CEPRATE® Systems Related Abstracts for the Thirty-eighth Annual Meeting of the **American Society of Hematology** December 6-10, 1996 Orlando, FL

PRESENCE OF BETA 1 INTEGRINS PREVENTS APOPTOSIS AND PROMOTES DNA SYNTHESIS OF CD34 SELECTED (EARLY) BUT NOT UNFRACTIONATED (LATE) HEMATOPOIETIC CELLS IN STROMAL CULTURES. M.W.-I. Wang*, R. Champlin, and A.B. Deisseroth. U.T. M.D. Anderson Cancer Center, Houston, TX and the Yale University School of Medicine, New Haven, CT.

Early hematopoietic precursor cells (HPC) require interaction with stromal cells (SC) and extracellular matrix protein (ECMP) ligands to survive in *in vitro* culture. As these hematopoietic precursor cells mature, or become neoplastic, they lose their dependency of these interactions and become free to circulate in the bloodstream. We studied the effects of monoclonal antibodies to surface cytoadhesion molecules on the binding, DNA synthesis and survival of early and later HPC cells grown on SC. A monoclonal antibody for the alpha 4 beta 1 (A4B1) integrin receptor dramatically reduced the binding and DNA synthesis of CD34 selected (immature) but not unfractionated (mature) HPC, and also increased the percentage of cells undergoing apoptosis from 12 to over 50% in cocultures of these CD34 selected cells with SC. Addition of the soluble VCAM-1 ligand, which binds to the A4B1 vector, also produced the same effect, showing that the A4B1 integrin receptor was functional. Addition of the VCAM-1 monoclonal antibody to these cocultures had the same effect showing that the VCAM-1 ligand engages the A4B1 receptor for this apoptosis rescue function. AML cells were not sensitive to this apoptosis rescue function, nor were mature normal cells. Measurement of the level of the A4B1 receptor on immature and mature hematopoietic cells showed that this loss of dependency on SC for binding was not due to downregulation of the receptor levels. This suggests that a functional change in the A4B1 receptor occurs during hematopoietic differentiation, and during the evolution of AML. These results may lead to methods of controlling the survival of these early cells for *in vitro* expansion and growth.

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EVALUATION OF DENDRITIC CELL (DC) LINEAGE COMMITMENT OF HUMAN CD 34$^+$ CELLS WITH DC-SPECIFIC mAb CMRF-44. T. Monji,* M.A. Rehse,* Grant Risdon,* T.C. Keenan, and K. Auditore-Hargreaves* CellPro, Inc., 22215 26th Ave. SE Bothell, WA.

CD 34$^+$ haematopoietic stem cells were isolated from mobilized leukapheresis products by using CEPRATE SC stem cell concentration system, followed by depletion of contaminating macrophages/monocytes by using CD14 magnetic beads. CD34$^+$ cells were then incubated in the presence of GM-CSF alone or GM-CSF plus TNF-alpha in the medium supplemented with 10% FCS, and their maturation into DC was followed by flow cytometry using mAb CMRF-44, which recognizes a DC-specific differentiation Ag. Following 3-day incubation in the presence of GM-CSF plus TNF-alpha, CD34^{bright}, but not CD34^{dull}, population quickly acquires CMRF-44 Ag expression forming CD34^{bright}CMRF-44^{bright} population. These double positive cells, which represented approximately 40% of the total population, then gradually lost CD34 expression by day 8, resulting in non-adherent CD34$^-$CMRF44$^+$CD14$^+$ cells. Incubation of CD34$^+$ cells in the absence of cytokines or in the presence of GM-CSF alone did not induce the expression of CMRF-44 Ag in CD34$^+$ cells. CMRF-44$^+$ cells also expressed high levels of surface class II molecules. Consistent with the maturation of CD34$^+$ cells into DC surface phenotypes, incubation of CD34$^+$ cells in the presence of GM-CSF plus TNF-alpha also induced changes in their cellular morphology and functional activities. After incubation with GM-CSF plus TNF-alpha for 6 days, small, blast-like CD34$^+$ cells acquired characteristic DC appearance: large, irregular-shaped cells with abundant cellular projections and irregular-shaped nuclei. These cells were extremely stimulatory in primary MLR when compared with CD34$^+$ cells before incubation or with CD34$^+$ cells after incubation in the absence of cytokines. Abilities of these CMRF-44$^+$ cells to present Ags and their surface phenotypes such as the expression of accessory molecules are currently under investigation. The ability to generate DC by culturing stem cells ex vivo may lead to immunotherapeutic applications of these cells.

