Results
Median FU was 36 months and 25% of patients had a FU of at least 60 months. The 5y-CIf of cGVHD was 58%. Sex mismatch (F>M) increased risk of cGVHD (HR: 1.41, *P*=.02). The 5y-CIf of relapse was 34% and was higher with MRD than MUD (39% *vs.* 24%, *P*=.038). Only MRD=60y resulted in significant higher risk of relapse than MUD (HR 2.46, *P*=.006) while MRD <60y had similar risk. The 5y-NRM was 26%. MUD *vs.* MRD was associated with higher NRM (HR: 1.84, *P*=.005). The 5y-OS was 46% and was similar with MUD and MRD. MRD=60y appeared to have notable low 5y-OS (6%, 6%). Transplantation from MRD=60y was associated with higher risk of late (=18 months) mortality (HR: 4.36, *P*=.007) than MUD (Fig. 1).

Conclusion
After PB SCT, MUD provided higher NRM but better disease control and similar OS than MRD. A sex mismatched donor (F>M) was associated with higher risk of cGVHD. We observed notable poor outcome for patients transplanted with MRD=60y. One may thus question HCT with old MRD when a younger MUD is available.

O.4 Combination of regulatory T cells injection with rapamycin for treatment of chronic Graft-versus-Host disease

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Background
Chronic graft-versus-host disease (cGvHD) occurs up to 50 % in long-term survivors and is one of the main complications after allo-HSCT. Donor $CD4^+$ and regulatory T cells (Tregs) are the key-players in its pathogenesis. Moreover, rapamycin, a mTor inhibitor, could suppress activation and proliferation of effector T cells and expand *in vitro* Tregs.

Aims
To assess the combined treatment of Tregs and rapamycin injections *in vivo* for cGvHD.

Results
Lethally irradiated Balb/C mice were injected with $10x10^6$ bone marrow cells and $70x10^6$ splenocytes from B10.D2 donor mice. Twenty-one days later, the treatments were started (PBS, rapamycin 1 mg/kg/Day, Tregs 1.10^6 cells or rapamycin 1 mg/kg/Day + Tregs 1.10^6 cells). No significant differences were observed between survival of PBS-treated (Median: 40 days) compared to rapamycin alone or Tregs alone (Median: 46 days, *p*=0.1390; Median: 46 days, *p*=0.2450 respectively) while survival of mice receiving rapamycin and Tregs was increased (*p*=0.0074). Twenty-one days after starting the treatment, number of $CD4^+$ T cells was significantly decreased in Tregs (37.0010.00; *p*=0.0303) and Tregs + rapamycin-treated (27.0056.00; *p*=0.0293) mice compared to PBS mice (95.00100.50). Proliferation of $CD4^+$ T cells (assessed by flow cytometry unsing Ki67) was only significantly decreased in Tregs + rapamycin-treated mice (19.609.17 versus 36.8012.40; *p*=0.0043). Number of cells per micorliters and proliferation of both effector and central memory $CD4^+$ T cells were significantly decreased in Tregs + rapamycin-treated mice compared to PBS mice. Number of $CD8^+$ T cells was significantly decreased in rapamycin (56.0063.00; *p*=0.0082) and Tregs + rapamycin-treated (58.5077.80; *p*=0.0082) mice compared to PBS mice (144.00103.80). Despite a significant increase in the percentage of Tregs in rapamycin 1 mg/kg/Day (20.3043.15; *p*=0.0519), Tregs 1.106 cells (95.6559.75; *p*=0.0190) or rapamycin 1 mg/kg/Day + Tregs 1.106 cells groups (29.6066.30; *p*=0.0043) compared to PBS-treated mice (15.353.80), no significant differences were seen in the number per micorliter.

Conclusion
Regulatory T cells injection combined with rapamycin daily administration seems to treat cGvHD *in vivo* by combining the beneficial effect of these treatments.

O.5 In vitro generation of antigen-specific T-cells from hematopoietic progenitor cells: a new and promising immunotherapeutic strategy

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Introduction
Transfer of high-affinity tumor-specific T-cell receptor (TCR) genes into polyclonal peripheral blood T-cells is an attractive immunotherapeutic strategy against malignancies and viruses. However, inappropriate crosspairing between introduced and endogenous TCR chains can result in suboptimal activity and unpredicted, potentially harmful antigen-specificities. Efficient *in vitro* generation of antigen-specific T-cells from $CD34^+$ hematopoietic progenitor cells (HPCs) may eliminate these restrictions, based on the hypothesis that early introduction of rearranged TCRa and TCR chains might result in allelic exclusion of the endogenous TCRa and/or TCR locus. We and others have previously shown that HPCs commit to the T-cell lineage and become $CD4^+CD8^+$ double positive (DP) precursors when cultured on OP9-DL1 stromal cells.

Results
$CD34^+$ HPCs from human postnatal thymus were retrovirally transduced to express the TCRa and TCR chains of HLA-A2 restricted TCRs recognising epitopes of cytomegalovirus (CMV pp65) or Wilms' tumour 1 (WT1). Differentiation in transduced cultures was studied. We confirmed earlier reports showing that terminal maturation of TCR-transduced DP cells to mature CD8 single positive (SP) cells occurs, albeit at low efficiency. We hypothesised that the observed maturation involved selection by TCR binding to HLA class I /peptide complexes present in culture. Therefore, we added the respective agonist peptide to the cultures. This induced rapid phenotypical maturation to $CD27^+CD1^-$ of the majority of $TCRa^+$ DP cells. Antigen presentation by $HLA\text{-}A2^+$ dendritic cells, HLA-A2+ tumour cell lines, and even cross-presentation by $HLA\text{-}A2^+$ T-cell progenitors, but not by $HLA\text{-}A2^-$ cells, induced this maturation process. The mature cells are $CD8a^+$ or $CD8aa^+$SP and $CD4^-CD8^-$ cells. These T-cells expanded upon culture with PHA and IL-2 on irradiated feeders, indicating functionality. Upon activation, specific killing of T2 cells loaded with agonist peptide, was observed. *In vitro* generated T-cells showed clearly higher percentages of tetramer-positive cells compared with TCR-transduced peripheral blood T-cells. Spectratyping revealed major inhibition of endogenous TCRa and TCR gene rearrangements.

Conclusion
In vitro generation of functional antigen-specific T-cells from $CD34^+$ HPCs is a promising new immunotherapeutic strategy.

O.6 Infusion of CliniMACS® (Miltenyi Biotec) enriched regulatory T-cells delays experimental xenogeneic graft-versus-host disease.

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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-