipient pairs were genotyped for the CTLA-4c.49A>G (rs231775) and CT60G>A (rs3087243) SNPs using the TaqMan (R)SNP Genotyping Assays. In this study, it was shown that the recipients' CT60G>A [GG] genotype, the myeloablative conditioning regimen, and HSCT from an unrelated donor were independent aGvHD risk factors (odds ratio (OR) 2.63, 95% confidence intervals (95% CI) 1.45-4.59, p = 0.001; OR 2.68, 95% CI 1.65-4.07, p = 0.00003; and OR 1.87, 95 % CI 1.02-3.24, p = 0.04, respectively). Moreover, haplotype analysis revealed that possessing allele A in both of the SNPs decreased the risk of aGvHD approximately 1.5-fold (RR 0.69, p = 0.008). Our data suggest that the CT60G>A [GG] genotype in the recipient has an impact on aGvHD development, especially in patients receiving transplants from unrelated donors together with the myeloablative conditioning regimen.

Karasawa, M., et al. (2005). "Long-term persistence of host cells detected by X-chromosome gene-based assay in patients undergoing gender-mismatched hematopoietic stem cell transplantation." Am J Hematol **80**(2): 101-105.

Using our recently developed human androgen receptor (HUMARA) gene-based chimerism assay, long-term chimerism was investigated in female patients who underwent hematopoietic stem cell transplantation (HCT) with cells from male donors. After restriction digestion of samples, we detected a small number of female-derived cells within a large population of male-derived cells, with a sensitivity from 0.1% to 0.05%. Chimerism was examined in four patients with myeloid malignancies: two patients with acute myeloid leukemia (AML) from myelodysplastic syndrome (MDS), and one patient each with AML M3 and AML M4. All patients underwent myeloablative conditioning regimens and exhibited good clinical results during a median follow-up period of 6.6 years (range, 3.4-7.5 years). Female-derived cells were detected throughout the entire follow-up period in all bone marrow samples, but they became undetectable in the peripheral blood samples of 3 patients. Moreover, the HUMARA band pattern suggests that these residual host cells were normal cells. This study confirms the usefulness of the HUMARA gene-based assay, which showed that patients undergoing HCT frequently show mixed chimerism (MC) for a long period, especially in bone marrow, although the possibility of contamination by host stromal cells cannot be excluded.

Karimi, M. H., et al. (2014). "Association of IL-17 gene polymorphisms and serum level with graft versus host disease after allogeneic hematopoietic stem cell transplantation." Cytokine **69**(1): 120-124.

BACKGROUND: Cytokines are important factors determining the outcome of transplantation. Host ability in cytokine production may be affected by cytokine genes polymorphisms. The aim of the present study was to investigate the effect of IL-17 gene polymorphisms on outcome of Hematopoietic stem cell transplantation. MATERIALS AND METHODS: A total of 60 bone marrow recipients were included in this study. Twenty-five recipients (41.66%) underwent a GVHD. IL-17 gene polymorphisms were evaluated by PCR-RFLP method and the serum levels were also checked by ELISA. RESULTS: No significant differences in distribution of the IL-17(A/G) (rs3819025) genotypes and alleles were observed between two groups. But, IL-17 (A/G, -197) GG genotype was found to be significantly higher in GVHD group compared to those of non-GVHD group (P = 0.04). Interestingly, after stratification of patients according severity of GVHD, IL-17 (rs3819025) G allele was remained significantly higher in GVHD grade (0-I) group compared to those of grade (II-IV) group (P = 0.05). In addition, after categorization of patients according to their sex, IL-17-197 GG genotype showed a significant association with non-GVHD in male patients (P = 0.05). IL-17 serum levels did not show any significant difference between GVHD and non GVHD groups. CONCLUSION: Results indicated that IL-17197 GG genotype, G allele of (rs3819025) and its serum level have predictive values for severity of GVHD. Also, IL-17-197 GG genotype is a sex dependent genetic risk factor for development of GVHD, but this subject need to be studied in different population.

Kim, I., et al. (2007). "Polymorphisms of the methylenetetrahydrofolate reductase gene and clinical outcomes in HLA-matched sibling allogeneic hematopoietic stem cell transplantation." Ann Hematol **86**(1): 41-48.

To evaluate whether the C677T and A1298C polymorphisms of 5,10-methylenetetrahydrofolate reductase (MTHFR) are related to the toxicity of methotrexate (MTX) used in allogeneic stem cell transplantation, we performed association analysis between these genetic polymorphisms and the clinical outcomes of patients treated using human leukocyte antigen-matched sibling stem cell transplantation. Patients (n=72) with hematological malignancy or aplastic anemia were given a short course of MTX as a

graft-versus-host disease prophylaxis. Patients with the 677TT genotype showed higher total bilirubin levels (677TT vs 677CT vs 677CC, 14.5 vs 8.6 vs 3.8 mg/dl, respectively; p=0.07) and higher aspartic transaminase levels (677TT vs 677CT vs 677CC, 678.9 vs 156.6 vs 111.8 IU/l; p=0.04). Platelet recovery to 20,000/mul was slower for patients with the 677TT genotype than for patients with other genotypes (677TT, 59 days; 677CT, 26 days; 677CC, 26 days; p=0.0075). The influences of the C677T polymorphism on treatment-related mortality (TRM) were also analyzed. One-year cumulative TRMs for patients with the TT genotype and the other genotypes were 66 and 30% (p=0.04) and their respective 1-year overall survivals were 30 and 56% (p=0.11). No association was observed between the A1298C polymorphism and clinical outcome for any of the different genotypes. Therefore, patients at high risk of developing hepatic toxicity and with a poor likelihood of survival could be selected by genotyping MTHFR C677T before allogeneic stem cell transplantation.

Kim, Y. J., et al. (2002). "Comprehensive comparison of FISH, RT-PCR, and RQ-PCR for monitoring the BCR-ABL gene after hematopoietic stem cell transplantation in CML." Eur J Haematol **68**(5): 272-280.

The reverse transcriptase-polymerase chain reaction (RT-PCR) was compared with fluorescence in situ hybridization (FISH) and real-time quantitative RT-PCR (RQ-PCR) for minimal residual disease (MRD) monitoring in 266 post-transplant bone marrow samples from 78 patients with chronic myelogenous leukemia (CML). The sensitivities of FISH to BCR-ABL positive samples determined by first-round (1st) RT-PCR, second-round (2nd) RT-PCR, and RQ-PCR were 64.2%, 25.8%, and 20.7%, respectively. The BCR-ABL/ABL ratio by RQ-PCR had a mean of 0.000 13 in the 1st RT-PCR-negative samples and 1.42 in the 1st RT-PCR-positive samples (P<0.001), and means of 0.000 39 and 0.51 in the 2nd RT-PCR-negative and -positive samples (P< 0.001). The mean ratios of BCR-ABL/ABL by RQ-PCR were significantly different in N/N (1st/2nd RT-PCR) or N/P and P/P (P<0.001), but not in N/N and N/P, which showed that the discriminative power of RQ-PCR is confined to the 1st RT-PCR level. In this respect, monitoring of the 1st RT-PCR might be useful for estimating normalized BCR-ABL levels after transplantation. Nested RT-PCR was of limited use, as RQ-PCR quantified the BCR-ABL transcripts in 60 (91%) of 66 samples determined to be negative by 2nd RT-PCR. FISH was significantly correlated with RQ-PCR in FISH-positive samples (n=24, r=0.79, P=0.001). An increase of FISH preceded that of RQ-PCR in a few cases with molecular relapse. By analyzing a large number of samples post-transplant, we found that RQ-PCR might be the most useful assay for MRD monitoring; however, FISH and RT-PCR were found to be useful complementary tools.

Klein, C., et al. (2003). "Gene therapy for Wiskott-Aldrich syndrome: rescue of T-cell signaling and amelioration of colitis upon transplantation of retrovirally transduced hematopoietic stem cells in mice." Blood **101**(6): 2159-2166.

The Wiskott-Aldrich syndrome (WAS) is an X-linked primary immunodeficiency that is caused by mutations in the recently identified WASP gene. WASP plays an important role in T-cell receptor-mediated signaling to the actin cytoskeleton. In these studies we assessed the feasibility of using retroviral gene transfer into WASP-deficient hematopoietic stem cells (HSCs) to rescue the T-cell signaling defect that is characteristic of WAS. Upon transplantation of WASP-deficient (WKO) HSCs that have been transduced with WASP-expressing retroviruses, mature B and T cells developed in normal numbers. Most importantly, the defect in antigen receptor-induced proliferation was significantly improved in T cells. Moreover, the susceptibility of colitis by WKO HSCs was prevented or ameliorated in recipient bone marrow chimeras by retrovirus-mediated expression of WASP. A partial reversal of the T-cell signaling defect could also be achieved following transplantation of WASP-deficient HSCs expressing the WASP-homologous protein N-WASP. Furthermore, we have documented a selective advantage of WT over WKO cells in lymphoid tissue using competitive repopulation experiments and Southern blot analysis. Our results provide proof of principle that the WAS-associated T-cell signaling defects can be improved upon transplantation of retrovirally transduced HSCs without overt toxicity and may encourage clinical gene therapy trials.

