SSI use was 152 (13-981) days. At 6 months and 1 year, 63% and 83% of patients were off all immunosuppressive therapy, respectively. With a median follow-up of 26 months, cumulative incidence of chronic GVHD was 10%. Only 3 patients have died with refractory GVHD. These results extend our previous observations that post-transplantation Cy is effective single agent strategy for prophylaxis of acute GVHD with both a low rate of grade III-IV and more than half of the patients never requiring additional SSI. The limited use of SSI may be responsible for low infectious rate and excellent immune reconstitution seen in these patients. This approach also provides novel platform to facilitate the use of post-transplant immunotherapy aimed at reducing relapse.

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HIGH IMPACT OF HUMAN LEUKOCYTE ANTIGEN MATCHING ON OVERALL SURVIVAL AND TRANSPLANT RELATED MORTALITY IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR CLL: LONG-TERM STUDY FROM THE EBMT REGISTRY

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Objective: To evaluate the impact of HLA matching and difference in matching degree among transplants from unrelated donors (UD) on different outcomes in chronic lymphocytic leukemia (CLL).

Materials & Methods: We have analyzed 370 CLL patients who underwent an allogeneic HSCT reported to the EBMT registry. There were 280 males (75%) and 90 females with a median age of 53 years (24-69). At transplant, 294 among 317 evaluated patients had a good performance status (PS) (93%), 43 patients were in CR (12%), 160 in PR (46%), 44 in SD (13%) and 103 in PD (29%) among 349 evaluated patients. Two hundred and sixty six patients received a reduced intensity conditioning regimen (RIC) and 103 a standard (Std) conditioning; 313 patients received PBSC, 56 BM and 2 cord blood cells from 198 HLA siblings, and 172 unrelated donors (UD).According to the registry, there were 198 HLA siblings, 135 matched UD (MUD) and 37 mismatched UD (MMUD). We focused on UD and re-analyzed all HLA typings for patients and donors, after classification we found: 31 well matched (10/10, 8/8) in high resolution), 30 matched in low resolution and 111 mismatched in high resolution.

We found a high significant difference in term of OS between the siblings, well & partially matched groups versus low & MMUD groups (p = 0.002) (figure2). [OS at 3 & 5 years: Siblings: 68.3% (61.8-75.5) and 57.2% (49.8-65.6); well matched: 60.8% (43.1-85.8) from 3 to 5 years, low resolution & MMUD: 50.5% (43.1-60.1) and 39.6% (29.5-50.2) respectively]. We observed also a high significant difference in term of transplant related mortality (TRM) between the same groups (p = 0.0024) (figure4). The multivariate analysis using Cox model studying age, pre-transplant status, gender, PS, cells source, ABO compatibility, conditioning and different HLA groups, showed a significant impact of 3 factors on OS: age [HR = 1.04 (1.02-1.6) p = 0.001], PS [HR = 2.75 (1.5-5.1) p = 0.001] and HLA MMUD + Low resolution group [HR = 1.43 (1.01-2.01) p = 0.04]. The same factors were also highly significant in multivariate analysis in term of TRM (age: HR = 1.04 (1.001-1.07) p = 0.0033; PS: HR = 2.47 (1.1-5.4) p = 0.009 and HLA MMUD group: HR = 1.8 (1.07-3.37) p = 0.004.

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REDUCED-INTENSITY CONDITIONING (RIC) ALLOGENEIC STEM CELL TRANSPLANTATION (ALLO-SCT) FOR PATIENTS AGED ≥60 YEARS: A RETROSPECTIVE ANALYSIS OF 629 PATIENTS FROM THE SOCIETE FRANCAISE DE GREFFE DE MOELLE ET DE THERAPIE CELLULAIRE (SFGM-TC)

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This retrospective report assessed the outcome of 629 patients aged ≥60 years and who received RIC allo-SCT, with a special emphasis on the comparison of the outcome of patients aged 60-65 and patients aged >65 y.

