improved RFS (P = 0.019) and OS (P = 0.033) compared to the A/G or G/G genotype. Multivariable analysis, adjusted for stem cell source (peripheral blood vs. bone marrow), GVHD subtype (classic/overlap vs. acute subtypes/none), and disease risk (low vs. intermediate vs. high) were performed to test the association of the significant SNPs with RFS and OS. rs4553808 A/A genotype (HR 2.44, P = 0.015), classic/overlap GVHD (HR 2.98, P = 0.002) and low/intermediate disease risk (HR 2.16, P = 0.03) were independent predictors of superior OS. rs4553808 A/A genotype (HR 2.39, P = 0.016), and classic/overlap GVHD (HR 3.84, P = 0.001) were independent predictors of superior RFS.

Our study confirms reports that genetic variation in donor CTLA-4 is associated with outcome after allo-HCT and may allow for identification of patient subsets that may benefit from pre-emptive modulation of immunosuppressive therapy. Further investigation into the CTLA-4/ICOS/CD28 and B7 regulation of T cell homeostasis is warranted to design a risk adapted approach to decrease the mortality for patients with a high risk of relapse.

Table 1. Patient characteristics and survival of all patients. (N=164)

Variable	N(%) Total Cohort (N=164)	GVHD subtype: Classic plus Overlap cGVHD N(%) (N=101)	GVHD subtype: aGVHD subtypes plus No GVHD N (%) (N=63)
Age, median in years (range)	47(18-69)	49(21-67)	46(18-69)
Gender			
Male (R)	80(49)	49(49)	31(49)
Female (R)	84(51)	52(51)	32(51)
Female D to male R	31(19)	21(21)	10(19)
Race			
Caucasian (R/D)	156(95)/153(93)	94(93)/92(91)	62(98)/61(97)
Diagnosis			
Acute Leukemia	86(52)	49(49)	37(59)
Chronic Leukemia	19(12)	11(11)	8(13)
Lymphoma	54(33)	38(37)	16(25)
other	5(93)	3(3)	2(3)
Disease Risk #			
Low	65(40)	38(38)	27(43)
Intermediate	30(18)	21(20)	9(14)
High	40(24)	25(25)	15(24)
Missing	29(18)	17(17)	12(19)
Stem Cell Source			
Marrow	44(27)	23(22)	21(33)
Peripheral Blood	120(73)	78(78)	42(67)
Regimen Intensity			
Myeloablative	111(68)	69(68)	42(67)
Other	53(32)	32(32)	21(33)
Donor Type			
Related	109(67)	69(68)	40(63)
Unrelated	55(33)	32(32)	23(37)
HLA Match			
HLA identical sibling	108(66)	68(67)	40(63)
HLA matched unrelated	55(33)	32(32)	23(37)
Other	1(1)	1(1)	0
GVHD Prophylaxis			
CSA/MTX	112(68)	72(71)	40(63)
CSA/MMF	50(31)	28(28)	22(36)
Other	2(1)	1(1)	1(1)
TBI			
Yes	79(48)	52(51)	27(43)
No	85(52)	49(49)	36(57)
CMV			
R/D (+/+)	63(38)	35(34)	28(44)
R/D (+/-)	19(12)	12(12)	7(11)
R/D (-/+)	49(29)	29(29)	18(29)
R/D (-/-)	35(21)	25(25)	10(16)
Post-transplant Characteristics			
aGVHD€			
Grade 0-2 vs. 3-4	179(78) vs. 32(20)	78(77) vs. 21(21)	51(81) vs. 11(7)
Grade 0-1 vs. 2-4	39(24) vs. 122(74)	39(24) vs. 75(74)	15(24) vs. 47(75)
PLT (X10(9)/L at day 100, median (range)	110(10-356)	105(17-244)	112(10-356)
Total Bilirubin (mg/dL) at day 100, median(range)	0.8(0-3.6)	0.8(0-2.4)	0.9(0.2-3.6)
Survival			
2 year OS: (95% CI)	63%(56-72)	73%(64-83)	48%(36-64)
2 year RFS¥: (95% CI)	61%(54-70)	73%(65-83)	41%(30-57)

Disease risk-based on Committee of International Bone Marrow Transplant Registry (CIBMTR) risk criteria; € aGVHD-3 cases missing; ¥ RFS analyses includes death from non-relapse causes as competing risk. GVHD-graft-versus-host disease; cGVHD-chronic GVHD; aGVHD-acute GVHD; R-recipient; D-donor; HLA-Human Leukocyte Antigen; CSA-cyclosporine; MTX-methotrexate; MMF-mycophenolate moefitil; TBI-Total body irradiation; CMV-cytomegalovirus; PLT-platelet; OS-overall survival; RFS-relapse free survival; CI-confidence interval.

