P = 0.029). Non-relapse death was not observed in patients with standard-risk disease in the SD-MTX group (n = 22). There was no significant difference between the two groups in the 2-year incidence of relapse: 27.0% (95% CI, 13.9%-42.0%) for the LD-MTX group and 15.3% (95% CI, 5.4%-29.7%) for the SD-MTX group (P = 0.55).

Conclusion: Our study demonstrated that the GVHD prophylaxis consisting of SD-MTX and TAC led to a significantly lower incidence of severe acute GVHD and/or TRC-EC than the combination of LD-MTX and TAC without increasing the risk of graft failure and relapse. Thus, while our findings need to be further verified by multicenter studies with larger cohorts of patients, for now SD-MTX is optimal in combination with TAC for GVHD prophylaxis in UCBT.

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Treatment of Severe Acute GvHD with Alemtuzumab Is Effective without Increased Relapse Rates

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Introduction: Steroid-refractory acute graft versus host disease (GvHD) is still lacking a therapeutic standard and has a high mortality rate, thus presenting a major obstacle for allogeneic stem cell transplantation. Previously, we and others have demonstrated that the CD52 antibody alemtuzumab is able to significantly improve grade III and IV steroid-refractory acute GvHD (Schub et al., BMT 46, 143, 2011). However, this therapy results usually in complete lymphocyte depletion at least in the peripheral blood. This raises the question whether this may not also lead to a loss in tumor control. Here, we update and extend our previous monocentric observations in patients with severe steroid-refractory acute GvHD treated with alemtuzumab.

Methods/ Patients: Fifty-five consecutive patients (median age 52 years, range from 14 to 69 years)with lymphatic (n= 16) or myeloid (n= 39) malignancies that had received allogeneic stem cell transplantation from a family or unrelated donor developed severe acute GvHD that was refractory to high-dose corticosteroids. Patients were treated between the years 2004 and 2018 with alemtuzumab. While initially total doses up to 191 mg of alemtuzumab were applied, it became obvious that smaller doses were sufficient. Usually doses of 3 to 10 mg (first application limited to 3 mg) were given initially and alemtuzumab was repeated in intervals of 7 to 14 days for 3 to 4 times. In addition, shortly after alemtuzumab application immune suppressive drugs other than calcineurin inhibitors could be discontinued.

Results: Treatment complications were infections, particularly in CMV-positive patients. Thirty-four patients, despite many temporarily improving, eventually died due to infections and/or other complications of acute GvHD. However, 15 patients, almost 30%, achieved a long-term survival often with limited restraints and remain free of their initial malignant disease. Only 6 patients (10.9%) relapsed later.

Conclusions: These data indicate, that despite additional severe alemtuzumab-mediated immunosuppression and subsequent “rebooting” of the immune system, immunosurveillance against foreign hemato-lymphatic cells was sufficient to prevent relapse in most patients

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Treg/Tcon Immunotherapy and High Dose Marrow Irradiation Ensure Full Control of Leukemia Relapse in Haploidentical Transplantation