Kobayashi, A., et al. (2007). "Allogeneic MHC gene transfer enhances an effective antitumor immunity in the early period of autologous hematopoietic stem cell transplantation." Clin Cancer Res **13**(24): 7469-7479.

PURPOSE: In autologous hematopoietic stem cell transplantation (HSCT), lymphopenia-induced homeostatic proliferation of T cells is driven by the recognition of self-antigens, and there is an opportunity to skew the T-cell repertoire during the T-cell recovery by engaging tumor-associated antigens, leading to a break of tolerance against tumors. However, the homeostatic proliferation-driven antitumor responses seem to decline rapidly in association with tumor growth. We hypothesized that a tumor-specific immune response induced by an

immune gene therapy could enhance and sustain homeostatic proliferation-induced antitumor immunity. EXPERIMENTAL DESIGN: The antitumor effect of allogeneic MHC (alloMHC) gene transfer was examined at the early phase of the immune reconstitution after syngeneic HSCT. RESULTS: Syngeneic HSCT showed significant tumor growth inhibition of syngeneic colon cancer cells within a period of 30 days; however, the tumor then resumed rapid growth and the survival of the mice was not prolonged. In contrast, when the alloMHC plasmid was intratumorally injected at the early phase after syngeneic HSCT, the established tumors were markedly regressed and the survival of recipient mice was prolonged without significant toxicities, whereas no survival advantage was recognized in recipient mice injected with a control plasmid. This tumor suppression was evident even in the other tumors that were not injected with the alloMHC plasmid. The antitumor response was characterized by the development of tumor-specific T cell- and natural killer cell-mediated cytotoxicities. CONCLUSION: The results suggest the efficacy and safety of integrating intratumoral alloMHC gene transfer with an autologous HSCT for the treatment of solid cancers.

Koh, K., et al. (2015). "Early use of allogeneic hematopoietic stem cell transplantation for infants with MLL gene-rearrangement-positive acute lymphoblastic leukemia." Leukemia **29**(2): 290-296.

Sixty-two infants with MLL gene-rearrangement-positive acute lymphoblastic leukemia (MLL-r ALL) were treated with the MLL03 protocol of the Japanese Pediatric Leukemia/Lymphoma Study Group: short-course intensive chemotherapy followed by early allogeneic hematopoietic stem cell transplantation (HSCT) within 4 months of the initial induction. The 4-year event-free survival and overall survival rates were 43.2% (95% confidence interval (CI)=30.7-55.1%) and 67.2% (53.8-77.4%), respectively. A univariate analysis showed younger age (<90 days at diagnosis), central nervous system disease and poor response to initial prednisolone therapy significantly associated with poor prognosis (P<0.05). In a multivariate analysis, younger age at diagnosis tended to be associated with poor outcome (hazard ratio=1.969; 95% CI=0.903-4.291; P=0.088). Although the strategy of early use of HSCT effectively prevented early relapse and was feasible for infants with MLL-r ALL, the fact that substantial number of patients still relapsed even though transplanted in their first remission indicates the limited efficacy of allogeneic HSCT for infants with MLL-r ALL. Considering the risk of severe late effects, indications for HSCT should be restricted to specific subgroups with poor risk factors. An alternative approach incorporating molecular-targeted drugs should be established.

Kosaka, Y., et al. (2004). "Infant acute lymphoblastic leukemia with MLL gene rearrangements: outcome following intensive chemotherapy and hematopoietic stem cell transplantation." Blood **104**(12): 3527-3534.

Forty-four infants with acute lymphoblastic leukemia (ALL) characterized by MLL gene rearrangements were treated on a protocol of intensive chemotherapy followed by hematopoietic stem cell transplantation (HSCT) between November 1998 and June 2002. The remission induction rate was 91.0%, and the 3-year overall survival and event-free survival (EFS) rates, with 95% confidence intervals, were 58.2% (43.5%-72.9%) and 43.6% (28.5%-58.7%), respectively. Univariate analysis of EFS by presenting features indicated a poorer outcome in patients younger than 6 months of age with high white blood cell counts (>/= 100 x 10(9)/L; EFS rate, 9.4% versus 55.1% for all others, P =.0036) and in those with central nervous system invasion (EFS rate, 10.0% versus 56.9% for all others, P =.0073). The 3-year posttransplantation EFS rate for the 29 patients who underwent HSCT in first remission was 64.4% (46.4%-82.4%). In this subgroup, only the timing of HSCT (first remission versus others) was a significant risk factor by multivariate analysis (P <.0001). These results suggest that early introduction of HSCT, possibly with a less toxic conditioning regimen, may improve the prognosis for infants with MLL (+) ALL. Identification of subgroups or patients who respond well to intensified chemotherapy alone should have a high priority in future investigations.

Ligeiro, D., et al. (2016). "KIR genotypic diversity in Portuguese and analysis of KIR gene allocation after allogeneic hematopoietic stem cell transplantation." HLA **87**(5): 375-380.

The diversity of killer-cell immunoglobulin-like receptors (KIR) genes was evaluated in Portuguese and the observed genotypic profiles were found related to the ones reported in European populations. The KIR repertoire after hematopoietic stem cell transplantation is determined by these gene frequencies and the KIR group B motifs are the less common. We estimated donor-KIR/recipient-ligand interactions in transplants with related donors and unrelated donors found in a local registry or from abroad. A large fraction of transplants had all three ligands of inhibitory receptors, and therefore, in theory were not prone to natural killer cell (NK) mediated alloreactivity. Furthermore, the distribution of KIR alloreactive interactions was found independent of the donor-recipient genetic proximity,

probably because of different gene segregation and comparable KIR frequencies in the donor pools.

Liu, Q. F., et al. (2004). "Changes in the T-cell receptor V beta gene repertoire after allogeneic hematopoietic stem cell transplantation." Chin Med J (Engl) **117**(3): 413-418.

BACKGROUND: We distinguished graft-versus-host disease (GVHD) from graft-versus-leukemia (GVL) effects and to investigate the distribution of T-cell receptor (TCR) V beta gene repertoire in individuals with leukemia before and after allogeneic hematopoietic stem cell transplantation (allo-HSCT). METHODS: Peripheral blood mononuclear cells (PBMC) were obtained from 10 normal individuals, 8 donors and 11 patients with leukemia before and after transplantation. Polymerase chain reaction (PCR) amplification of complementarity-determining region 3 (CDR3) of 24 TCR V beta genes was used to examine serial samples of PBMC. The PCR products were further analyzed by genescan to evaluate clonality of T cells. RESULTS: The 24 TCR V beta gene repertoire displayed highly diverse and polyclonal spectratypes in all normal individuals and 4 of 8 donors. Another 4 donors expressed part of the 24 TCR V beta subfamily and 1 donor had oligoclonality. The expressions of the 24 TCR V beta subfamilies were skewed and restricted in 11 leukemia patients before and after transplantation. Some absences of 24 TCR V beta subfamily expression were quite similar between the recipients pro-transplantation and related donors. The number of subfamilies expressed increased over time post-transplantation, but the restricted expressions of the subfamily could last 6 - 30 months after transplantation. All patients with GVHD and some without GVHD exhibited T cell clonal expansion. The expansive T cell clone was distributed in V beta 2-3, 16-17, 18-19, 21 and V beta 23 in patients with GVHD and in V beta 7, 9, 16 and 19 in patients without GVHD. One patient with syngeneic-HSCT (syn-HSCT) had V beta 15 and 16 T cell expansion after transplantation. One patient displayed V beta 18 T cell expansion after donor lymphocyte infusion (DLI). CONCLUSIONS: Normal individuals express the entire 24 TCR V beta gene repertoire and have polyclonal distribution. However, the TCR V beta gene repertoire is only partially expressed in some donors. The TCR V beta gene repertoire is restrictedly expressed in a skew fashion in patients with leukemia before and after transplantation. The number of TCR V beta gene subfamilies increases over time post-transplantation. GVHD and GVL effects may induce the proliferation of T cell clones. Clinical GVL response may be distinguished from GVHD alloreactivity through the host MHC antigen.

Lupo-Stanghellini, M. T., et al. (2010). "Clinical impact of suicide gene therapy in allogeneic hematopoietic stem cell transplantation." Hum Gene Ther **21**(3): 241-250.

