could be successfully re-engrafted. Two patients with MDS or AML died for transplant related complications. Conclusion I: These results are rather promising especially for patients with alternative donors and advanced disease.

Autologous HSC were harvested in 15 patients for reinfusion in case of graft failure, cancer treatment or gene therapy. BM was stored in 14 patients, containing 59 x 10E8 TNC (range: 15 – 378); 21.0 x 10E6 CD34 cells (range: 1 – 62); and a viability of 72.4 % (range: 40 – 94). CB was harvested in one patient, containing 5.2 x 10E8 TNC; and 2 x 10E6 CD34 cells. Quality of residual HSC and absence of clonal aberrations are depending on the interval between donor’s diagnosis and HSC harvest. Autologous BM has been reinfused in one leukemic FA-patient resulting in a partial reconstitution. Conclusion II: The preservation of autologous HSC in FA is ingenious but should be performed early after diagnosis. BM cells are favored.

O353
Hemopoietic stem cell transplantation (HSCT) for paroxysmal nocturnal hemoglobinuria (PNH) and acquired severe aplastic anaemia (SAA): a report from the Aplastic Anaemia Working Party of the European Blood and Marrow Transplant group (EBMT)

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Severe aplastic anaemia (SAA) and paroxysmal nocturnal haemoglobinuria (PNH) share many clinical and possibly pathogenetic features. Allogeneic haemopoietic stem cell transplantation (HSCT) is a curative treatment for both disorders. In the present study we compare the outcome of PNH and SAA patients allografted in Europe in the past 3 decades. The patient population included 2894 patients with severe aplastic anaemia (SAA) and of 113 patients with PNH. The donor was an HLA identical sibling in 2309 SAA and 92 PNH patients respectively, an identical twin in 67 and 0, a mismatched family or matched unrelated donor in 469 and 23 patients. The median time from diagnosis to transplant was 111 days for SAA and 946 for PNH (p<0.0001) and the median age of patients 18 and 29 respectively (p<0.0001). Radiation was used in days for SAA and 946 for PNH (p<0.0001) and the median age of patients allografted in Europe in the past 3 decades. The patient population consisted of 2894 patients with severe aplastic anaemia (SAA) and of 113 patients with PNH. EBMT Aplastic Anaemia Working Party

EBMT Aplastic Anaemia Working Party

The overall actuarial survival for HLA identical sibling HSCT 10 years after transplant is 63% in SAA vs 52% in PNH (p<0.04). Patients with PNH had more acute GVHD (p<0.04) as compared to SAA patients, also when adjusted for age and excluding radiation based conditioning. There is a strong overall effect of patient age, interval diagnosis-HSCT and year of HSCT on outcome: older age is a significant negative predictor in both SAA and PNH, whereas year of HSCT and duration of the disease appears to be significant only in SAA. The small number of PNH patients undergoing an alternative donor transplant (n= 23) did relatively well with 14 patients surviving. Probability of survival in this group was not different from patients grafted from HLA-identical siblings.

Conclusion. This study confirms the feasibility of HSCT in patients with PNH. When compared to the SAA patients, there are differences in patient characteristics, disease duration and outcome of the transplant. Patients receiving a non radiation based conditioning and bone marrow as the source of stem cells, seem to be doing better.

O354
High-dose administration of allogenic mesenchymal stem cells to immunocompetent baboons

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Mesenchymal stem cells (MSCs) are rare cells found in bone marrow and other tissues that have the capacity to be expanded to large numbers for tissue repair. We have previously demonstrated that MSCs from humans, rats, and baboons do not elicit an alloreactive T cell response in vitro as evaluated by peripheral blood cell responses to MSCs in one-way mixed lymphocyte reactions (MLR). Furthermore, MSCs from these species were shown to suppress MLRs induced against immunogenic stimulator cells. In studies of bone repair, transplantation of relatively low numbers of allogenic MSCs (approximately 0.5 million MSCs/kg body weight) to immunocompetent recipients did not induce immune responses that result in the rejection of these cells.