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IMMUNOMONITORING OF TRANSPLANTED PATIENTS INFUSED WITH MESENCHYMAL STROMAL CELLS (MSC) FOR TREATING STEROID-REFRACTORY GvHD

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Although the usage of mesenchymal stromal cells (MSC) as therapy for Graft versus Host Disease (GvHD) is constantly increasing, studies on MSC efficacy have been scarcely corroborated by biological analysis of patient response to cell infusion. We report the immunological monitoring of 9 patients with steroid-refractory GvHD, receiving multiple doses of MSCs. GvHD presented as acute in 5 cases and chronic in 4 cases. After MSC therapy, 2 patients showed complete response (CR), 4 patients showed partial response (PR) whereas 3 patients did not respond (NR) to MSC infusion.

To better comprehend the immunomodulatory effects of MSC infusions, we studied GvHD plasmatic markers, inflammatory cytokines and CD4+ T-cell subsets circulating in the peripheral blood (PB) of enrolled patients before MSC infusion and at day 7, 14 and 28 after cell therapy.

In accordance with clinical observations, in CR patients we observed a dramatic decrease of three validated GvHD plasmatic markers TNFRI, IL2Rα and elafin (Paczesny S et al. Blood 2009) to the mean levels of Healthy Donors (HD). In particular, at day 28 after therapy, TNFRI decreased of 2 times, IL2Rα levels decreased of 1.9 times and elafin decreased of 2.3 times. Moreover, investigating the effect of MSC infusion on lymphocyte counts, we observed in both CR patients a significant decrease in CD3+ and CD4+ lymphocyte counts in the PB. Interestingly, after MSC infusions, CD4+ T-cell subsets changed significantly: Tregs increased and Th1 and Th17 populations decreased. In particular, Th1/Treg ratio decreased of 3.5 times and Th17/Treg ratio decreased of 3.6 times. Correspondingly, patient symptoms also gradually improved,

suggesting an association between GvHD clinical course and CD4+ T-cell imbalance. In accordance with the decrease of Th1 CD4+ T cells in the PB of CR patients, we observed a valuable decrease of IFNγ plasma concentrations, which reached the levels typical of HD. Contrary to CR patients, in PR patients we observed a transient decrease of GVHD plasmatic markers and Th1/Treg, Th17/Treg ratios, while NR patients showed stable or even increasing levels of all analysed plasmatic and cellular markers.

In summary, despite its limited size, the present study suggests that MSCs, upon infusion, are able to convert an inflammatory environment to a more physiological one, both at a cellular level, promoting the expansion of circulating Tregs, and at a molecular level, diminishing inflammatory cytokines.

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PROGNOSTIC FACTORS IN ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION FROM MATCHED UNRELATED DONORS: LESSONS FROM EXTENDED FOLLOW UP OF A RANDOMIZED TRIAL ON GVHD PROPHYLAXIS WITH OR WITHOUT ANTI T-CELL GLOBULIN ATG-FRESENIUS (ATG-F)

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GvHD is a major problem in allogeneic hematopoietic cell transplantation (HCT) fom unrelated donors (UD). In our prospective randomized multicenter trial we could show the efficacy of additional ATG-F to standard GvHD prophylaxis with cyclosporine A and Mtx in reducing all grades of acute and chronic GvHD without negatively affecting NRM, relapse rate or DFS in 201 adult patients (median age 40 (range 18-60) years) with leukemia or MDS in early (n = 107) and advanced (n = 94) disease transplanted after myeloablative conditioning with marrow (n = 37) or blood (n = 134). (Finke et al., Lancet Oncol, 2009).

Risk factors for the outcome after UD-HCT have been postulated from retrospective analyses of registry data, however data from randomized trials are lacking. With an extended follow of median 3 years we present mature data on outcome and multivariate analysis of risk factors: Incidence of grade III-IV aGvHD was 11.7% in the ATG-F group and 25.5% in the control group ($p = 0.039$), the incidence of extensive chronic GvHD (cGvHD) after three years was 12.2% versus 45.0% ($p < 0.0001$), DFS was 48.0% and 38.4%, ($p = 0.71$), incidence of relapse was 32.6% and 28.2% ($p = 0.47$), incidence of NRM was 19.4% and 33.5% ($p = 0.18$), and OS was 55.2% and 43.3% in the ATG-F and control groups, respectively ($p = 0.39$).