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KINETICS OF LONG TERM BONE MARROW CULTURE-INITIATING CELL (LTCIC) AMPLIFICATION DURING CULTURE OF CD34+ BLOOD PROGENITOR CELLS (BPC) AT A CLINICAL SCALE. D. Möbest,* J. Winkler,* A. Garbe,* G. Schulz,* W. Lange,* R. Mertelsmann, and R. Henschler. Experimental Hematology Group. Department of Hematology and Oncology. University Medical Center. Freiburg. Germany.

Results from animal studies indicate that both short term and long term hematopoietic reconstitution from bone marrow aplasia are mediated only by a small subset of very primitive progenitors. These cells should therefore be maintained or, optimally, amplified in ex vivo cultured transplants. In this study, BPC from cancer patients mobilized by combination chemotherapy and G-CSF were enriched for CD34+ cells by immunoaffinity selection and subsequently cultured in serum free medium at 37°C for 14 days. Cultures were supplemented with 100 ng/ml rh SCF, 100 ng/ml IL-3 and in addition either FLT3 Ligand (FL) at 300 ng/ml, or IL-6 (100 ng/ml). Small scale experiments (3 x 10E4 CD34+ cells in total, n=6) were cultured in 1 ml cultures, clinical scale cultures (3 x 10E7 CD34+ cells in total, n=2) in 100 ml flask cultures with no feeding. Ex vivo expanded cells were analyzed for LTCIC content by seeding on Dexter type preformed irradiated allogeneic stroma using limiting dilution into 20 to 25 100µl replicate cultures per dilution step and readout on day 42 (Pettengell et al, Blood 84, 3653). Time course analysis of LTCIC during ex vivo culture of CD34+ cells in the presence of SCF, IL-3 and FL revealed an initial decline of LTCIC to values of one third of input LTCIC by day 2 of ex vivo expansion, concomitant with a decline in the number of granulocyte-macrophage colony forming cells (GM-CFC) and total nucleated cells (TNC). Subsequently, LTCIC expanded by a factor of 7.4 to 26 in small scale cultures by day 10 to 14. In clinical scale cultures, LTCICs amplified 6.9 and 9.5 fold, reaching plateau levels between days 6 and 8 after initiation, and declined to input values by day 10 to 14. In parallel, from day 2 to day 10, GM-CFC expanded 82 fold and TNC 27 fold in the clinical scale cultures. Parallel clinical scale cultures using SCF, IL-3 and IL-6 revealed a similar time course of LTCIC, GM-CFC and TNC, however the absolute numbers of LTCIC generated was 3 fold lower than in SCF, IL-3 and FL supplemented cultures. These data indicate that amplification of LTCIC for transplantation purposes is feasable at large scale. However, depending on the cytokines used, up to two thirds of input LTCIC may be lost within the first 48 hours of ex vivo expansion and only a subfraction therefore contributes to the expanded LTCIC.

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SEQUENTIAL CD34+ AND CD4+ CELL SELECTION FROM LEUKAPHERESIS COMPONENTS. L.J. Wang, M. Beach*, J.F. Daley*, K.C. Anderson. Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA

Allogeneic peripheral blood progenitor cell (PBPC) transfusions restore hematopoiesis after myeloablative therapy, and allogeneic donor lymphocyte infusions (DLI) have been shown to produce a graft-versus-leukemia effect. CD34+ cell enriched components reconstitute hematopoiesis as rapidly as unselected PBPCs, while CD4+ T lymphocytes have been implicated in the pathogenesis of the graft-versus leukemia effect. We tested the feasibility of sequential selection of CD34+ and CD4+ cell enriched fractions from aliquots of five autologous leukapheresis components. All patients had received GCSF with or without chemotherapy for PBPC mobilization. CD34+ cells were selected from a median 8.0 x 10^9 mononuclear cells using the CellPro (Bothell, WA) Ceprate LC cell separation system. Without washing or other manipulation, all cells in the CD34 depleted fraction (median 4.8x10^9, range 0.6-10.0x10^9) were then incubated with the appropriate antibodies for CD4 selection and passed through a second LC column.

	Start fraction Purity (%)*	CD34 enriched Purity (%)	CD34 depleted** Purity (%)
CD34	4.4 (0.1-11.0)	90.5 (1.4-95.5)	1.3 (0.1-5.4)
CD3	3.2 (0.9-62.9)	0.4 (0.4-0.8)	3.2 (0.7-80.3)
CD4	7.8 (2.9-70.0)	0.4(0.2-0.8)	6.1 (0.0-69.0)
CD8	2.0 (0.7-12.5)	0.1 (0.0-0.3)	2.5 (0.2-12.0)
NKH1	2.5 (0.8-8.6)	1.0 (0.2-1.2)	3.1 (1.0-9.0)