O355
Pediatric bone marrow mesenchymal stem cells favour the expansion of primitive over committed progenitor CD34+ cells

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The proliferation of repopulating human stem cells (HSC) and primitive or committed human progenitors (HPP-CFC and HPC) proceeds through the regulated amplification of a series of gradually more restricted compartments. A complete understanding of this process and the clinical application of ex vivo expansion protocols require the development of model systems that support the proliferation of HSC/HPC with no or minimal differentiation. We now report that mesenchymal stem cells (MSC) from human pediatric bone marrow (BM) support the hematopoiesis of human HPP-CFC/HPC and favour the proliferation of primitive over committed HPC. Magnetic activated cell sorted CD34+ cells (>98% phenotypically pure) were isolated from term CB and cultured on pediatric BM irradiated MSC (CD34-, CD14-, CD45-, SH2+, SH3+) in serum-containing and serum-free conditions with or without supplementation of exogenous early acting cytokines (FL, TPO, SCF). Over a period of 1 week, in the presence of MSC, observed changes included (n=7): higher number of nucleated and CD34+ cells and amplification of the blast compartment with lower differentiation. Interestingly, in
comparison with MSC-free cultures, MSC sustained a modest amplification of committed HPC, coupled with a high expansion of primitive progenitors (high proliferative potential-colony forming cells, HPP-CFC). Interestingly, the presence of MSC allows one-half to one-fourth reduction of CD34+/CD38+ inoculated to obtain similar levels of HPC expansion. These results indicate an influence of MSC on expansion of primitive HPC and possibly repopulating HSC and indicate the use of MSC in clinical transplantation strategies, in particular when an insufficient harvest of HPC/HSC is obtained.

**O356**

**Multilineage differentiation of CD133+ bone marrow cells**

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A subset of bone marrow and mobilized peripheral blood CD34+ cells, co-expressed with CD133, the stem cell marker, recently designated CD133. The potential of CD34+/CD133+ cells to give rise to hematopoietic clonogenic precursors has already been investigated. The aim of this study was to determine their ability in generating both endothelial (ECs) and mesenchymal cells (MCs). Bone marrow samples from five healthy donors were separated by means of immunomagnetic selection with -CD133 antibodies. FACSCalculations of the co-expression of CD34 or CD133 of the CD133+ cells and endothelial and stromal markers (VEGF receptor VEGFR-2/KDR, NGFR p75 and SH2 CD105) on 3%. The cells were then grown on fibronectin-coated flasks in M199, 10% FBS and in the presence of VEGF, bFGF and IGF-1 to promote endothelial cells differentiation: after 3-4 weeks of culture, the cells formed a monolayer with a typical EC morphology. These cells were further purified with Ulx eupeus agglutinin-1 (UEA-1)-FITC and anti-FITC microbeads and expanded with VEGF for a further 3 weeks: all of the cells expressed endothelial markers and typical Weibel-Palade bodies. In the attempt to generate fibroblastic cells, CD133+ cells were also grown in IMDM supplemented with FBS. After 3-4 weeks of culture, these cells gave rise to an adherent layer of fibroblastic cells (SH2+, TE-7+), and hematopoietic clonogenic precursors (CFU-GM, BFU-E) were detected in the supernatant for up to 6 days. Furthermore, fibroblastic clonogenic precursors (CFU-F) were present in the supernatant for up to four weeks. The multipotential differentiation capacity of CD133+ bone marrow cells was further confirmed by the formation of an adherent layer clearly showing an increase in calcium accumulation (Alizarin red S positive) when the cells were cultured in media promoting osteoblastic differentiation. These data suggest the existence of CD133+ bone marrow precursors capable to differentiate into the hematopoietic, endothelial and mesenchymal lineages.

**O357**

**Long term culture of human hematopoietic stem cells - influence on homing, organ selectivity, survival and proliferation early after transplantation into NOD/SCID mice**

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Long-term culture of human hematopoietic stem cells (HSC) is clinically important for retrospective gene therapy and for expansion of umbilical cord blood (UCB) HSC to enable transplantation of older children and adults. However, reports in literature on long-term engraftment analysis of cultured HSC in NOD/SCID mice are mostly disappointing, suggesting a decrease in HSC activity after culture. We wanted to investigate the behavior of these cells early after transplantation, and therefore introduced UCB HSC that were cultured for 2 weeks in serum-free medium supplemented with SCF, TPO and Flt-3L, into our short-term in vivo trafficking assay (T. Kerre et al, J. Immunol. 2001;167(7): 3692-8). In this model, fresh and anti-FITC microbeads were injected into the murine bone marrow and spleen, but only in the bone marrow proliferation exceeds apoptosis and cells expand, with kinetics depending on the source (UCB>mPB>BM) and the expression of CD38. In comparison, cultured CD34+ cells show an impaired homing capacity (prolonged presence in the circulation) and altered organ selectivity (homing to spleen and bone marrow, but also to liver and lung). Apoptosis on the other hand, is less pronounced. Markedly, the bone marrow homed CD34+ cells show a reduction in both the early expansion (day 3 - week1: fresh 8x vs cultured <1x) as well as the late expansion (day 3 - week4: fresh 350x vs cultured 50x). All these data show that long-term culture of HSC affects all stages in the early transplantation phase. The homing defect could be partially explained by the downregulation of CXCR4, an important homing receptor of CD34+ cells to the bone marrow on cultured CD34+ cells, or the increased expression of other adhesion molecules on these cells, which results in homing to inappropriate sites.