The median age for the whole cohort was 62 (range, 60-71) y. 378 patients (55%) had a myeloid malignancy, while 240 (38%) had lymphoid malignancies, and 11 (2%) had other diseases. 386 patients (61%) received allo-SCT from an HLA-matched related donor, while 199 patients (32%) received the graft from a MUD, and 44 (7%) from mismatched donors. The conditioning regimen consisted of Fludarabine and Busulfan in 280 cases (44.5%), Fludarabine and low dose TBI in 150 cases (24%). The remaining 199 patients (32%) received other so-called RIC regimens.

With a median follow-up of 9 (range, 1-90) m., grade II-IV and grade III-IV acute GVHD occurred in 29% (n = 182) and 12% (n = 76) of patients, respectively. Chronic GVHD was observed in 145 patients (23%; limited: n = 67; extensive: n = 72; unknown stage: n = 6). 180 patients died of transplant-related causes (TRM: 29%). The estimates of overall survival (OS) at 1 and 2 years were 57% (95%CI, 53-62%) and 47% (95%CI, 42-52%), respectively.

In order to assess the applicability of RIC allo-SCT to the older age group, we compared the outcome of patients aged from 60 to 65 y. (n = 516) and those aged >65 y. (n = 113). Except for age, in univariate analysis, these 2 groups were not statistically different in terms of demographic, disease or transplant characteristics. The incidences of grade II-IV and grade III-IV acute GVHD were comparable between both groups (29% vs. 30%, p = NS; and 12% vs. 12%; p = NS). The TRM incidence was 29% in the younger group vs. 27% in the older group (p = NS). The estimates of OS at one and 2 years were 58% (95%CI, 53-62%) and 47% (95%CI, 42-52%) in the younger age group and 55% (95%CI, 44-65%) and 48% (95%CI, 37-60%) in the older age group (p = NS). In a Cox multivariate analysis accounting for all relevant factors, age >65 y. was not found to be a statistically significant factor associated with worsened survival.

In all, this data support the use of RIC-allo-SCT in patients >60 y. Outcome of patients aged >65 y. appears to be comparable to that of patients aged 60-65 y. Physiologic aging is likely more important than chronologic aging. With the refinement of comorbidities scoring systems, age per se (at least up to 70 y.) should not be a contraindication to perform RIC allo-SCT.

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ENGRAFTMENT FAILURE AFTER NONMYELOABLATIVE HEMATOPOIETIC STEM CELL TRANSPLANTATION: DONOR CD4 CELLS INTERFERE WITH RESIDUAL HOST CELLS AND CAN TRIGGER GRAFT REJECTION

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While non-myeloablative conditioning significantly reduces morbidity and mortality after allogeneic hematopoietic cell transplantation (HCT), the risk of allograft rejection is increased. Persistent host cells mediate host-vs-graft alloreactivity or occupy niches in the hematopoietic microenvironment. Here we studied engraftment and hematopoietic reconstitution in BALB.K mice given non-myeloablative radiation and purified hematopoietic stem cells (HSC: cKIT + Thy1.1loSca1 + Lin-) +/- T cells (TC) from AKR/J donors, a MHC-identical (H-2 K) model with high barriers to engraftment. Recipients of pure HSC regularly achieved stable mixed chimerism. However, if grafts contained CD4 TC (but not CD8 TC) donor lymphopoiesis was completely abolished in ~80% of hosts, even long-term. This suppression was associated with bone marrow (BM) lymphopenia (<5% vs >40% after HSC alone), and hypocellularity (median 3.8 vs 13.3 x10^6 cells/2 legs; p = 0.0003) at 2 weeks post-HCT. B cell reconstitution was the most severely affected (6% vs 75% of lymphocytes). At this early time point there was expansion of donor and host CD4 TC, each comprising ~6% of BM cells (vs

