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Background
Graft-Versus-Host-Disease (GVHD) is a life-threatening complication of allogeneic hematopoietic stem cell transplantation (HSCT). Animal models have demonstrated that Treg infusion could prevent otherwise lethal GVHD in mice given grafts from MHC-disparate donors. Here, we assessed the ability of clinical-grade isolated human Treg to attenuate experimental xenogeneic GVHD.

Material and methods
Human Treg were isolated from cytapheresis products with the Miltenyi CliniMacs system using a two steps procedure (CD8 and CD19 depletion followed by CD25 positive selection) in six independent experiments with six different healthy volunteer donors. Sub-lethally (2.5 Gy) irradiated NSG mice were given $2x10^6$ cytapheresis product cells i.v. without (PBMC group) or with $1x10^6$ Tregs (PBMC+Treg group), while other NSG mice received only $2x10^6$ Treg (also in i.v.; Treg group). Mice in terminal stage GVHD were euthanised.

Results
After the selection, we obtained a CD25 enriched fraction including a median of 1.81 x108 cells and containing 59 +/- 6% or 66 +/- 6% Treg defined as either $CD45^+CD4^+CD25^{High}FoxP3^+$ cells or $CD45^+CD4^+CD25^{high}CD127^{low}$ cells. In all experiments but the last (a technical problem dramatically impacts the efficiency of this selection), Treg co-transfusion significantly delayed death from xenogeneic GVHD. Specifically, median survivals in PBMC versus PBMC+Treg mice were 30 *vs* 56 days (p=0.015), 123.5 *vs* >162 days (p=0.23), 25.5 *vs* 70 days (p=0.012), 13 *vs* 16 days (p=0.038), 27 *vs* 49 days (p=0.061), and 46 *vs* 47 days (p=0.338) respectively. Further, none of the mice given only Treg experienced signs of GVHD, while, interestingly, the $CD4^+$ cells found in these mice 27 days after transplantation were mainly conventional T cells ($CD25^+FoxP3^+$ cells in human $CD4^+$ total cells were only 2.1%, 3.1% and 17.7% in spleen, bone marrow and blood, respectively while 80.2% were grafted).

Conclusion
Treg infusion delayed the occurrence of xenogeneic GVHD without showing any toxicity in this murine model.

O.7 Erythropoietin therapy after allogeneic hematopoietic cell transplantation : a prospective randomised trial

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Based on the impairment of erythropoietin production after allogeneic hematopoietic cell transplantation (HCT), we previously reported in a phase-2 trial that recombinant human erythropoietin (rhEPO) therapy was very efficient when started one month after transplantation. We also demonstrated that anemia after non-myeloabalative (NM) HCT was less sensitive to rhEPO therapy than after conventional allogeneic HCT. This prompted us to confirm these findings in a prospective randomised trial.
One hundred and thirty-one patients were randomised (1:1) between no treatment (arm 1) or erythropoietin (Neorecormon) at the dose of 500 U/kg/week (arm 2). Once the target Hb (13g/dL) has been attained, the dose of rhEPO was reduced by half, while it was withheld when Hb was = 14g/dL. Cohort A included 42 patients on day 28 after myeloablative HCT, cohort B 39 patients on day 28 after NMHCT, and cohort C 50 patients on day 0 of NMHCT. Primary endpoints included proportion of complete correctors (i.e. patients reaching Hb = 13g/dL) and median time to achieve Hb correction in each arm.
The proportion of complete correctors before day 126 post-transplant was 0% in group 1A *vs* 52.4% in group 2A, 0% in group 1B *vs* 69.5% in group 2B and 19.1% in group 1C *vs* 70.2% in group 2C. Median time to achieve Hb = 13g/dL was not reached in group 1B *vs* 49 days in group 2B; 363 and 59 days in groups 1A and 1B respectively and 363 and 87 days in groups 3A and 3B respectively (figure 1). Hb evolution in each group is shown in figure 2. Seventy-one patients (47/62 in control groups and 24/57 in treated groups, p=0.0003) required red blood cell transfusions. The difference was most pronounced in cohort B. There was no difference in rates of thrombo-embolic events or other complications between the two arms. In conclusion, this is the first trial to demonstrate that EPO therapy hastens erythroid recovery and decreases transfusion requirements when started one month after allogeneic HCT.

O.8 The value of asparaginase intensification for children with low and average risk acute lymphoblastic leukemia (ALL) and non-Hodgkin lymphoma (NHL) in the EORTC-CLG Randomized Phase III Trial 58951

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Background
Asparaginase (ASP) is an essential component in combination chemotherapy for childhood ALL. However, the optimal number of ASP-administrations is still unknown. We conducted a randomised phase III trial comparing conventional *E.coli*-ASP-regimen (short-ASP, 12 doses) with prolonged *E.coli*-ASP-therapy (long-ASP, 24 doses).

Methods
The EORTC-CLG 58951 trial was open to de novo ALL or NHL patients (pts) <18y. This study addressed two main randomised questions. The first evaluated the value of dexamethasone (DEX, 6mg/m/d) *vs* prednisolone (PRED, 60mg/m/d) in induction for all patients. In the second question all non-VHR pts were randomised for either short- or long-ASP. All patients received 8x10000U/m in induction. In the short-ASP-arm patients received 4x10000U/m in reinduction; patients in the long-ASP-arm received 8x5000U/m *E. coli*-ASP-injections in consolidation and eight (4x10000U/m + 4x 5000U/m) in reinduction. Patients with grade =2 allergy to *E.coli*-ASP were switched to equivalent doses of *Erwinia* or PEG-ASP.

Results
Between 12/1998 and 08/2008, 1552 patients were randomly assigned to receive long-ASP (n=775) or short-ASP (n=777). The 8-year DFS rate was 87.0% in the long-ASP and 84.2% in short-ASP-group (hazard ratio (HR) = 0.87, 95% CI 0.66-1.14, 2-sided logrank p=0.30). The 8-year OS rate was comparable in both treatment arms: 92.6% in the long-ASP-group and 91.3% in the short-ASP-group (HR = 0.89, 95% CI 0.61-1.29, p=0.53). Similar treatment differences were observed in each risk group, in PRED *vs* DEX arm, and B- and T-lineage ALL-patients. The incidence of grade 3-4 infection was higher in the long- versus short-ASP-group during consolidation (25.2% *vs* 14.5%) and re-induction (22.6% *vs* 15.9%). This difference was more pronounced in patients who received DEX in induction (27.3% *vs* 11.6%). During the whole treatment period, the incidence of grade 2-4 allergy was 32.8% in the long-ASP-arm and 21.8% in the short-ASP-arm.

Conclusion
At long follow-up prolonged *E.coli* asparaginase therapy in conso-