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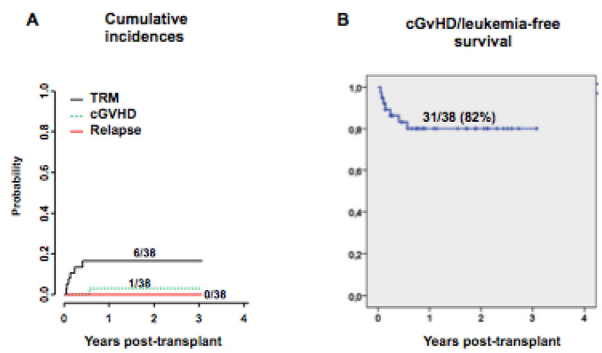
Allogeneic hematopoietic stem cell transplantation (HSCT) is the most powerful therapy for patients with high risk of relapse. In spite of that, no matter the donor source or conditioning regimen used, leukemia relapse is still the leading cause of HSCT failure. In HLA-haploidentical HSCT, we recently applied a clinical protocol consisting of total body irradiation (TBI)-based conditioning regimen and a peripheral blood CD34+ cell graft combined with the adoptive transfer of naturally occurring regulatory T cells (Tregs) and conventional T cells (Tcons). No post-transplant pharmacologic GvHD prophylaxis was given. Such protocol was associated with low GvHD and relapse rate (Martelli et al., Blood 2014). To further reduce leukemia relapse in Treg/Tcon-based haploidentical HSCT (Treg/Tcon haplo-HSCT) we used high dose hyper-fractionated TBI (HF-TBI) in the conditioning regimen. We also extended Treg/Tcon haplo-HSCT to patients that are unfit (because of previous comorbidities) and/or too old to withstand high intensity regimens. In these patients the extra-hematologic toxicity of irradiation was reduced with the use of targeted total marrow and lymph node irradiation (TMLI).

40 patients with high risk acute leukemia (36 AML, 4 ALL) received Treg/Tcon haplo-HSCT. All but 3 patients were transplanted in complete remission. 12 younger patients (median age: 28, range: 20-43) received HF-TBI, while 28 older or unfit patients (59, 40-70) received TMLI in the conditioning regimen. HF-TBI (14.4 Gy) was administered in 12 fractions, 3 times a day for 4 days. TMLI was administered by means of Helical Tomotherapy HI-ART (9 fractions, 2 times a day for 4.5 days). Irradiation was followed by chemotherapy with Thiotepa, Fludarabine, and Cyclophosphamide. 2×10^6/kg freshly isolated CD4+CD25+FOXP3+ Tregs were transferred 4 days before the infusion of 1×10^6/kg Tcons and a mega-dose of CD34+ hematopoietic stem cells. No post-transplant pharmacologic GvHD prophylaxis was given.



38/40 patients engrafted. 12 (31%) developed aGvHD grade $^{3}2$ (10 are alive and off-therapy). 6 (16%) died because of transplant related complications (2 because of aGvHD, 2 infections, 1 veno-occlusive disease, 1 intracranial hemorrhage). Strikingly, despite the high risk diseases, no patient relapsed after a median follow up of 13 months (range 1-36, Fig. A). Further, only 1 patient developed cGvHD. Thus, cGvHD/Leukemia-free survival was 82% (Fig. B).

Treg adoptive transfer allows for the safe infusion of an otherwise lethal dose of donor alloreactive Tcons in the absence of any other form of immune suppression. Our results demonstrate that the potent graft versus leukemia effect of Treg/Tcon adoptive transfer was boosted by high dose marrow irradiation. Thus, this study proves that the right combination of haploidentical Treg/Tcon immunotherapy plus a powerful conditioning regimen can fully eradicate leukemia.

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Using Functional Performance Measures to Assess Clinical Response in Patients with Sclerotic Chronic Graft Versus Host Disease: An Exploratory Study

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Introduction: In allogeneic hematopoietic stem cell transplant survivors, sclerotic chronic graft-versus-host disease (ScGVHD) is associated with poor quality of life (QOL) and range of motion restrictions. The latter can limit patients' ability to perform important activities of daily living (ADLs). The NIH Consensus Development Project established a standard set of measures to monitor chronic GVHD (cGVHD) progression. This includes indicators of patient-reported QOL and symptom burden, which is defined as the subjective severity and impact of physiological symptoms. Adding measures of functional performance may allow clinicians to more fully characterize the impact of ScGVHD on daily life.

Objective: To determine whether change in functional capacity and QOL relate to changes in symptom burden and clinician-rated cGVHD severity.