<1% in mice given HSC), accompanied by a substantial increase of host NK cells (median 14% vs <1%; 6.3 vs <0.4 x10^5 cells). Donor CD4 TC in the BM expressed high levels of IFNγ (30-50%), which exceeded IFNγ secretion in control groups (3-15%). Similar findings of engraftment failure due to donor CD4 TC were noted when mice were conditioned with total lymphoid irradiation (TLI: 17×240 cGy): while recipients of HSC alone were mixed chimeras in all lineages, those given HSC + TC failed to engraft with T and B cells, had only marginal myeloid engraftment, but increased proportions of host NK cells in the PB. We conclude that after non-myeloablative conditioning transplantation of pure HSC can result in superior immune reconstitution and donor chimerism as compared to grafts supplemented with CD4 TC. These results are of broad significance, as it is generally believed that TC augment donor HSC engraftment. The way in which CD4 TC retard engraftment appears to be by induction of inflammation within the BM microenvironment and activation of host lymphoid populations that mediate resistance. IFNγ, which is a known regulator of innate and acquired immune responses, may be central in activating host CD4 TC, enhancing NK cell mediated rejection of the graft and/or suppression of donor hematopoiesis.

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CCR5 EXPRESSION ON CIRCULATING BLOOD DC POST-ALLOGENEIC HEMOPOIETIC CELL TRANSPLANT IS HIGHLY PREDICTIVE FOR THE DEVELOPMENT OF CLINICALLY SIGNIFICANT ACUTE GRAFT VERSUS HOST DISEASE

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Introduction: Dendritic cells (DC) are central to the development of acute graft-versus-host disease (GvHD) following allogeneic hematopoietic cell transplantation (alloHCT). Because DC migration is tightly regulated by the interaction between chemokine receptors on DC and their ligands, we investigated the relationship between the severity of acute GvHD and the expression of the chemokine receptors CCR5 and CCR7 on $CD11c^+$ myeloid and $CD11c^-$ plasmacytoid DC from the peripheral blood of 32 patients post alloHCT.

Methods: Peripheral blood was collected twice weekly up to day 100 post transplant. The expression of each chemokine receptor on CD11c+ and CD11c- DC was calculated using multiparameter flow cytometry. The percentage of CD11c+ DC expressing a given receptor was added to the percentage of CD11c- DC expressing the same receptor to give a maximum score that could vary from 0 to 200%.

Results: Eleven of 32 patients developed moderate to severe acute GvHD (grade II-IV), the remaining 21 patients developed either no GvHD or only grade I GvHD. The percentage of DC expressing either CCR5 or CCR7 was correlated with the development of acute GvHD. CCR7 expression was detected in 13 of 32 patients post-HCT with a median of 2.3% of DC positive (range 0 to 39%). CCR7 expression on DC showed no association with the severity of acute GvHD ($p = 1.0$). In contrast, higher CCR5 expression was detected on DC in patients developing grade II-IV GvHD (median 98.0%, SEM 9.1%) than in those with grade 0-I GvHD (median 5.2%, SEM 5.1%) $p < 0.0001$. All eleven patients with grade II-IV GvHD expressed CCR5 at a level of >35% of myeloid and plasmacytoid DC. Only two of 21 patients with grade 1 GvHD expressed CCR5 at the same level (66% and 94%). Most importantly, the expression of CCR5 preceded the development of moderate to severe GvHD in all patients by a median of 19 days (range 2 to 47 days, SEM 4.3 days). Using a receiver operator curve analysis, CCR5 expression above 35% demonstrated a sensitivity of 100% and a specificity of 90.5% for predicting grade II-IV GvHD.

Conclusion: Expression of CCR5 on circulating DC post allo-HCT predicts for the development of moderate to severe GvHD, and detection could allow earlier therapeutic intervention prior to the development of clinical GVHD.