Studies are now ongoing to identify the phenotype of the long-term repopulating cell after culture, and to further improve homing of these cells, by investigating the influence of culture on other homing molecules in this model.

**O358**

**Factors inherent to a human cord blood stem cell graft determine telomere dynamics after transplantation to NOD/SCID mice**

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Telomere length dynamics of a cell population after stem cell transplantation is determined by multiple factors. E.g. induction of telomerase or the recruitment of immature precursors with longer telomeres might result in an increase of telomere length while the excessive expansion of stem cells following transplantation might cause telomere shortening. The net result of these factors might therefore depend on intrinsic properties of the graft as well as on extrinsic factors inherent to the recipient. To differentiate between such intrinsic and extrinsic factors, we performed xenotransplantations of human progenitor cells into NOD/SCID mice. Hematopoietic progenitor cells (CD34+) were isolated from 4 umbilical cord blood samples and transplanted into cohorts of 3 – 4 recipient NOD/SCID mice. Six to eight weeks after transplantation, the mice were sacrificed and human progenitor cells (CD45+ /34+) as well as B cells (CD45+/19+) were isolated from the bone marrow. Using FlowFish analysis, we compared the telomere length of these expanded cell populations with the telomere length of the transplanted progenitor cell populations. The expanded B cell populations invariably showed longer telomeres than the transplanted progenitor cells. However, transplantation of progenitor cells from 3 out of 4 different umbilical cord blood sources resulted in longer telomeres in the expanded progenitor cell populations, while transplantation of progenitor cells from 1 out of 4 umbilical cord blood sources resulted in shorter telomeres in all 4 recipients. Our data indicate that the change in telomere length during the expansion phase after transplantation in the recipient mice depends on the specific progenitor cell source. It is unlikely that the increase in telomere length that was observed in the majority of the expanded progenitor (CD34+) and B cell populations is due to the recruitment of immature precursors (CD34+/38-) with longer telomeres since in a direct comparison between the telomere lengths of CD34+/38- and CD34+/38+ umbilical cord progenitor cells no difference was found. The differential telomere elongation/shortening cannot be explained by differences in graft size, since the grafts contained similar numbers of CD34+ cells. Our data indicate that factors that are intrinsic to the graft, e.g. the capacity of the transplanted cells to engraft and upregulate telomerase expression, influence the telomere dynamics in the recipient.
The common lymphoid progenitor protects against lethal MCMV infection in a murine model of matched unrelated hematopoietic stem cell transplantation

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Cytomegalovirus (CMV) reactivation after allogeneic hematopoietic cell transplantation (HCT) is one of the leading causes of infectious morbidity and mortality despite available antiviral agents. Recently, a population of cells committed to lymphoid lineage development was phenotypically identified and isolated from mouse bone marrow (BM). These cells termed common lymphoid progenitors (CLP) comprise 0.02% of total BM cells and rapidly give rise to T, B and NK cells. The goal of our study was to assess the capacity of CLP to restore functional immunity when co-transplanted with purified hematopoietic stem cells (HSC) as compared to mice that were transplanted with HSC alone. CLP activity was studied in a pre-clinical murine model of CMV infection in the setting of a matched unrelated donor HCT. HSC and CLP were isolated by fluorescence activated cell sorting from C57BL/6.CD45.2 (H-2b) mice (for HSC) or C57BL/6.CD45.1 (H-2b) mice (for CLP) and transplanted into lethally irradiated BALB.B (H-2b) mice. The recipients of either group were challenged with 5x10^6 pfu (i.p.) of murine CMV on day 14 post transplantation and examined daily for signs of CMV disease and acute graft-versus-host disease (GVHD) through day 35. Infected mice that were co-transplanted with HSC and CLP demonstrated a higher survival rate as compared to infected mice that had received HSC alone (80% vs. 27.8%; p=0.002). The severity of CMV disease reflected by mouse body weight as well as the virus load in the liver was decreased in CLP co-transplanted animals. In addition, no signs of clinical GVHD were observed in infected or in uninfected mice. In conclusion, these data suggest that (1) CLP do not induce clinical GVHD and (2) co-transplantation of CLP with HSC protects against lethal CMV infection in a matched unrelated donor HCT model.

Processing of preterm cord blood for transfusion of red blood cells in combination with stem and progenitor cells

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Objectives: Low-birth-weight preterm infants are among the most heavily transfused of patient groups during the early weeks of life. The risks associated with donor blood exposure are largely unknown. Possible immunomodulatory effects with consequences for infection and organ failure have not been studied. The infants might benefit from autologous red blood cells (RBC) transfusion. Whereas expanded stem and progenitor cells to different hematopoietic lineages can support the therapy against anemia and leucopenia. We started a pilot study to investigate the possibility of collecting and processing cord blood (CB) from preterm infants.