Allogeneic hematopoietic stem cell transplantation (allo-SCT) from an HLA-matched related or unrelated donor is a curative option for patients with high-risk hematological diseases. In the absence of a matched donor, patients have been offered investigational transplantation strategies such as umbilical cord blood SCT or family haploidentical SCT. Besides the activity of the conditioning regimen, most of the antileukemic potential of allo-SCT relies on alloreactivity, promoted by donor lymphocytes reacting against patient-specific antigens, such as minor and major histocompatibility antigens, ultimately translating into cancer immunotherapy. Unfortunately, alloreactivity is also responsible for the most serious and frequent complication of allo-SCT: graft-versus-host-disease (GvHD). The risk of GvHD increases with the level of HLA disparity between host and donor, and leads to impaired quality of life and reduced survival expectancy, particularly among patients receiving transplants from HLA-mismatched donors. Gene transfer technologies are promising tools to manipulate donor T cell immunity to enforce the graft-versus-tumor effect, to promote functional immune reconstitution (graft vs. infection), and to prevent or control GvHD. To this purpose, several cell and gene transfer approaches have been investigated at the preclinical level, and are being implemented in clinical trials. Suicide gene therapy is to date the most extensive clinical application of T cell-based gene therapy. In several phase I-II clinical studies conducted worldwide this approach proved highly feasible, safe, and effective in promoting a dynamic and patient-specific modulation of alloreactivity. This review focuses on this approach.

Marktel, S., et al. (2003). "Immunologic potential of donor lymphocytes expressing a suicide gene for early immune reconstitution after hematopoietic T-cell-depleted stem cell transplantation." Blood **101**(4): 1290-1298.

We have previously shown that the infusion of donor lymphocytes expressing the herpes simplex virus thymidine kinase (HSV-tk) gene is an efficient tool for controlling graft-versus-host disease (GVHD) while preserving the graft-versus-leukemia (GVL) effect. In addition to the GVL effect, the administration of donor HSV-tk (+) cells could have a clinical impact in promoting immune reconstitution after T-cell-depleted stem cell transplantation (SCT). To explore this hypothesis, we have investigated whether in vitro polyclonal activation, retroviral transduction, immunoselection, and expansion affect

the immune competence of donor T cells. We have observed that, after appropriate in vitro manipulation, T cells specific for antigens relevant in the context of SCT are preserved in terms of frequency, expression of T-cell receptor, proliferation, cytokine secretion, and lytic activity. A reduction in the frequency of allospecific T-cell precursors is observed after prolonged T-cell culture, suggesting that cell manipulation protocols involving a short culture time and high transduction efficiency are needed. Finally, the long-term persistence of HSV-tk (+) cells was observed in a patient treated in the GVL clinical trial, and a reversion of the phenotype of HSV-tk (+) cells from CD45RO (+) to CD45RA (+) was documented more than 2 years after the infusion. Based on all this evidence, we propose a clinical study of preemptive infusions of donor HSV-tk (+) T cells after SCT from haploidentical donors to provide early immune reconstitution against infection and potential immune protection against disease recurrence.

Masetti, R., et al. (2015). "Impact of inflammatory cytokine gene polymorphisms on developing acute graft-versus-host disease in children undergoing allogeneic hematopoietic stem cell transplantation." J Immunol Res **2015**: 248264.

Single nucleotide polymorphisms (SNPs) in gene encoding pro- and anti-inflammatory factors have been associated with the occurrence of aGvHD. We retrospectively tested a wide panel of 38 polymorphisms in 19 immunoregulatory genes, aiming to first establish, in a pediatric HSCT setting, which SNPs were significantly associated with the development of aGvHD. A significant association was found between aGvHD grades II-IV and SNPs of donor IL10-1082GG, and Fas-670CC + CT and recipient IL18-607 TT + TG genotype. aGvHD grades III-IV resulted associated with donor IL10-1082GG, Fas-670CC + CT, and TLR4-3612TT as well as the use of peripheral CD34+ cells as stem cell source. The multivariate analysis confirmed the association between donor IL10-1082GG and Fas-670CC + CT and aGvHD grades II-IV and between donor IL10-1082GG and TLR4-3612TT and aGvHD grades III-IV. In conclusion we found an association between IL10, FAS, and TLR4 in the donor and IL18 in the recipient and an increased risk of developing aGvHD in transplanted children. Knowledge of the SNPs of cytokine genes associated with aGvHD represents a useful tool for an integrated pretransplantation risk assessment and could guide the physicians to an optimal and more accurate HSCT planning.

Mayor, N. P., et al. (2007). "Single nucleotide polymorphisms in the NOD2/CARD15 gene are associated with an increased risk of relapse and death for patients with acute leukemia after hematopoietic stem-cell transplantation with unrelated donors." J Clin Oncol **25**(27): 4262-4269.

PURPOSE: Hematopoietic stem cell transplantation (HSCT) is an important option in the management of acute leukemia, but the risk of disease relapse and death remains appreciable. Recent studies have suggested that nucleotide-binding oligomerization domain 2 (NOD2)/caspase recruitment domain 15 (CARD15) gene single nucleotide polymorphisms (SNPs), implicated in innate immunity and Crohn's disease, may also affect immune function post-HSCT. PATIENTS AND METHODS: NOD2/CARD15 genotypes were analyzed in 196 patients diagnosed with acute leukemia and their unrelated donors. The pairs are part of a previously well-characterized cohort with a median follow-up of 2.2 years (range, 0.42 to 6.61 years). T-cell depletion was used in 83% of pairs. RESULTS: NOD2/CARD15 SNPs were associated with a reduction in overall survival (44% v 22%; log-rank P =.0087) due to an increase in disease relapse (32% v 54%; Gray's test P =.001) as compared with wild-type pairs. In multivariate analyses, the two most significant factors impacting outcome were transplantation in relapse and the presence of SNPs. The incidence of acute graft-versus-host disease was low and there was no significant difference due to the presence of SNPs. CONCLUSION: These data indicate an unrecognized role for the NOD2/CARD15 gene in unrelated donor HSCT for acute leukemia. The increased risk of disease relapse suggests that the wild-type gene product may contribute to a graft-versus-leukemia effect. These data suggest that NOD2/CARD15 genotyping before transplantation may contribute to prognosis and influence clinical management.

Miller, C. L., et al. (2002). "Feasibility of using autologous transplantation to evaluate hematopoietic stem cell-based gene therapy strategies in transgenic mouse models of human disease." Mol Ther **6**(3): 422-428.

Histoincompatibility between murine donors and recipients of bone marrow (BM) transplants reduces engraftment, and this compromises assessment of hematopoietic stem cells (HSCs) in certain transgenic mice. To study HSCs in the S+S-Antilles mouse model of human sickle cell disease (SCD), we developed an autotransplant protocol. Initial experiments showed no differences between S+S-Antilles mice and normal C57BL/6 (+/+) mice in their radiosensitivity or baseline hematopoietic progenitor numbers. The kinetics of red blood cell (RBC) replacement post-transplant in +/+ recipients of mixtures of transgenic and +/+ BM cells also showed

no competitive advantage of the +/+ cells. BM cells were then aspirated from mice 4 days after 5-fluorouracil treatment, transduced with a green fluorescent protein (GFP)-encoding retrovirus, and transplanted into the same recipients that, just before transplant, were irradiated with 800 cGy. We subsequently detected high levels of GFP (+) RBCs (21-79%) and white blood cells (WBCs; 35-88%) in the blood for 11 months and showed that transduced HSCs regenerated in the primary mice also repopulated secondary mice. These findings provide a generally applicable protocol for performing autotransplants in mice and forecast the potential utility of this approach in assessing HSC-based gene therapy protocols in transgenic mouse models of many human diseases.

Ohashi, M., et al. (2006). "Allogeneic MHC gene transfer enhances antitumor activity of allogeneic hematopoietic stem cell transplantation without exacerbating graft-versus-host disease." Clin Cancer Res **12**(7 Pt 1): 2208-2215.

Enhancement of the specific antitumor activity of allogeneic hematopoietic stem cell transplantation (alloHSCT) against solid cancers is a major issue in the clinical oncology. In this study, we examined whether intratumoral allogeneic MHC (alloMHC) gene transfer can enhance the recognition of tumor-associated antigens by donor T cells and augment the antitumor activity of alloHSCT. In minor histocompatibility antigen-mismatched alloHSCT (DBA/2-->BALB/c: H-2(d)) recipients, alloMHC gene (H-2K (b)) was transduced directly into a s.c. tumor of CT26 colon cancer cells. Because CT26 cells have an aggressive tumorigenicity in syngeneic BALB/c mice, an H-2K (b) gene transfer provides only a limited antitumor effect after syngeneic (BALB/c-->BALB/c) HSCT. By contrast, the H-2K (b) gene transfer caused significant tumor suppression in the alloHSCT recipients, and this suppression was evident not only in the gene-transduced tumors but also in simultaneously inoculated distant tumors without gene transduction. In vitro cytotoxicity assay showed specific tumor cell lysis by donor T cells responding to the H-2K (b) gene transfer. Graft-versus-host disease was not exacerbated serologically or clinically in the treated mice, demonstrating that alloMHC gene transfer enhances the antitumor effects of alloHSCT without exacerbating graft-versus-host disease. This combination strategy has important implications for the development of therapies for human solid cancers.

Oshima, K., et al. (2015). "Hematopoietic Stem Cell Transplantation for X-Linked Thrombocytopenia With Mutations in the WAS gene." J Clin Immunol **35**(1): 15-21.