	CD4 enriched Purity (%)	CD4 enriched Yield (%)	CD4 enriched Cell number (x10^6)
WBC			9.9 (7.5-25.2)
CD34	0.4 (0.0-1.6)	0.1 (0.0-3.1)	0.0 (0.0-0.2)
CD3	83.3 (69.7-97.3)	47.9 (0.0-109.5)	8.8 (5.2-24.5)
CD4	86.0 (76.7-97.2)	55.7 (13.3-68.0)	8.8 (7.2-24.5)
CD8	0.7 (0.4-4.1)	1.3 (0.5-5.4)	0.1 (0.1-0.3)
NKH1	0.5 (0.1-6.0)	0.8 (0.0-4.4)	0.1 (0.0-0.3)

*All values are median (range) **Start fraction for CD4 selection

This study demonstrates that high purity CD34+ cells and CD4+ cell enriched fractions can be isolated sequentially from leukapheresis components. In addition, CD8+ lymphocytes, implicated in graft-versus-host disease, are depleted in the course of both positive selection procedures. This approach could decrease the number of donor procedures by providing separate CD34+ and CD4+ enriched populations for PBPC transplantation and donor lymphocyte infusion from the same leukapheresis component.

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A MULTI-CENTER PHASE II TRIAL OF CD-34+ PERIPHERAL BLOOD STEM CELLS (PBSC) AND HIGH DOSE CYCLOPHOSPHAMIDE (CY), VP-16, CARBOPLATIN (CB) AND TOTAL BODY IRRADIATION (TBI) FOR RECURRENT NON-HODGKIN'S LYMPHOMA (NHL). T. Shea, L. Perry, R. Brown, S. Yanovich, J. Wiley, D. Hurd, J. DiPersio, B. Popat, K. Claxton, and C. Jacobs. UNC School of Medicine, Chapel Hill, NC (TS,JW,BP), Bowman Gray School of Medicine, Winston Salem, NC (RB,DH) Washington University School of Medicine, St. Louis, MO (RB,JD), Medical College of Virginia, Richmond, VA (SY) and Cell-Pro, Inc, Bothell, WA (KC,CJ).

High-dose therapy with PBSC support for recurrent NHL is curative for a proportion of patients. Despite this treatment, 50% or more of such patients relapse, necessitating the development of more effective conditioning regimens and, possibly, methods for PBSC selection and tumor cell purging. This report describes an intensive treatment regimen that includes Cy 6 g/m^2, VP-16 1.8 g/m^2, Cb 1 g/m^2, and TBI 1200 cGy with CD-34 selected PBSC using the CEPRATE^2 SC Stem Cell Concentration System. All but 2 patients underwent PBSC mobilization with C 2.5 g/m^2, Prednisone 100 mg/d x 4 and G-CSF 10 ug/kg/d. Up to 6 collections were permitted to obtain a minimum of 2 x 10^5 selected (post-processing) cells/kg for reinfusion. Unprocessed reserve PBSC or marrow were also obtained. 50 patients with low (25), intermediate (22), or high grade (3) NHL were enrolled at 4 institutions between 8-95 and 7-96. Median age was 53, # prior regimens, 2, (range, 1-6), and 35 pts had marrow involvement. 31 pts have been mobilized, received the conditioning regimen, and were infused with a median of 3.86 x 10^6 CD-34 cells/kg. 17 pts (34%) did not proceed with conditioning and were inevaluable due to poor mobilization and CD-34 yields less than 2 x 10^6/kg. One pt was unable to receive TBI, but mobilized adequately and is evaluable for engraftment with a non-TBI regimen and one patient with a low cardiac ejection fraction did not proceed with this regimen. The good and poor mobilizers were not significantly different (unpaire d t-test) in amount of prior chemotherapy or XRT, age, incidence of marrow involvement, or histology. Serious toxicities included 2 treatment related pulmonary deaths (ARDS, alveolar hemorrhage), one grade 4 sepsis syndrome, and one culture negative septic death which occurred after neutrophil engraftment in a man who was discharged and readmitted 52 days post transplant. All patients recovered adequate neutrophil counts (median 11 days to ANC>500, range 9-20), and all but two recovered platelets to > 20,000 (median 23 days, range 10-154). These 2 patients received unprocessed PBSC or back-up marrow 90 and 154 days post initial reinfusion. Four pts required more than 50 days to achieve platelet counts > 20,000/ul. A Spearman rank correlation of .06 was observed between the # of infused CD-34 cells and platelet recovery. With a median follow-up of 6 months, 3 pts have progressed and 25 of 31 remain progression-free. This approach is feasible in the majority of pts with NHL, including those with extensive prior therapy and marrow involvement. Mobilization is difficult in up to a third of patients, however, and delayed platelet recovery can be seen despite infusion of adequate #s of CD-34 cells.