The following factors were analyzed with regard to OS, DFS, risk of relapse, aGvHD III/IV, extensive cGvHD and NRM: patient age ([> / <] 40 y), donor age (> / <] 40 y), male patient/ female donor v. other, CMV negative v. seropositive, HLA-C mismatch, type and status of disease, conditioning regimen (TBI v. no TBI), source of stem cells (marrow v. PBSC), mean cyclosporine trough levels during the first months (> / < median 220ng/ml), graft cell count in PBSC (> / <] median 7.5x106 CD34/kg). In multivariate analyses advanced disease was a negative factor for aGvHD III-IV (HR = 2.1, $p = 0.018$), DFS (HR 1.7, $p = 0.004$), relapse (HR = 1.7, $p = 0.038$), and OS (HR = 1.9, $p = 0.002$). Patient age 40 years or more negatively affected NRM (HR = 1.8. $p = 0.041$). Interestingly, donor age 40 years or more adversely affected the risk of aGvHD III-IV (HR = 2.6, $p = 0.009$), extensive cGvHD (HR = 2.1, $p = 0.021$) and OS (HR = 1.7, $p = 0.016$), whereas CMV status, male patient/female donor, HLA-C mismatch, conditioning, graft source, CD34 count or cyclosporine levels had no influence.

Conclusion: ATG-F significantly reduces acute and chronic GvHD. By choosing younger donors outcome can be improved in unrelated donor transplantation.

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ABROGATION OF DONOR T CELL IL-21 SIGNALING LEADS TO TISSUE-SPECIFIC MODULATION OF IMMUNITY AND SEPARATION OF GVHD FROM GVL

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Interleukin 21 (IL-21) is a pro-inflammatory cytokine produced by Th17 helper T cells, and abrogation of IL-21 signaling has recently been shown to reduce GVHD while retaining GVL. However, mechanisms by which IL-21 may lead to a separation of GVHD and GVL are incompletely understood. To characterize its effect on GVH and GVL T cell responses, we compared wild type (WT) and IL-21 receptor knockout (IL-21R KO) donor T cells in a C57BL/6 into BALB/c murine MHC-mismatched bone marrow transplant (BMT) model. Lethally irradiated BMT recipients of IL-21R KO T cells demonstrated decreased GVHD-related morbidity ($p < .05$) and mortality ($p < .01$) and decreased histopathologic evidence of GVHD within the small bowel ($p < .05$). While this reduced GVHD was associated with increased donor regulatory T cells two to three weeks post-BMT ($p < .001$), transplanting selected T cell subsets indicated that IL-21 signaling in both donor CD4 and CD8 T cells contributed to GVHD mortality (CD4, $p < .01$; CD8, $p < .05$), although effects on CD8 T cells occurred only in the presence of CD4s. KO and WT donor T cells demonstrated equivalent alloactivation, as evidenced by proliferation ($p < .001$), upregulation of CD25 ($p < .001$), and downregulation of CD62L ($p < .01$ for CD8 T cells) in allogeneic vs. syngeneic recipients. However, IL-21R KO T cells demonstrated decreased infiltration within the small bowel ($p < .05$) and mesenteric lymph nodes (MLN; CD8, $p < .05$; CD4, $p < .001$), and decreased inflammatory cytokine-producing CD4 T cells within MLN (IFN-γ, $p < .01$; TNF-α, $p < .001$). Consistent with this, transplanted IL-21R KO donor T cells demonstrated decreased expression of α4β7 integrin (LPAM, $p < .05$), a molecule known to be involved in homing of GVHD-mediating donor T cells to the gut. However, in contrast to the reduced inflammatory cytokine-producing CD4 T cells observed in MLN, IL-21R KO helper T cell cytokine production was maintained in spleen and peripheral lymph nodes, and IL-21R KO T cells were able to protect recipient mice from lethality due to A20 lymphoma ($p < .001$). In summary, abrogation of IL-21 signaling in donor T cells leads to tissue-specific modulation of immunity, such that gastrointestinal GVHD is reduced, but peripheral T cell function and GVL capacity are retained. Targeting IL-21 for therapeutic intervention is an exciting strategy to separate GVHD from GVL, and this novel approach should be considered for clinical investigation to improve transplant outcomes and prevent malignant relapse.

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REG3α IS A BIOMARKER OF GRAFT VERSUS HOST DISEASE OF THE GASTROINTESTINAL TRACT

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There are no validated plasma biomarkers specific to graft versus host disease (GVHD) of the gastrointestinal (GI) tract. We have previously identified and validated elafin as a plasma biomarker for skin GVHD (Science Transl Med, 2:50-57). Using an unbiased proteomics