X-linked thrombocytopenia (XLT) is a mild form of the Wiskott-Aldrich syndrome (WAS) caused by mutations in the WAS gene. A recent retrospective study of the clinical outcome and molecular basis of a large cohort of XLT patients demonstrated that although overall survival is excellent, event free survival is severely affected with conservative treatment. To answer the question whether hematopoietic stem cell transplantation (HSCT) offers a viable alternative therapeutic option in XLT, we retrospectively investigated the outcome of HSCT in a cohort of 24 XLT patients who received HSCT between 1990 and 2011 at 14 transplant centers in the United States, Italy, Germany, Canada, and Japan. The engraftment rate was 100% and the overall survival rate was 83.3%. Of the four non-survivors, 2 underwent splenectomy prior to HSCT and died of sepsis, and two of aspergillus infections associated with severe GVHD. In all but one patient, pretransplant complications were resolved by HSCT. Our data indicate that HSCT following myeloablative conditioning is curative and associated with acceptable risks as a treatment option for XLT.

Palagano, E., et al. (2017). "Hematopoietic stem cell transplantation corrects osteopetrosis in a child carrying a novel homozygous mutation in the FERMT3 gene." Bone **97**: 126-129.

Osteopetrosis (OPT) is a rare skeletal disorder with phenotypic and genotypic heterogeneity: a variety of clinical features besides the bony defect may be present, and at least ten different genes are known to be involved in the disease pathogenesis. In the framework of this heterogeneity, we report the clinical description of a neonate, first child of consanguineous parents, who had osteoclast-rich osteopetrosis and bone marrow failure in early life, but no other usual classical features of infantile malignant OPT, such as visual or hearing impairments. Because of the severe presentation at birth, the patient received Hematopoietic Stem Cell Transplantation (HSCT) at 2months of age with successful outcome. Post-HSCT genetic investigation by means of exome sequencing identified a novel homozygous mutation in the Fermitin Family Member 3 (FERMT3) gene, which was predicted to disrupt the functionality of its protein product kindlin 3. Our report provides information relevant to physicians for recognizing patients with one of the rarest forms of infantile malignant OPT, and clearly demonstrates that HSCT cures kindlin 3 deficiency with severe phenotype.

Pihusch, M., et al. (2004). "Impact of thrombophilic gene mutations and graft-versus-host disease on thromboembolic complications after

allogeneic hematopoietic stem-cell transplantation." Transplantation **78**(6): 911-918.

BACKGROUND: Hemostatic events in patients undergoing allogeneic hematopoietic stem-cell transplantation (HSCT) increase the morbidity and mortality in this cohort. Little is known about the impact of graft-versus-host disease (GvHD) or of thrombophilic gene mutations/polymorphisms on these complications. STUDY DESIGN: Eighty-nine allogeneic stem-cell recipients and their donors were evaluated prospectively for the presence of the factor V G1691A mutation, the prothrombin G20210A mutation, the 5,10-methylenetetrahydrofolate-reductase (MTHFR) C677T mutation, the glycoprotein IIIa PI (a1/a2) polymorphism, the fibrinogen-beta-chain 455G/A polymorphism, the plasminogen activator inhibitor-1 -675 4G/5G polymorphism, and the angiotensin-converting enzyme intron 16 I/D polymorphism. These mutations/polymorphisms and GvHD parameters were correlated to hemostatic and toxic complications after transplantation. The data were compared with those of 128 healthy controls. RESULTS: The PAI-1 4G/4G polymorphism increases the risk for catheter thrombosis after HSCT 5.7-fold (32.2% vs. 71.4%, P<0.05). In patients with hepatic veno-occlusive disease, the frequency of the PAI-1 4G allele is also increased (83.3% vs. 55.1%, NS). Thrombophilic mutations/polymorphisms in donors do not influence complications in the corresponding recipients. The MTHFR TT genotype does not modify severity and duration of mucositis and aplasia in patients receiving methotrexate prophylaxis. Patients with chronic GvHD have a higher risk of thromboembolism (12.9% vs. 1.7%, P<0.05). CONCLUSION: Thrombophilic gene mutations have only a moderate influence on hemostatic complications in patients undergoing HSCT. This may be because of the overwhelming immunologic impact of GvHD on hemostasis in the allogeneic transplantation setting.

Poloni, A., et al. (2011). "Gene expression profile of cytokines in patients with chronic graft-versus-host disease after allogeneic hematopoietic stem cell transplantation with reduced conditioning." Cytokine **53**(3): 376-383.

There are no reliable markers useful to predict the onset or the evolution of chronic graft-versus-host disease (cGVHD) after allogeneic hematopoietic stem cell transplantation (HSCT), although several candidate biomarkers have been identified from limited hypothesis-driven studies. In this study we evaluated 14 patients who received a reduced intensity conditioning HSCT. Seven patients had cGVHD, whereas 7 never developed cGVHD during the period of observation. The expression of 114 cytokines in immunoselected cell populations was explored by microarray analysis and 11 cytokines were selected for further evaluation by real-time PCR. Differential gene expression measurements showed a significant up-regulation for INFgamma (interferon, gamma) in CD8+ and for TNFSF3 (tumor necrosis factor superfamily, member 3) and for TNFSF10 (tumor necrosis factor superfamily, member 10) in CD14+ cell population when comparing cGVHD with control samples. The expression levels were significantly decreased for TNFSF10 in CD8+ cell population and for TNFSF12 (tumor necrosis factor superfamily, member 12) and for PDGFbeta (platelet-derived growth factor, beta) in CD4+. Our data seem to suggest that different immune populations can play a role in cGVHD pathogenesis and the early detection of gene expression profile in these patients could be useful in the monitoring of GVHD. We hypothesized that PDGFbeta down-regulation could represent a negative feedback to compensate for enhanced expression of its receptor recently reported.

Ramzi, M., et al. (2017). "Association Between Cytotoxic T-Lymphocyte Antigen 4 Gene Polymorphisms and Torque Teno Virus Infection After Hematopoietic Stem Cell Transplantation." Exp Clin Transplant.

OBJECTIVES: An association between costimulatory molecule gene polymorphisms and viral infection after hematopoietic stem cell transplantation may be related to clinical outcomes, especially acute graft-versus-host disease. Cytotoxic T-lymphocyte antigen 4 has been suggested as a crucial negative regulator of the immune system. In this study, our objective was to investigate the association between cytotoxic T-lymphocyte antigen-4 gene polymorphisms (including -1722 T/C, -1661 A/G, -318 C/T, and +49 A/G) and torque teno virus infection after hematopoietic stem cell transplantation in patients with and without acute graft-versus-host disease. MATERIALS AND METHODS: Our study included 71 recipients. We evaluated cytotoxic T-lymphocyte antigen 4 gene polymorphisms using the polymerase chain reaction-restriction fragment length polymorphism method. RESULTS: Our results showed that the GG genotype of the cytotoxic T-lymphocyte antigen 4 +49 A/G was significantly more frequent in transplanted patients infected with torque teno virus, whereas the AG genotype was more common in transplanted patients who did not have this infection. In addition, the -1661 AA and GA genotypes and -318 TC genotypes were significantly more frequent in transplanted patients infected with the virus and who had low-grade (grades I and II) acute graft-versus-host disease. Among those with grade I graft-versus-host disease, the GG genotype of

the cytotoxic T-lymphocyte antigen 4+49 A/G was more frequent in transplanted patients with torque teno virus infection, whereas the AG genotype was higher in transplanted patients who did not have this infection. CONCLUSIONS: This is the first report indicating that cytotoxic T-lymphocyte antigen 4 gene polymorphism may be implicated in prevalence of torque teno virus infection after stem cell transplant. Further larger studies and evaluation of other costimulatory molecules are suggested.

Rocha, V., et al. (2009). "Association of drug metabolism gene polymorphisms with toxicities, graft-versus-host disease and survival after HLA-identical sibling hematopoietic stem cell transplantation for patients with leukemia." Leukemia **23**(3): 545-556.

Individual differences in drug efficacy or toxicity can be influenced by genetic factors. We investigated whether polymorphisms of pharmacogenes that interfere with metabolism of drugs used in conditioning regimen and graft-versus-host disease (GvHD) prophylaxis could be associated with outcomes after HLA-identical hematopoietic stem cell transplantation (HSCT). Pharmacogenes and their polymorphisms were studied in 107 donors and patients with leukemia receiving HSCT. Candidate genes were: P450 cytochrome family (CYP2B6), glutathione-S-transferase family (GST), multidrug-resistance gene, methylenetetrahydrofolate reductase (MTHFR) and vitamin D receptor (VDR). The end points studied were oral mucositis (OM), hemorrhagic cystitis (HC), toxicity and venoocclusive disease of the liver (VOD), GvHD, transplantation-related mortality (TRM) and survival. Multivariate analyses, using death as a competing event, were performed adjusting for clinical factors. Among other clinical and genetic factors, polymorphisms of CYP2B6 genes that interfere with cyclophosphamide metabolism were associated with OM (recipient CYP2B6(*)4; P=0.0067), HC (recipient CYP2B6(*)2; P=0.03) and VOD (donor CYP2B6(*)6; P=0.03). Recipient MTHFR polymorphisms (C677T) were associated with acute GvHD (P=0.03), and recipient VDR TaqI with TRM and overall survival (P=0.006 and P=0.04, respectively).Genetic factors that interfere with drug metabolisms are associated with treatment-related toxicities, GvHD and survival after HLA-identical HSCT in patients with leukemia and should be investigated prospectively.