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HIGH FREQUENCY OF COMPLETE DONOR CHIMERISM FOLLOWING ALLOGENEIC TRANSPLANTATION OF CD34- SELECTED PERIPHERAL BLOOD PROGENITOR CELLS (ALLO PBPCT/CD34-)

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Ex vivo T-cell depletion (TCD) of the graft has been associated with a high rate (up to 30%) of mixed chimerism. The dose of transplanted progenitor cells is considered to be an important factor in promoting complete donor chimerism in the TCD setting. An advantage of G-CSF mobilized PBPC for allogeneic transplantation is the high number of progenitor cells obtained. We have prospectively studied, using a highly sensitive technique such as the PCR amplification of polymorphic short tandem repeats sequences (PCR-STR), the chimeric status in 8 patients receiving alloPBPCT/CD34- from HLA-identical sibling donors. Eight patients (5 male) with a median age of 32 years (range, 22-47), with AML (n=3), ALL (n=1), CML (n=2), CLL (n=1), and RAEBt (n=1), were conditioned with cyclophosphamide (120 mg/kg) and TBI (13 Gy in four fractions). The apheresis product was partially T-cell depleted by the immunoadsorption avidin-biotin method (Ceprate SC). The median number of $CD34^+$ cells and $CD3^+$ cells infused was 4.3 x 10^6/kg (range, 1.9-6.9), and 0.5 x 10^6/kg (range, 0.3-1.0), respectively. Molecular analysis of engraftment was done using PCR-STR in peripheral blood samples. Six STRs were analysed: FES, vWFA31, HUMTH01, F13A1, CYP19, and ApoA2. The sensitivity for detecting the recipient cell population was $\leq$0.1%. The follow-up was between 6 and 13 months, and at least three samples after transplant were analysed for each patient. Mixed chimerism was detected in 2 of 8 (25%) patients; these two patients relapsed at 8 and 10 months after transplant, respectively. The remaining 6 patients show complete donor chimerism and are in clinical remission after a maximum follow-up of 13 months (range, 6-13). These results suggest that in patients receiving alloPBPCT/$CD34^-$ a high frequency of complete donor chimerism is achieved probably because of the high number of progenitor cells administered.

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AUTOLOGOUS TRANSPLANTATION (AT) OF CD34+ PERIPHERAL BLOOD PROGENITOR CELLS (PBPC) AFTER DOUBLE (D) HIGH DOSE CHEMOTHERAPY (HDC) IN MULTIPLE MYELOMA (MM) IS FOLLOWED BY SEVERE IMMUNODEFICIENCY (ID) AND HIGH PRODUCTION OF INTERLEUKIN-6 (IL-6) RELATED-C REACTIVE PROTEIN (CRP) REQUIRING ADDITIVE IMMUNOTHERAPY (IT). J.F. Rossi, S. Legouffe,* N. Fegueux,* R.X. Sun,* C. Exbrayat,* P. Laury,* G. Paige,* J. de Voos,* V. Candevila,* Z.Y. Lu,* B. Klein. Hematology-Oncology, University Hospital, Montpellier France.

In MM, HDC with AT compared to conventional C represents a major advance in this disease, with better complete response (CR) (22 vs 5%) and survival (52 vs 12% at 5 years, y). With DHDC, CR reached 50% or more with potential benefit on survival. A major problem of AT is the infusion of tumor cells. Dramatic reduction of tumoral contamination has been obtained with CD34 purification of PBPC. 42 unpreviously treated patients (pts) with advanced MM (23 males, 19 females) were included in this study (30 stages (s)III, 5 sII, 2 sI, 5 plasma cell dyscrasias). 20 pts under 50 y had DAT and 22 pts between 60-65 y had single (S) AT, all with CD34 PBPC. All the pts had 3 cycles of VAD, followed by HD cyclophosphamide (4 g/m^2) with G-CSF for mobilization. 1-2 VAD cycles before HDC.melphan (M) HD (140mg m^2)=Total Body Irradiation (TBI:8Gy) for SAT pts, and MHD followed by a 2nd HDC (MHD=TBI) for DAT pts. Actually, 19 pts ended treatment (=14 in the next 2 months, m) and were evaluable. 8x10^6 CD34+/kg (range :5-42x10^6 CD34+/kg) were collected after mobilization (median=1.6 apheresis (A), range:1-4) for the 2 groups of pts. For optimizing collection, circulating PB CD34+ cells were checked every day when pt was in aplasia. Predictive number of CD34+ cells in A was calculated for a 5-hour period of A (median=5 blood volumes). A was made as soon as the predictive number of collected CD34+ cells was superior to 10.10^6/kg in 2 A. Purification of CD34+ cells was made with CEPRATE columns (CellPro) and CD34 purity after process ranged from 65% to 95% (mean=85.45%) with a median of 3x10^6 CD34+ reinfused cells. The log-reduction in AT of tumoral cells was 3-4. Hematologic recovery was 8.3 days for 500 neutrophils/mm^3 (7.3 for MHD, 10.4d for MHD+TBI) and all the pts achieved an untransfused platelet count greater than 20x10^9/L. Days of hospitalization ranged from 19.8d for SAT, 19.1d for MHD/ DAT and 25.4d for MHD+TBI/DAT. The 2nd AT was made after hematologic recovery (median=3m between the 2 HDCs). 6 pts (31%) were in CR (3 in each group). Very good response (>90% reduction) was seen in 4 additional pts. Severe viral infections were observed for 4/9 pts after the 2nd AT at median d35 with rehospitalization. Circulating CD4+ cell count was reduced (lower count=11/mm^3, reaching 100/mm^3 after 120d). CRP peaked at 44mg/mL after the 1st MHD and at 56 mg/mL after the 2nd AT. T cell repertoire was evaluated for 11 pts, at diagnosis and every 3m, showing important variation. ID, high IL-6 production occuring on tumoral residual disease required IT.