Methods: Between December 2008 and February 2011, patients with ScGVHD enrolled in a single-arm prospective clinical trial assessing the efficacy of imatinib mesylate. At baseline and 6 months, patients completed measures of functioning [Disabilities of the Arm, Shoulder, and Hand (DASH); Human Activity Profile (HAP); Manual Ability Measure (MAM-36)] and QOL [Short Form 36 version 2 (SF 36); Lee Symptom Scale (Lee)]. Clinicians rated patients' ADL ability [Assessment of Motor and Process Skills version 7 (AMPS)] and cGVHD symptom severity [Provider Global Score (PGS)]. Spearman's rank correlation tests evaluated the association among measures. The alpha level was set to 0.01.

Results: Twenty patients with ScGVHD enrolled in the trial; 13 patients were assessable for primary endpoint analysis. At study entry, patients were a median of 53 years old, a median of 53 months post-transplant, and had a median of 5 cGVHD-affected organs. Change in PGS was not correlated with change of any measures. Reduced symptom burden (Lee) correlated with improved SF 36 physical component scores ($r = -0.72$, $p = 0.008$), AMPS ADL motor skill scores ($r = -0.84$, $p < 0.001$), and DASH scores ($r = 0.71$, $p = 0.010$). No measures showed evidence of ceiling or floor effects.

Conclusion: Our results suggest the value of supplementing the Lee scale and the SF 36 with functional measures to assess clinical response to ScGVHD treatment. The association between changes in symptom burden and changes in ADL ability (AMPS and DASH scores) indicate the potential utility of these measures in this clinical population. Such scales may allow for a better understanding of patients' functional limitations, thereby promoting individualized clinical care and rehabilitative efforts. Clinician-reported cGVHD severity may not have been related to functional changes due to insufficient sensitivity of the measure or the small size of this sample. Our findings highlight the potential relevance of the AMPS and DASH in patients with cGVHD, though further exploration in larger and more diverse samples is necessary.

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$\alpha 4\beta 7$ Integrin Is Upregulated on CD8+ Effector Memory T-Cells in Children with Gut Gvhd Prior to Clinical Symptoms and Represents a Therapeutic Target in Pediatric Allogeneic HSCT Patients

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Background: $\alpha 4\beta 7$ integrin is expressed on T-cells which migrate to gut-associated lymphoid tissues. Expression of $\alpha 4\beta 7$ integrin is elevated on memory T-cells in adult hematopoietic stem cell transplant (HSCT) recipients at diagnosis of GI GVHD. No pediatric data exist regarding $\alpha 4\beta 7$ integrin expression before or at onset of GVHD. We hypothesized that $\alpha 4\beta 7$ integrin expression on effector memory T-cells (TEMs) would be elevated in patients before acute GI GVHD symptoms compared to patients without GVHD and may be a useful therapeutic target for the prevention of acute GI GVHD.

Methods: Cryopreserved T-cells were characterized in 48 children who developed acute GI GVHD (n=22), isolated skin GVHD (n=12) and no GVHD (n=14). In patients with GVHD, T-cells were characterized prior to HSCT, one week before GVHD and at GVHD diagnosis. In patients without GVHD, samples were analyzed at baseline and day+30 after HSCT. Samples were incubated with fluorochrome conjugated monoclonal antibodies directed against CD3, CD8, CCR7, $\alpha 4\beta 7$ integrin and CD45RA. TEMs were defined as CD3+, CCR7-, CD45RA- lymphocytes. Co-expression of trafficking markers implicated in GI GVHD (CCR5, CCR9), and liver GVHD (CXCR6 and CCR5) were also assessed. Samples were analyzed on an LSR Fortessa flow cytometer (BD Biosciences). Data were analyzed using FCS Express (De Novo Software). Surface expression of all markers were compared using the Mann Whitney U-test. Patients with skin GVHD and no GVHD were grouped for analysis. Patients with at least 200 absolute CD8+ T-cell events were deemed evaluable.

Results: Twenty patients with GI GVHD, 8 with skin GVHD and 10 patients without GVHD had at least 200 absolute CD8+ events and were evaluable. Demographics of patients are shown in Figure 1. Expression of $\alpha 4\beta 7$ integrin on CD8+ TEMs