Ruf, S., et al. (2014). "EBV load in whole blood correlates with LMP2 gene expression after pediatric heart transplantation or allogeneic hematopoietic stem cell transplantation." Transplantation **97**(9): 958-964.

BACKGROUND: Epstein-Barr virus (EBV) is associated with posttransplant lymphoproliferative disease (PTLD), and EBV load measurement is an important tool to monitor transplant patients. Although EBV DNA quantification has high sensitivity to identify patients at risk for PTLD, it lacks specificity. We examined whether EBV gene expression in peripheral B cells can increase specificity or correlates with EBV load. METHODS: Altogether, 220 blood samples were collected from pediatric patients after heart transplantation (HTx, n=57), renal transplantation (n=1), or hematopoietic stem cell transplantation (n=21). In each blood sample, EBV load was quantified in whole blood, plasma, and B cells using qPCR. Additionally EBV gene expression (EBNA2, LMP1, LMP2, and BZLF1) in B cells was analyzed using relative quantitative RT-qPCR. RESULTS: Positive expression of at least one gene was detected in 112 (51%) of 220 samples. Patients with PTLD or chronic high viral loads after solid organ transplantation exhibited no homogeneous EBV gene expression pattern. Expression of LMP2, LMP1, or EBNA2 was only observed when EBV load exceeded 1000 copies/mL. A high correlation between the level of LMP2 expression and EBV load in B cells or whole blood was observed (rho=0.72 or rho=0.6, HTx population). CONCLUSION: The analysis of EBV gene expression in peripheral B cells does not provide additional information about patients' risk of developing PTLD. As EBV load in whole blood correlates well with LMP2 gene expression in EBV-infected B cells, EBV DNA quantification in whole blood alone seems to be a sufficient tool to monitor these patients.

Sarashina, T., et al. (2016). "Hematopoietic stem cell transplantation for pediatric mature B-cell acute lymphoblastic leukemia with non-L3 morphology and MLL-AF9 gene fusion: three case reports and review of the literature." Int J Hematol **104**(1): 139-143.

Mature B-cell acute lymphoblastic leukemia (B-ALL) is typically associated with French-American-British (FAB)-L3 morphology and MYC gene rearrangement. However, rare cases of mature B-ALL with non-L3 morphology and MLL-AF9 fusion have been reported, and such cases are characterized by a rapid and aggressive clinical course. We here report three such cases of pediatric mature B-ALL in female patients respectively aged 15 months, 4 years, and 4 months. Bone marrow smears at diagnosis showed FAB-L1 morphology in all patients. Immunophenotypically, they were positive for cluster of differentiation (CD)10, CD19, CD20 (or CD22), Human Leukocyte Antigen-DR, and surface immunoglobulin lambda. No evidence of MYC rearrangement was detected in any of the cases by fluorescent in situ hybridization (FISH) analysis. However, MLL rearrangement was detected by FISH,

and MLL-AF9 fusion was confirmed by reverse transcriptase-polymerase chain reaction. All patients achieved complete remission after conventional chemotherapy and subsequently underwent hematopoietic stem cell transplantation as high-risk ALL; patient 3 for infantile ALL with MLL rearrangement and the others for ALL with MLL rearrangement and hyperleukocytosis (white blood cell count at diagnosis >50 x 10(9)/L). At the latest follow-up for each case (12-98 months post-transplantation), complete remission was maintained. Moreover, we discuss the clinical, genetic, and immunophenotypic features of this rare disease.

Sasazuki, T., et al. (2016). "Gene Map of the HLA Region, Graves' Disease and Hashimoto Thyroiditis, and Hematopoietic Stem Cell Transplantation." Adv Immunol **129**: 175-249.

The human leukocyte antigen (HLA) genomic region spanning about 4 Mb is the most gene dense and the polymorphic stretches in the human genome. A total of the 269 loci were identified, including 145 protein coding genes mostly important for immunity and 50 noncoding RNAs (ncRNAs). Biological function of these ncRNAs remains unknown, becoming hot spot in the studies of HLA-associated diseases. The genomic diversity analysis in the HLA region facilitated by next-generation sequencing will pave the way to molecular understanding of linkage disequilibrium structure, population diversity, histocompatibility in transplantation, and associations with autoimmune diseases. The 4-digit DNA genotyping of HLA for six HLA loci, HLA-A through DP, in the patients with Graves' disease (GD) and Hashimoto thyroiditis (HT) identified six susceptible and three resistant HLA alleles. Their epistatic interactions in controlling the development of these diseases are shown. Four susceptible and one resistant HLA alleles are shared by GD and HT. Two HLA alleles associated with GD or HT control the titers of autoantibodies to thyroid antigens. All these observations led us to propose a new model for the development of GD and HT. Hematopoietic stem cell transplantation from unrelated donor (UR-HSCT) provides a natural experiment to elucidate the role of allogenic HLA molecules in immune response. Large cohort studies using HLA allele and clinical outcome data have elucidated that (1) HLA locus, allele, and haplotype mismatches between donor and patient, (2) specific amino acid substitution at specific positions of HLA molecules, and (3) ethnic background are all responsible for the immunological events related to UR-HSCT including acute graft-versus-host disease (GVHD), chronic GVHD, graft-versus-leukemia (GvL) effect, and graft failure.

Shaw, B. E., et al. (2004). "Polymorphisms in the TNFA gene promoter region show evidence of strong linkage disequilibrium with HLA and are associated with delayed neutrophil engraftment in unrelated donor hematopoietic stem cell transplantation." Tissue Antigens **63**(5): 401-411.

Sustained myeloid engraftment is an important determinant of outcome in hematopoietic stem cell transplantation (HSCT). Human tumor necrosis factor (TNF)-alpha is encoded by a gene, TNFA, located in the class III region of the major histocompatibility complex on chromosome 6, flanked by the human leukocyte antigen (HLA) class I and II regions. A number of polymorphisms in the promoter region of the TNFA gene have been associated with increased production of TNF-alphain vivo. Additionally, raised TNF-alpha levels have been reported to have a detrimental effect on the outcome in HSCT, in particular on early complications such as acute graft vs host disease, failure to engraft, and transplant-related mortality. There is evidence of linkage disequilibrium (LD) between TNFA promoter polymorphisms and extended HLA haplotypes. We have genotyped 73 cell lines and 189 donor/recipient pairs (undergoing HSCT) for their TNFA polymorphism, all of which had been well characterized with respect to their HLA genes. We found evidence of strong LD between HLA genes and TNFA; however, there was also evidence for recombination events having taken place, as we found that a number of transplant pairs who were matched for their HLA haplotypes were not matched for their TNFA alleles. We analyzed early outcomes in the transplant recipients and found a significant delay in engraftment in those pairs where both donor and recipients possessed an AG allele (associated with higher TNF-alpha levels). Our results suggest a functional effect of TNFA polymorphisms on myeloid engraftment in unrelated HSCT.

Sivula, J., et al. (2012). "Toll-like receptor gene polymorphisms confer susceptibility to graft-versus-host disease in allogenic hematopoietic stem cell transplantation." Scand J Immunol **76**(3): 336-341.

Graft-versus-host disease (GvHD) is a major complication in hematopoietic stem cell transplantation (HSCT). The immune response against gut microbes is thought to be an important factor in the beginning of GvHD. Toll-like receptors (TLR) recognize molecular structures of microbes and viruses and play central part in the innate immunity. We studied whether genetic variation in the TLR1, TLR2, TLR4, TLR5, TLR6 and TLR10 genes confers susceptibility to GvHD in 305 human leucocyte antigen-identical sibling donor HSCT's performed in a single Finnish centre. The results showed that the genetic markers rs4833079 (P = 0.035) in TLR1,

rs4837656 (P = 0.032) and rs17582214 (P = 0.029) in TLR4, rs10737416 (P = 0.048) in TLR5, rs6531656 (P = 0.035) in TLR6, and rs337629 (P = 0.005) in TLR10 were associated with the occurrence of acute GvHD. Interestingly, two markers in the TLR5 gene, rs2800230 (P = 0.010) and rs2800237 (P = 0.017), were associated with chronic GvHD. These results indicate that many genes of the TLR system are involved in the overall genetic risk for GvHD and emphasize the role of innate immunity in GvHD.

Sizzano, F., et al. (2012). "Genotypes and haplotypes in the 3' untranslated region of the HLA-G gene and their association with clinical outcome of hematopoietic stem cell transplantation for beta-thalassemia." Tissue Antigens **79**(5): 326-332.