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GENERATION OF NOVEL CELL SURFACE TRANSMEMBRANE-CONTAINING SUICIDE FUSION GENES: STRUCTURE, FUNCTION AND EXPRESSION IN NIH3T3 CELLS, T CELLS AND T CELL LINES. A Harrelson*, B Boecker*, M Sands*, and J DiPersio. Washington Univ School of Medicine, St Louis, MO. 63110.

The use of suicide gene (SG)-containing T cells to control GvHD has been problematic due to inefficient methods of T cell transduction, selection of transduced T cells and to a lack of informative animal model systems. Since selection of transduced T cells with neomycin is extremely inefficient, some investigators have attempted to co-express SG's with a surface epitope tag. Unfortunately, the subsequent selections usually require sterile cell sorting, resulting in low yields. In addition, cellular expression of SG and surface epitopes often becomes uncoupled due to 'promoter silencing.' In order to overcome these obstacles we have constructed a series of fusion SG's in which the human CD34 extracellular and transmembrane domains have been joined in-frame via a 17 AA linker to the first (CD34-TK) or second ATG (CD34-ΔTK) of HSV thymidine kinase (TK) and to the first amino acid of E. coli cytosine deaminase (CD34-CDA). After ligation into the LXSN retroviral vector, high titer ecotropic and amphotropic packaging extracts were generated and used to transduce NIH3T3 cells, as well as primary T cells and T cell lines from both mouse (CTLL-2) and man (Jurkat). Transduction efficiencies in primary murine and human T cells ranged between 1-25%. The extracellular domains of these fusion SG's are strongly expressed on the cell surface, and cells expressing these fusion SGs can be selected with high yield and purity on a Ceprus™ CD34 affinity chromatography selection column or by sterile cell sorting, without the need for neomycin selection.

Clones of transduced NIH3T3 cells selected for high expression of CD34-TK by FACS die rapidly in response to the prodrug gancyclovir (GCV) (Id_{50} = 5 uM). Cells transduced with the CD34-CDA fusion SG's are equally sensitive to the killing effects of 5FC (Id_{50} 50 uM), as are cells transduced with the wild type E. coli CDA. Although all three fusion constructs are strongly expressed on the cell surface of NIH3T3 and T cells, the CD34-ΔTK construct is 100-fold less sensitive to GCV. Approximately 0.0001-0.0002% of NIH3T3 cells overexpressing CD34-TK consistently survive the effects of GCV. These resistant cells continue to express high levels of the fusion SG, as detected by FACS. The mechanism of resistance remains unknown. In order to further improve the functional activity of these fusion SG's, we have mutated the active site of TK in a similar fashion to that previously described (Black et al. PNAS, 93: 3525, 1996).

In addition to testing these constructs in murine bone marrow transplant models we are also attempting to direct the high level expression of these fusion SG's in the T cell compartment of transgenic mice using the human CD2 and human granzyme B T cell targeting vectors. These mice can then be used as donors for both syngeneic and allogeneic transplantation, ensuring that 100% of the T cells are genetically manipulated. These mouse models will allow us to test the function, trafficking, efficiency of prodrug-induced killing and immunologic clearance of the fusion SG-expressing T cells. The generation of single fusion cDNA's which function as both a selectable surface epitope tag as well as potent SG's may overcome many of the obstacles inherent in attempting to control GvHD via genetic manipulation and prodrug-induced ablation of allogeneic T cells.

