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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