Polymorphisms in the 3' untranslated region (3'UTR) of HLA-G, an important player in immunological tolerance, could be involved in post-transcriptional expression control, and their association with different clinical immune-related conditions including autoimmunity and transplantation is of mounting interest. Most studies have focused on a 14 base pair (bp) insertion/deletion (ins/del), while additional single-nucleotide polymorphisms (SNPs) in the HLA-G 3'UTR have been described but not extensively investigated for their clinical relevance. Here we have comparatively studied the association between 3'UTR haplotypes of HLA-G, or the 14 bp ins/del, with clinical outcome of HLA-identical sibling hematopoietic stem cell transplantation (HSCT) in 147 Middle Eastern beta-thalassemia patients. Sequence based typing of 3'UTR HLA-G polymorphisms in the patients and in 102 healthy Italian blood donors showed strong linkage disequilibrium between the 14 bp ins/del and five 3'UTR SNPs, which together could be arranged into eight distinct haplotypes based on expectation-maximization studies, with four predominant haplotypes (UTRs1-4). After HSCT, we found a moderate though not significant association between the presence of UTR-2 in double dose and protection from acute graft versus host disease (hazard ratio (HR) 0.45, 95% confidence intervals (CI): 0.14-1.45; P = 0.18), an effect that was also seen when the corresponding 14 bp ins/ins genotype was considered alone (HR 0.42, 95% CI: 0.16-1.06; P = 0.07). No association was found with rejection or survival. Taken together, our data show that there is no apparent added value of considering entire 3'UTR HLA-G haplotypes for risk prediction after allogeneic HSCT for beta-thalassemia.

Srivastava, A. and R. V. Shaji (2017). "Cure for thalassemia major - from allogeneic hematopoietic stem cell transplantation to gene therapy." Haematologica **102**(2): 214-223.

Allogeneic hematopoietic stem cell transplantation has been well established for several decades as gene replacement therapy for patients with thalassemia major, and now offers very high rates of cure for patients who have access to this therapy. Outcomes have improved tremendously over the last decade, even in high-risk patients. The limited data available suggests that the long-term outcome is also excellent, with a >90% survival rate, but for the best results, hematopoietic stem cell transplantation should be offered early, before any end organ damage occurs. However, access to this therapy is limited in more than half the patients by the lack of suitable donors. Inadequate hematopoietic stem cell transplantation services and the high cost of therapy are other reasons for this limited access, particularly in those parts of the world which have a high prevalence of this condition. As a result, fewer than 10% of eligible patients are actually able to avail of this therapy. Other options for curative therapies are therefore needed. Recently, gene correction of autologous hematopoietic stem cells has been successfully established using lentiviral vectors, and several clinical trials have been initiated. A gene editing approach to correct the beta-globin mutation or disrupt the BCL11A gene to increase fetal hemoglobin production has also been reported, and is expected to be introduced in clinical trials soon. Curative possibilities for the major hemoglobin disorders are expanding. Providing access to these therapies around the world will remain a challenge.

Sun, J. F., et al. (2008). "[Dynamic detection of chimerism and fusion gene in chronic myeloid leukemia patients relapsed after allogeneic hematopoietic stem cell transplantation]." Zhongguo Shi Yan Xue Ye Xue Za Zhi **16**(4): 833-837.

This study was aimed to investigate the chimerism and fusion gene expression in patients with CML after allo-HSCT, to analyse engraftment and minimal residual disease by using STR-PCR combined with RT-PCR qualitative and quantitative assays, and to evaluate their clinical value for predicting disease relapse. 4 relapsed patients with CML after allo-HSCT were dynamically investigated. Qualitative analysis of donor chimerism was performed by multiplex PCR amplification of STR markers and capillary electrophoresis with fluorescence detection, qualitative detection of bcr/abl transcripts was performed by RT-PCR. The results showed that the 100% donor chimerism appeared in 4 patients on day 28 after transplantation and bcr/abl expression was negative, but the 4 patients were in status of unstable mixed chimerism (DC: 0% - 80.4%) at the different time points during the following up with bcr/abl gene positive. 2 patients of them were continuously mixed chimerism after relapse of CML, the other 2 changed

from MC to CC by intervention of clinical treatment. Decreasing values of donor chimerism were detected prior to the occurrence of graft rejection and CML relapse, and bcr/abl gene expression was positive. It is concluded that the results of STR-PCR in the range of its sensitivity fully correspond with bcr/abl tests in patients. The combination of STR-PCR with RT-PCR will provide a highly sensitive and valuable tool for evaluating engraftment, graft rejection, and relapse and predicting GVHD. Furthermore, it can provide a basis for early intervention of clinical treatment, and can identify these high risk patients with molecular or cytogenetic relapse after allo-HSCT.

Taira, C., et al. (2012). "Application of allele-specific quantitative PCR using genomic DNA to monitor minimal residual disease based on mutant gene levels following allogeneic hematopoietic stem cell transplantation in patients with hematological malignancies: comparison of mutant levels with autologous DNA percentage by short tandem repeat-PCR." Clin Chim Acta **413**(3-4): 516-519.

BACKGROUND: Quantitative evaluation of minimal residual disease (MRD) following hematopoietic stem cell transplantation (HSCT) is indispensable for patients with hematological malignancies. In addition to established MRD markers such as immunoglobulin and T-cell receptor gene rearrangements, fusion genes, or aberrantly expressed genes, single nucleotide mutations are considered one of the MRD markers that reflect the malignant cell clone. METHODS: We compared the quantity of mutant genes by allele-specific quantitative polymerase chain reaction (AS-qPCR) for single nucleotide mutations (TP53 410T>A and PTPN11 1508G>A) with the percentage of autologous DNA by short tandem repeat (STR)-PCR. RESULTS: Following HSCT, the quantity of mutant genes detected by AS-qPCR correlated with the percentage of autologous DNA assessed by the STR-PCR. Moreover, mutant DNAs were detected at a quantifiable level before relapse, whereas the percentage of autologous DNA was less than 5%, that is, complete chimerism. CONCLUSIONS: The AS-qPCR approach for single nucleotide mutations was accurate and highly sensitive for monitoring pre-transplantation as well as post-transplantation MRD. AS-qPCR for single nucleotide mutation is suitable for monitoring MRD in patients who lack previously established MRD markers.

Takahashi, H., et al. (2000). "Contribution of TNF-alpha and IL-10 gene polymorphisms to graft-versus-host disease following allo-hematopoietic stem cell transplantation." Bone Marrow Transplant **26**(12): 1317-1323.

Some cytokines are believed to play a role in the development of acute and chronic GVHD after allo-hematopoietic stem cell transplantation. It has been reported that TNF-alpha and IL-10 gene polymorphisms are associated with the production of those cytokines and the development of graft failure after organ transplantation and systemic lupus erythematosus. We examined whether TNF-alpha and IL-10 gene polymorphisms affect the severity of acute GVHD (aGVHD) and chronic GVHD (cGVHD). Sixty-two and 54 patients were available for the analysis of aGVHD and cGVHD, respectively. We analyzed the gene polymorphisms derived from pre- and post-transplant blood cells. Donor-derived TNF2 allele (A) was more frequently detected in patients with aGVHD III/IV than those aGVHD 0-II (2/6 vs 2/56) (P = 0.04). The donors of the patients with cGVHD more frequently possessed a greater number of alleles (allele 13 or more which contain 26 or more CA repeats) in IL-10.G than those without (13/26 vs 5/28) (P = 0.02), and the patients with cGVHD had more CA repeats in donor-derived IL-10.G than those without (mean = 25.2 vs 23.4) (P = 0.01). Donor-derived TNF-308 and IL-10.G alleles may contribute to severe aGVHD and cGVHD, respectively, and will help us distinguish those patients at high risk for GVHD.

Tesi, B., et al. (2016). "Successful Hematopoietic Stem Cell Transplantation in a Patient with LPS-Responsive Beige-Like Anchor (LRBA) Gene Mutation." J Clin Immunol **36**(5): 480-489.

PURPOSE: Autosomal recessive mutations in LRBA, encoding for LPS-responsive beige-like anchor protein, were described in patients with a common variable immunodeficiency (CVID)-like disease characterized by hypogammaglobulinemia, autoimmune cytopenias, and enteropathy. Here, we detail the clinical, immunological, and genetic features of a patient with severe autoimmune manifestations. METHODS: Whole exome sequencing was performed to establish a molecular diagnosis. Evaluation of lymphocyte subsets was performed for immunological characterization. Medical files were reviewed to collect clinical and immunological data. RESULTS: A 7-year-old boy, born to consanguineous parents, presented with autoimmune hemolytic anemia, hepatosplenomegaly, autoimmune thyroiditis, and severe autoimmune gastrointestinal manifestations. Immunological investigations revealed low immunoglobulin levels and low numbers of B and NK cells. Treatment included immunoglobulin replacement and immunosuppressive therapy. Seven years after disease onset, the patient developed severe neurological symptoms resembling acute disseminated encephalomyelitis, prompting allogeneic

hematopoietic stem cell transplantation (HSCT) with the HLA-identical mother as donor. Whole exome sequencing of the patient uncovered a homozygous 1 bp deletion in LRBA (c.7162delA:p.T2388Pfs*7). Importantly, during 2 years of follow-up post-HSCT, marked clinical improvement and recovery of immune function was observed. CONCLUSIONS: Our data suggest a beneficial effect of HSCT in patients with LRBA deficiency.