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THE EFFECT OF FLT3 LIGAND AND/OR C-KIT LIGAND ON THE GENERATION OF DENDRITIC CELLS FROM HUMAN CD34+ BONE MARROW. E.Maraskovsky*, E. Roux*, M. Teepe*, H.J. McKenna*, K. Brasel*, S.D. Lyman*, D.E. Williams. (Intro. by D.E. Williams) Immunex Corporation, Seattle, WA.

There is growing interest in generating dendritic cells ex vivo for use as tumor or infectious disease vaccine adjuvants. However, the isolation of large numbers of functionally mature dendritic cells has been hampered by their low frequency in blood, accessibility of lymphoid organs and their terminal state of differentiation. Flt3 ligand (flt3L) is a recently cloned hematopoietic growth factor which affects enriched populations of hematopoietic stem and progenitor cells, whilst having little apparent effect on more mature cell types. The effects of flt3L compared to c-kit ligand (c-kitL) on the ex vivo generation of functionally mature dendritic cells from CD34+ human bone marrow precursors was investigated. Functionally mature dendritic cells can be generated in vitro from CD34+ bone marrow progenitors after 2 weeks of culture using GM-CSF, IL-4 and TNF-α. Approximately 60-70% of the resultant cells are HLA-DR+, CD86+, with 40-50% of cells also expressing CD1a; markers suggestive of the dendritic cell lineage. The addition of either flt3L or c-kitL increased the total number of cells obtained with GM-CSF, IL-4 and TNF-α a further 6-7-fold; whilst the combination of flt3L and c-kitL resulted in a 12-13-fold increase. The addition of flt3L or c-kitL did not significantly affect the percentage HLA-DR+, CD86+, CD1a+ dendritic cells; but did increased the total number of CD1a+ cells by 5-fold with flt3L, 6.7-fold with c-kitL and 11-fold when used in combination. Dendritic cells generated in the presence of flt3L and/or c-kitL presented allo-antigen as efficiently to allo-donor T cells or Tetanus Toxoid (TTX) to TTX-specific autologous T cells as efficiently as dendritic cells expanded with GM-CSF, IL-4 and TNF-α alone. This suggests that flt3L can be used in combination with GM-CSF, IL-4 and TNF-α as well as c-kitL to generate large numbers of functionally mature dendritic cells ex vivo.

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TRANSFORMING GROWTH FACTOR (TGF)-BETA 1 INHIBITS GROWTH OF MAMMARY CARCINOMA CELLS DURING EX VIVO CULTURE OF CD34+ CELLS. A. Spyridonidis,* A. Garbe,* D. Behringer,* R. Mertelsmann, R. Henschler. Department of Hematology/Oncology, University Medical Center, Freiburg, FRG.

Although the incidence of contaminating mammary carcinoma cells in blood progenitor cells (BPC) transplants has been reported to be lower than in bone marrow, data from several groups indicate the presence of residual malignant cells within autologous BPC preparations. We assessed the potential of TGF-beta 1 to influence proliferation and survival of mammary carcinoma cells in the presence of a combination of Interleukin-(IL)-1 beta, IL-3, IL-6, c-kit Ligand (KL) and Erythropoietin (EPO), which can be used for ex vivo culture of BPC. Hematopoietic cytokines IL-1 beta, IL-3, IL-6, KL and EPO (136KE), added to the optimal growth medium of MCF-7, 401X, MDA-MB-468, SK-BR-3, T-47D, MDA-MB-453 and MDA-MB-231 mammary carcinoma cell lines reduced cell growth in 6/7 lines over a culture period of 2 weeks by a median of 61.5% (range, 41.5 - 96.5%). However, this effect was reversible since in 3/3 cell lines that were subsequently allowed to regrow in single cell cultures in optimal growth medium, clones reemerged from about 80% of cells seeded. If TGF-beta 1 (30 ng/ml) was present alone during a 14 day culture period, cell growth was inhibited by 57% or in 4/7 cell lines. If both TGF-beta 1 and 136KE were present, growth was reduced in 7/7 cell lines by 72% (range, 31% to 97%). Moreover, the surviving cells displayed a more differentiated morphology, with an decreased nucleus to cytoplasm ratio (5/5 lines). In single cell clonogenic assays subsequent to exposure against hematopoietic growth factors plus TGF-beta 1, only 7% of the surviving MCF-7 cells were able to regrow over periods of 20 to 30 days. At the same time, TGF-beta 1 permitted ex vivo expansion of colony forming cells granulocyte-macrophage (GM-CFC) from CD34+ BPC at similar rates as control cultures using 136KE, and maintenance of long term bone marrow culture initiating cells (LTCIC) as assayed by limiting dilution analysis in Dexter type long term bone marrow cultures. These data indicate that selection of growth factors during ex vivo purging cultures of CD34+ BPCs may influence the survival clonogenicity, and differentiation status of contaminating breast cancer cells.