Methods: By using the collection system with a incorporated syringe of our CBBank we collected CB from preterm infants. Modification of the bag and the needles were made. CB was separated in RBC, stem cell (SC) and a plasma fraction by using a Sepax device (Biosafe). The RBC fraction was stored in SAG-M, AS3 and autologous plasma for at least 14 days and during this period concentrations of ATP, pH and MCV were measured. In addition different cell counts in the different fraction were performed. Our first aim was to evaluate the storage possibilities of RBC.

Results: Mean volume of collected cord blood (excl. anticoagulation) is 287.7 ml. By using the syringe in 42% of the collections, we collected an additional extra of 5.9 ml Sepax separation resulted in a mean recovery of RBC in the RBC fraction of 51% with a mean Hct of 0.53. The residual contamination of WBC in RBC fraction was still 29%, whereas 24% of WBC were collected in the SC fraction, which still contained a Hct of 0.22. The first storage results in SAG-M, AS3 or autologous plasma are shown in table 1.

Storage results

| Day  | 0   | 7   | 14  | 21  | 28  |
|------|-----|-----|-----|-----|-----|
| ATP  | SAG-M | 5.22 | 4.13 | 3.34 |     |
|      | AS3  | 5.34 | 3.22 | 3.14 |     |
|      | plasma | 3.82 | 2.67 | 3.27 |     |
| pH   | SAG-M | 6.71 | 6.54 | 6.49 | 6.44 | 6.38 |
|      | AS3  | 6.52 | 6.43 | 6.38 | 6.36 | 6.44 |
|      | plasma | 6.68 | 6.55 | 6.52 | 6.54 | 6.68 |
| MCV  | SAG-M | 112.6 | 110.7 | 116.5 | 117.1 | 117.6 |
|      | AS3  | 113.6 | 111.1 | 115.6 | 115.5 | 114.1 |
|      | plasma | 112.9 | 108.5 | 115.4 | 113.8 | 115.0 |

Conclusion: In all instances a RBC volume (10 ml/kg) at least sufficient for 1 transfusion can be obtained. Separation by Sepax shows promising results. Recovery of RBC and Hct can be increased by making adaptions. RBC can be stored for a period of 15-20 days in AS3. It remains to be evaluated whether expansion of committed CB cells can be further applied to support transfusion needs of the neonate.

Immune reconstitution after autologous PBPC transplantation: effect of IL-15 on T-cell survival and function

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Objective: To evaluate the occurrence of T-cell spontaneous apoptosis (Aspont) and its modulation in vitro by the IL-2 receptor (IL-2R) gamma-chain-signaling cytokine IL-15 in patients transplanted with autologous PBPC for hematological malignancies.

Patients and Methods: Patients were examined on days 30-60, 60-90 and 90-120 after PBPC infusion. The dissipation of mitochondrial transmembrane potential, a hallmark of T-cell apoptosis, has been detected with the fluorescent probe 3,3'-dihexyloxacarbocyanine iodide, following short-term T-cell culture in the absence or in the presence of exogenous cytokines. The expression of Bcl-2 family members has been studied by flow cytometry and reverse transcriptase polymerase chain reaction. T-cell proliferative responses to recall antigens have been estimated in autologous mixed leukocyte cultures.

Results: Aspont could be evidenced in 45±6% of CD4+ and 55±6% of CD8+ T cells cultured in the absence of cytokines; of interest, IL-15 and to a lesser extent its structural cousin IL-2 counteracted T-cell Aspont by a) inhibiting the processing of caspases and b) upregulating Bcl-2 mRNA and protein levels. Cell division tracking confirmed that IL-15 did not rescue T cells from Aspont by promoting proliferation but rather it acted as a genuine survival factor. The addition of a gamma-chain-blocking antibody to cytokine-conditioned cultures abrogated both apoptosis inhibition and Bcl-2 induction by IL-15, suggesting the involvement of the IL-2R gamma-chain signal transduction pathway. Whereas cytokine-unprimed posttransplant T cells mounted inadequate responses to recall antigens, T cells conditioned with IL-15 expanded vigorously, indicating a restoration of antigen-specific proliferation.

Conclusions: T cells recovering after autologous PBPC transplantation are highly susceptible to spontaneous apoptosis in vitro; this phenomenon can be counteracted by the gamma-chain-signaling cytokine IL-15. These findings suggest that IL-15 might be a promising immunomodulating agent to improve postgrafting T-cell function.