Trop-Steinberg, S., et al. (2013). "Early cell-cycle gene expression in T-cells after hematopoietic stem cell transplantation." Transpl Immunol **29**(1-4): 146-154.

Regeneration of the immune system after hematopoietic stem cell transplantation (HSCT) is a slow process. Early cell cycle proto-oncogenes are key players in the first events of the proliferative process of T-cell immune response. To identify the causes of the prolonged immuno-suppression after transplantation we evaluated the expression of early T-cell cycle genes c-myc, c-jun and c-fos in peripheral blood T-cells from post-transplant patients versus healthy controls before and after phytohemagglutinin (PHA) incubation. Results show that c-jun and c-fos expression continued to increase during the first 18 months post-transplant, and eventually decreased at two years post-transplant. C-myc, c-jun and c-fos expression values also showed a time-dependent increase in surviving patients. In non-surviving patients, however, there was a time-dependent decrease in gene expression. Significant correlation was shown in c-jun expression values of all patients up to 2 years post transplant between resting state and 3h post-PHA incubation when compared to healthy controls. A positive correlation was found in c-fos gene expression between values at resting state and 3h post PHA incubation in patients with Graft versus Host Disease (GVHD) compared patients with no GVHD or healthy controls. A positive correlation was observed between white blood cell count and c-fos expression among non-surviving patients and patients who suffered from GVHD. Our results show that molecular monitoring of the immune reconstitution using c-jun, and c-fos expression combined with clinical parameters may improve the early detection of immune-function failure. This follow-up system may allow for the development of more efficient treatments and new interventions designed to hasten desirable T-cell regeneration.

Udagawa, T., et al. (2012). "Syngeneic hematopoietic stem cell transplantation enhances the antitumor immunity of intratumoral type I interferon gene transfer for sarcoma." Hum Gene Ther **23**(2): 173-186.

Sarcoma at advanced stages remains a clinically challenging disease. Interferons (IFNs) can target cancer cells by multiple antitumor activities, including the induction of cancer cell death and enhancement of immune response. However, the development of an effective cancer immunotherapy is often difficult, because cancer generates an immunotolerant microenvironment against the host immune system. An autologous hematopoietic stem cell transplantation (HSCT) is expected to reconstitute a fresh immune system, and expand tumor-specific T cells through the process of homeostatic proliferation. Here we examined whether a combination of autologous HSCT and IFNs could induce an effective tumor-specific immune response against sarcoma. First, we found that a type I IFN gene transfer significantly suppressed the cell growth of various sarcoma cell lines, and that IFN-beta gene transfer was more effective in inducing cell death than was IFN-alpha in sarcoma cells. Then, to examine the antitumor effect in vivo, human sarcoma cells were inoculated in immune-deficient mice, and a lipofection of an IFN-beta-expressing plasmid was found to suppress the growth of subcutaneous tumors significantly. Finally, the IFN gene transfer was combined with syngeneic HSCT in murine osteosarcoma models. Intratumoral IFN-beta gene transfer markedly suppressed the growth of vector-injected tumors and inhibited formation of spontaneous lung and liver metastases in syngeneic HSCT mice, and an infiltration of many immune cells was recognized in metastatic tumors of the treated mice. The treated mice showed no significant adverse events. A combination of intratumoral IFN gene transfer with autologous HSCT could be a promising therapeutic strategy for patients with sarcoma.

Ueda, K., et al. (2004). "High-level in vivo gene marking after gene-modified autologous hematopoietic stem cell transplantation without marrow conditioning in nonhuman primates." Mol Ther **10**(3): 469-477.

The successful engraftment of genetically modified hematopoietic stem cells (HSCs) without toxic conditioning is a desired goal for HSC gene therapy. To this end, we have examined the combination of intrabone marrow transplantation (iBMT) and in vivo expansion by a selective amplifier gene (SAG) in a nonhuman primate model. The SAG is a chimeric gene consisting of the erythropoietin (EPO) receptor gene (as a molecular switch) and c-Mpl gene (as a signal generator). Cynomolgus CD34+ cells were retrovirally transduced with or without SAG and returned into the femur and humerus following irrigation with saline without prior conditioning. After iBMT without SAG, 2-30% of colony-forming cells were gene marked over 1 year. The marking levels in the peripheral blood, however, remained low (<0.1%).

These results indicate that transplanted cells can engraft without conditioning after iBMT, but in vivo expansion is limited. On the other hand, after iBMT with SAG, the peripheral marking levels increased more than 20-fold (up to 8-9%) in response to EPO even at 1 year posttransplant. The increase was EPO-dependent, multilineage, polyclonal, and repeatable. Our results suggest that the combination of iBMT and SAG allows efficient in vivo gene transduction without marrow conditioning.

Vago, L., et al. (2012). "T-cell suicide gene therapy prompts thymic renewal in adults after hematopoietic stem cell transplantation." Blood **120**(9): 1820-1830.

The genetic modification of T cells with a suicide gene grants a mechanism of control of adverse reactions, allowing safe infusion after partially incompatible hematopoietic stem cell transplantation (HSCT). In the TK007 clinical trial, 22 adults with hematologic malignancies experienced a rapid and sustained immune recovery after T cell-depleted HSCT and serial infusions of purified donor T cells expressing the HSV thymidine kinase suicide gene (TK+ cells). After a first wave of circulating TK+ cells, the majority of T cells supporting long-term immune reconstitution did not carry the suicide gene and displayed high numbers of naive lymphocytes, suggesting the thymus-dependent development of T cells, occurring only upon TK+ -cell engraftment. Accordingly, after the infusions, we documented an increase in circulating TCR excision circles and CD31+ recent thymic emigrants and a substantial expansion of the active thymic tissue as shown by chest tomography scans. Interestingly, a peak in the serum level of IL-7 was observed after each infusion of TK+ cells, anticipating the appearance of newly generated T cells. The results of the present study show that the infusion of genetically modified donor T cells after HSCT can drive the recovery of thymic activity in adults, leading to immune reconstitution.

Verner, J., et al. (2012). "Gene expression profiling of acute graft-vs-host disease after hematopoietic stem cell transplantation." Exp Hematol **40**(11): 899-905 e895.

Acute graft-vs-host disease (aGVHD) is a frequent, life-threatening complication after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Despite that, there are no reliable molecular markers reflecting the onset or clinical course of aGVHD. We performed a pilot study on gene expression profiling in peripheral blood mononuclear cells taken from 15 patients with hematological malignancies who underwent allo-HSCT and developed aGVHD. Based on survival rates after aGVHD, patients were divided into two groups-favorable (all patients alive; median follow-up 40 months) vs unfavorable group (all patients died; median survival 2 months). Two-hundred and eighty genes differentially expressed between these two groups were identified; among them, genes responsible for cytokine signaling, inflammatory response, and regulation of cell cycle were over-represented; interleukin-8, G0S2, ANXA3, and NR4A2 were upregulated in the unfavorable group, CDKN1C was downregulated in the same group. Interestingly, the same genes were also described as overexpressed in connection with autoimmune diseases. This indicates an involvement of similar immune regulatory pathways also in aGVHD. Our data support use of gene expression profiling at aGVHD onset for a prediction of its outcomes.

Wang, J. B., et al. (2002). "[Frequency of donor TNF-alpha gene polymorphism in patients with graft versus host disease following hematopoietic stem cell transplantation]." Zhongguo Shi Yan Xue Ye Xue Za Zhi **10**(2): 133-137.

To study the effect of donor TNF-alpha gene polymorphism on severe acute and extensive chronic graft versus host disease (GVHD), TNF-alpha gene polymorphism was analyzed by denaturing high performance liquid chromatography (DHPLC) and DNA sequencing in twenty-one patients with III/IV degree acute GVHD and twenty-seven patients with extensive chronic GVHD. The results showed that the frequency of TNF-alpha-308 (G/A) significantly increased in patients with III/IV degree acute GVHD compared to 0/I degree aGVHD patients (8/21 vs 1/28) ($P < 0.01$) and the frequency of TNF-alpha-308 (G/A) is significantly higher in patients with extensive chronic GVHD than in patients without chronic GVHD (7/23 vs 1/17) ($P < 0.05$). However, the frequency of TNF-alpha-238 (G/A) does not significantly changed in patients with III/IV degree acute GVHD and extensive chronic GVHD. In conclusion, the TNF-alpha-308 (G/A) is likely to contribute to high risk for III/IV degree acute GVHD and extensive chronic GVHD.

Wang, L., et al. (2016). "[Reduced intensity conditioning allogeneic hematopoietic stem cell transplantation in chronic lymphocytic leukemia (CLL) patients with the aberration of p53 gene]." Zhonghua Xue Ye Xue Za Zhi **37**(4): 308-312.