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COMPARATIVE ANALYSIS OF CD34+ SELECTED AND CAMPATH TREATED BONE MARROW (BM): EVALUATION OF T-CELL CONTENT AND PROGENITOR PROLIFERATIVE POTENTIAL. E. Clarke,[1] M.N. Porter,[2] J.M. Cornish,[2] A. Oakhill,[2] and D.H. Pamphilon.[1,2] [1]Institution of Transfusion Sciences and [2]The Royal Hospital for Sick Children, Bristol, U.K.

Depletion of T-cells from BM grafts reduces the incidence and severity of graft-versus-host disease (GVHD) but graft rejection and relapse is increased, implicating T-cells in the graft-versus-leukaemia (GVL) effect. The optimal T-cell concentration which can induce a GVL effect without the development of ≥ grade 2 acute (a)GVHD in man has not been established. We reasoned a procedure that selected CD34+ cells might provide adequate depletion of T-lymphocytes and tested this initially using a mini-column system (CEPRATE ® LC, CellPro). We evaluated 11 BM samples from normal donors following CD34+ cell selection and compared these with *in vitro* Campath-1M Mab treated samples (n=5). The recovery of CD34+ cells and the quality of the products was evaluated by measuring the content of primitive stem cells (LTCIC) and committed progenitors (CFU-GM and BFU-E) using standard long term and methylcellulose assays. T-cell depletion was estimated using flow cytometry and the frequency of proliferating T-cells determined using a limiting dilution assay (LDA). The recovery of CD34+ cells, CFU-GM, BFU-E and LTCIC was 55 ± 11%, 44 ± 12%, 42 ± 13% and 130 ± 70% respectively in the CD34+ cell enriched product (n=11) as compared to 50 ± 7%, 78 ± 19%, 78 ± 18% and 62 ± 49% in the Campath treated samples (n=5). Analysis of CD3+ cell content suggested equivalent T-cell depletions (99.8 ± 0.2% in the CD34+ cell enriched and 98.4 ± 1.9% in the Campath treated product) however the LDA confirmed a mean proliferative frequency of 1: 644 for the CD34+ cell enriched product (n=5) and 1: 2702 for the Campath treated samples (n=5). Clinical data obtained from 4 unrelated donor (UD) BM harvests processed on the CEPRATE ® SC system compared well with the laboratory model in terms of CD34+ cell recovery, purity and progenitor proliferative capacity. With a mean T-cell depletion of 99.2 ± 0.2%, the mean CD3+ cell infusion to recipients was 0.49 ± 0.40 x 10^6cells/kg. Three of 4 patients developed aGVHD ≥ grade 2. In comparison, with our standard T-cell depletion protocol (*in vitro* Campath-1M) for UD-BMT, of 15 patients (matched for age and disease status; mean CD3+ cell content 1.76 ± 2.04 x 10^5/kg - to 98.87 ± 2.98% T-cell depletion) 7 developed grade 1 and 1 developed grade 2 aGVHD. The discrepancy between flow cytometric analysis and LDA may be due to the continued destruction of Campath-coated lymphocytes either by opsonisation of cells or the modulation of proliferation which reduces the effective T-cell dose. This may be important clinically since T-cell depletion using CD34+ cell selection may be less effective at reducing the severity of aGVHD after UD-BMT.

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PAN T-CELL DEPLETION OF PBSC: ALLOGRAFT ENGINEERING WITH THE CEPRATE TCD SYSTEM. G. Risdon,* E.J. Read,* M. Poter,* L. Kang,* and K. Auditore-Hargreaves.* NHLBI and Dept. of Transfusion Medicine, NIH, Bethesda, MD, BMT Unit, Royal Hospital for Sick Children, Bristol, UK, Eberhard-Karls Univ., Tubingen, Germany and CellPro, Inc., Bothell, WA USA. Introduction by T. Keenan.

The CEPRATE TCD® system utilizes a biotinylated, murine monoclonal antibody to the pan-T-cell antigen, CD2, and a column containing 50 mL of avidin-conjugated gel to deplete residual T-cells from a CD34+ enriched product. The system runs on the CEPRATE instrument and adds 45 minutes to the total processing time. PBSC products obtained by apheresis of normal, volunteer donors mobilized with 7-10 μg G-CSF/kg/day, were used to evaluate the depletion of CD3+ T-cells, as measured by flow cytometry, obtained with this system. Processing a single apheresis with the CEPRATE TCD system resulted in a stem cell product containing a median of 2.7×10^6/kg CD34+ cells (range 4×10^5 - 4.4×10^6/kg) and a median of 3×10^4/kg T-cells (range 1.4×10^4 - 1.2×10^5/kg). This represents an overall CD34 cell recovery of 44%. Thus when processing 2 apheresis collections, the CEPRATE TCD system yields a graft whose T-cell content is below the threshold of clonable T-cells that leads to GVHD ($1\text{-}5 \times 10^5$/kg) yet contains large numbers of CD34 cells necessary to override graft rejection/failure in unrelated and mismatched allogeneic transplants.