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EX-VIVO EXPANDED AND FRESHLY ISOLATED $CD4^+CD25^+FOXP3^+$ REGULATORY T CELLS SUPPRESS MURINE ACUTE GVHD WITH DIFFERENT POTENCY

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Using murine models of acute GVHD in which allogeneic bone marrow cells are transplanted into lethally irradiated hosts, we and others have shown that donor $CD4^+CD25^+Foxp3^+$ regulatory T cells (Treg) are able to suppress GVHD induced by donor allogeneic conventional T cells (Tcon). The potential clinical use of Treg is limited by their low number. To overcome this problem, expansion of Treg has been performed, however, there has not been a head-to-head comparison of the function of expanded vs. fresh Treg. Highly purified $CD4^+CD25^+Foxp3^+$ T cells (>98% purity) were expanded using anti-CD3/CD28 dynabeads and 1000 U/ml IL-2. Treg cultured for five days expanded up to 13 fold while maintaining high Foxp3 expression levels (85-90%). The expanded Treg (eTreg) were evaluated in an *in vivo* acute GVHD mouse model in direct comparison with freshly isolated Treg (fTreg) using a novel bioluminescent imaging assay (BLI) that allowed for quantitative assessment of Tcon proliferation in addition to traditional metrics of GVHD severity. Expanded Treg were significantly more effective at suppressing the early proliferation of Tcon than an equal number of fTreg ($P < 0.0001$). On day 6, mice that received Tcon and eTreg had a mean BLI signal of 1.5×10^6 photons/sec/mouse, four times lower than the signal of mice receiving Tcon and fTreg (5.8×10^6 photons/sec/mouse). The difference in potency may be explained by the increased number of eTreg compared to fTreg that were re-isolated from peripheral lymph nodes (PLN) (40,600 total eTreg/PLN/mouse vs. 5,200 total fTreg/PLN/mouse) at day 5, suggesting that expanded Treg arrived and proliferated in PLN faster than the fresh Treg. However, over time, the eTreg lose their protective capabilities and, in order to suppress GVHD symptoms and improve survival, a greater number of eTreg were required compared to fTreg. On day 37, the mean GVHD score for the Tcon alone group was 8.6 ± 0.74. Fresh Treg added at 1:1 ratio with Tcon decreased GVHD score to 1.4 ± 0.25 ($p < 0.0001$). *Ex-vivo* expanded Treg demonstrated a dose-dependent decrease in GVHD score, although four times more eTreg were needed to obtain a similar reduction in GVHD score (0.33 ± 0.3, $p = 0.0002$). Although eTreg are less potent at suppressing GVHD than fTreg, the greater numbers achievable by short-term culture makes them viable option for treatment of GVHD, however, increased ratios of Treg:Tcon are likely to be required.

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ANALYSIS OF GRAFT FAILURE IN 168 PATIENTS WITH FANCONI ANEMIA SUBMITTED TO STEM CELL TRANSPLANTATION IN A SINGLE BRAZILIAN INSTITUTION

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Background: Graft failure (GF) is a serious and fatal complication that can occur in up to 30% of Fanconi anemia (FA) patients submitted to hemaopoietic stem cell transplantation. (HSCT). The goal of this study was the analysis of risks factors for GF in patients with FA.

Patient and Methods Between January 1985 and July 2008, 203 pts with FA were submitted to an HSCT and 168 were evaluable for engraftment .Thirty-one had graft failure (group A), while 140pts had an adequate engraftment (group B). Pts in group A had a longer duration of the disease when compared to pts in group B (40 versus 19 months – $p = 0{,}004$) and they were also more transfused (43 versus 8 – $p < 0{,}001$). Stem cell source: Group A: Bone marrow (BM) 65% and umbilical cord blood (UCB) 35%. Group B: 85% BM and 15% UCB. Unrelated donors (UD) were used in 68% of pts in group A and 28% in group B ($p < 0{,}001$); there were also less compatible transplant in group A (57 versus 79%- $p = 0{,}01$). A de-escalation dose of cyclophosphamide was used for the majority of pts receiving a related HSCT while a fludarabine based regimen was used in pts with UD. GVHD prophylaxis consisted of cyclosporine and methotrexate in the majority of pts.