OBJECTIVE: To investigate the effectiveness and safety of reduced intensity conditioning allogeneic hematopoietic stem cell transplantation (RIC allo-HSCT) in ultra high risk chronic lymphocytic leukemia (CLL) patients with the deletion of p53 to deepen the understanding of allo-HSCT in the treatment of CLL. METHODS: In this retrospective

study, a total of 4 ultra high risk CLL patients with the deletion of p53 in our center between July 2012 and Jan 2014 were enrolled. The RIC regimen was administered and the hematopoietic reconstitution, transplantation related mortality (TRM), overall survival (OS), progress free survival (PFS) were evaluated. RESULTS: We registered 4 patients with the median age of 56 years (49-61 years), including 3 males and 1 female. The median mononuclear cells (MNC) and CD34(+) cells were 6.54 (2.85-14.7) x 10(8)/kg (recipient body weight) and 5.81 (2.85-7.79) x 10(6)/kg (recipient body weight), respectively. The median time of the neutrophil recovery was 11 days (range of 9-12 days), and the median time of the platelet recovery 5.5 days (range of 0-11 days). Three patients (75%) attained a full donor chimerism at day 28 after transplantation and one (25%) got a mixed chimerism of donor and recipient. During the follow-up at a median time of 26.5 months (range of 21-39 months), 2 (50%) patients developed acute graft versus host disease (aGVHD) grade I and 2 (50%) patients got CMV infection. One patient got herpes zoster virus and EB virus infections. No transplantation related mortality was found in the 4 patients. One patient who was in partial response status progressed 5 months after transplantation, and the other 3 patients remained in durable remission after allo-HSCT. CONCLUSION: These results suggested that RIC allo-HSCT showed durable remission, good tolerance and acceptable toxicity, which could be a better option for the treatment of ultra high risk CLL patients with the deletion of p53 and was worth to be investigated and applied widely in future.

Wang, L. J., et al. (2002). "Evaluation of mixed hematopoietic chimerism in pediatric patients with leukemia after allogeneic stem cell transplantation by quantitative PCR analysis of variable number of tandem repeat and testis determination gene." Bone Marrow Transplant **29**(1): 51-56.

In order to monitor the clinical outcome of pediatric patients with leukemia following allogeneic hematopoietic transplantation, tests of variable number of tandem repeat (VNTR) and sex determination by quantitative polymerase chain reaction (PCR) were performed. PCR results combined with the blast counts from 21 leukemia patients were analyzed. Complete chimerism (100% donor cells) was found in 15 cases with remission, and incomplete chimerism in six cases with relapse. In the majority of cases, complete chimerism was always associated with no detectable blasts, while blasts were often detected in association with incomplete chimerism. There is significant correlation (P<0.0001) between the percentage of donor DNA and blast percentage in these patients. Early detection of incomplete chimerism may therefore predict a poor prognosis. In one patient (case 15), a differing percentage of donor DNA was observed between samples of bone marrow and peripheral blood collected on the same day. This may be due to the fact that allogeneic stem cells proliferate at different rates depending on their environment (bone marrow or peripheral blood). In addition, 100% donor cells found in the peripheral blood may not reflect the number of cells in the bone marrow. In case 17, asynchronous engraftment of donor cells was present between the white and red blood cell lineages, indicating that the degree of chimerism may not be the same in all cell lineages. At the time of this report, the significance of this observation is unknown and needs further investigation.

Wu, X. J., et al. (2013). "[The association of killer cell immunoglobulin like receptor gene polymorphism with cytomegalovirus infection after hematopoietic stem cell transplantation]." Zhonghua Nei Ke Za Zhi **52**(2): 161-165.

OBJECTIVE: To explore the influence of the killer cell immunoglobulin like receptor (KIR) gene polymorphism on cytomegalovirus (CMV) infection and pathogenesis after hematopoietic stem cell transplantation (HSCT). METHODS: The KIR genotype was determined by sequence-specific primer polymerase chain reaction (PCR-SSP) in 138 pairs of donors and recipients before HSCT during October, 2005 and May, 2011. Posttransplant monitoring for CMVpp65 antigen was performed by indirect immune histochemically assays since week 2 after transplantation. The differences between CMV positive group and negative group, inhibitive and active KIR of donors and recipients, and KIR haplotype frequency of donors and recipients were analyzed. RESULTS: There were no significant differences in frequency of KIR gene and haplotype AA, AB, BB between the donors and recipients. The frequencies of 2DS2 and 2DS4 * 003-007 of donors in CMV positive group were obviously lower than those in CMV negative group with significant differences (8% vs 16%, P = 0.0420; 3% vs 13%, P = 0.0050). There was no significant difference in KIR gene between CMV positive group and CMV negative group. The CMV infection rates of haplotype AA, BB, AB donors were 64.38%, 36.84% and 50.00%, while CMV infection rates of haplotype AA, BB, AB recipients were 53.73%, 46.15% and 51.72%, respectively. The CMV infection rate was higher in the patients received KIR haplotype AA donor than in those received KIR haplotype BB donor (36.84% vs 64.38%, P = 0.0299). 2DS4 x 003-007 and haplotype BB of donor were found associated with CMV infection in multifactor

analysis. CONCLUSION: KIR genotypes of donors are associated with CMV infection after HSCT.

Yanagimachi, M., et al. (2010). "Influence of CYP3A5 and ABCB1 gene polymorphisms on calcineurin inhibitor-related neurotoxicity after hematopoietic stem cell transplantation." Clin Transplant **24**(6): 855-861.

BACKGROUND: One severe side effect of calcineurin inhibitors (CNIs: such as cyclosporine [CsA] and tacrolimus [FK506]) is neurotoxicity. CNIs are substrates for CYP3A5 and P-glycoprotein (P-gp), encoded by ABCB1 gene. In the present study, we hypothesized that genetic variability in CYP3A5 and ABCB1 genes may be associated with CNI-related neurotoxicity. METHODS: The effects of the polymorphisms, such as CYP3A5 A6986G, ABCB1 C1236T, G2677T/A, and C3435T, associated with CNI-related neurotoxicity were evaluated in 63 patients with hematopoietic stem cell transplantation. RESULTS: Of the 63 cases, 15 cases developed CNI-related neurotoxicity. In the CsA patient group (n = 30), age (p = 0.008), hypertension (p = 0.017), renal dysfunction (p < 0.001), ABCB1 C1236T (p < 0.001), and G2677T/A (p = 0.014) were associated with neurotoxicities. The CC genotype at ABCB1 C1236T was associated with it, but not significantly so (p = 0.07), adjusted for age, hypertension, and renal dysfunction. In the FK506 patient group (n = 33), CYP3A5 A6986G (p < 0.001), and ABCB1 C1236T (p = 0.002) were associated with neurotoxicity. At least one A allele at CYP3A5 A6986G (expressor genotype) was strongly associated with it according to logistic regression analysis (p = 0.01; OR, 8.5; 95% CI, 1.4-51.4). CONCLUSION: The polymorphisms in CYP3A5 and ABCB1 genes were associated with CNI-related neurotoxicity. This outcome is probably because of CYP3A5 or P-gp functions or metabolites of CNIs.

Yoon, J. H., et al. (2015). "Wilms tumor gene 1 expression as a predictive marker for relapse and survival after hematopoietic stem cell transplantation for myelodysplastic syndromes." Biol Blood Marrow Transplant **21**(3): 460-467.

Relapse after allogeneic hematopoietic stem cell transplantation (HSCT) is a major concern in myelodysplastic syndromes (MDS), but the role of Wilms tumor gene 1 (WT1) as a predictive marker for post-HSCT relapse remains to be validated. We measured WT1 transcript levels by real-time quantitative PCR from marrow samples of 82 MDS patients who underwent transplantation between 2009 and 2013. Pre-HSCT WT1 expression weakly correlated with marrow blast counts or International Prognostic Scoring System scores and failed to predict post-transplantation relapse. Regarding post-HSCT WT1, transcript levels of relapsed patients were significantly higher in comparison to those in remission. Further analysis using receiver operating characteristics curves showed that higher (>154 copies/10(4)ABL) 1-month post-HSCT WT1 resulted in a higher 3-year relapse rate (47.2% versus 6.9%, P <.001) with poorer disease-free survival (DFS) and overall survival at 3 years (41.7% versus 79.0% and 54.3% versus 82.1%, P =.003 and P =.033, respectively). Multivariate analysis after adjusting for pre-HSCT karyotype and chronic graft-versus-host disease (GVHD) also revealed that higher 1-month post-HSCT WT1 was an independent predictive marker for subsequent relapse (P =.002) and poorer DFS (P =.010). In the higher 1-month post-HSCT WT1 subgroup, patients with chronic GVHD showed lower relapse rate and favorable survival outcome. One month post-HSCT WT1 expression was a useful marker for minimal residual disease and relapse prediction in association with chronic GVHD in the context of HSCT for MDS.

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