	Start	Post CD34+ Selection Step	Post TCD Depletion Step	Overall
Median # CD34+ Cells ($\times 10^6$) (n=11)	396 (72-1115)	227 (41-570)	190 (27-310)	-
Median # CD3+ Cells ($\times 10^6$) (n=10)	10352 (3800-24205)	31 (7-93)	1.9 (0.96-8.2)	-
Median # DFU-GM/10^5 Cells (n=4)	not done	not done	3050 (1200-4250)	-
Median CD3+ Log Depletion (n=10)	-	2.8 (2.1-3.1)	0.9 (0.3-1.9)	3.7 (3.4-4.4)

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DONOR LEUKOCYTE INFUSIONS (DLI) FOR THE TREATMENT OF RELAPSED HEMATOLOGICAL MALIGNANCIES AFTER ALLOGENEIC ELUTRIATED BONE MARROW TRANSPLANTATION (BMT). A Seber,* S J Noga, D Harris,* C Hattenburg,* E J Fuchs. Johns Hopkins Oncology Center, Baltimore, MD.

T cell depletion using elutriation-CD34+ stem cell selection has decreased the morbidity of transplants in our institution, but relapse remains an important cause of treatment failure. Since BMT preparative regimens are already used at maximally tolerated doses, adoptive immunotherapy, which overcomes drug resistance, is an attractive approach to improve disease free survival. In our institution DLI was used in 11 patients who relapsed after elutriated BMT, using escalating doses (10-100 million T-cells/kg). Among patients with acute lymphocytic leukemia (ALL; n=2), acute myelogenous leukemia (AML; n=2), myelodysplastic syndrome (MDS; n=2), and indolent lymphoma (n=1), those receiving DLI as consolidation after chemotherapy-induced remission (1 ALL, 2 AML) remain in remission at 9+, 20+, and 21+ weeks after the DLI, without aplasia or GVHD. The four patients not in remission had progression of their disease and three are alive receiving chemotherapy. One patient with Hodgkin's disease had resolution of the "B" symptoms and of adenopathy, despite progression of bony metastasis. Three patients with multiple myeloma were treated with one infusion of 10 million T-cells/kg. Of the two patients evaluable for response, both had progression of the disease within the first month post DLI; the first patient had an extraordinary response to chemotherapy with vincristine, adriamycin, and dexamethasone (VAD), but achieved complete remission at 12 weeks only after developing grade II acute GVHD. The second patient has an ongoing response and limited chronic GVHD. A twelfth patient with EBV-lymphoproliferative disease (LPD) is responding to 1 million T-cells/kg. Bone marrow aplasia was not observed in any patient. All responders had residual donor hematopoiesis prior to DLI. No side effects were observed during any of the infusions. No patient developed severe infections. Neither GVHD, nor response was observed earlier than four weeks after DLI. Study of the lymphocyte subsets (CD3, CD4, CD8, CD16, CD19, CD56) and NK-LAK function of the patients before and two weeks after DLI has not shown yet any consistent pattern or correlation with clinical response. This initial experience suggests that among patients relapsing following allogeneic elutriated BMT, DLI is a safe procedure which may consolidate chemotherapy-induced remission of acute leukemias, and induce responses in multiple myeloma and EBV-LPD with relatively low lymphocyte doses. In our experience, response is highly correlated with the occurrence of GVHD. Moreover, activated alloreactive lymphocytes appear to be resistant to chemotherapy and steroids (VAD), remaining capable of inducing both remission and GVHD. The mechanism of DLI-induced remissions is still unknown.

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OLIGODEOXYNUCLEOTIDE THERAPEUTICS FOR HUMAN MYELOGENOUS LEUKEMIA: INTERIM RESULTS. A.M. Gewirtz, S. Luger, D. Sokol, B. Gowdin, E. Stadtmauer, A. Reccio, M.Z. Ratajczak. Departments of Pathology and Internal Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA.

Inhibition of gene function with antisense (AS) oligodeoxynucleotides (ODN) has proved useful in research applications and is being investigated for potential therapeutic utility. We initiated clinical trials to evaluate the effectiveness of phosphorothioate modified ODN (P-ODN) AS to the c-myb gene as marrow purging agents for chronic phase (CP) or accelerated phase (AP) chronic myelogenous leukemia (CML) patients (pts), and a Phase I intravenous infusion study for blast crisis (BC) pts, and pts with other refractory leukemias. P-ODN purging was carried out for 24 hrs on CD34+ marrow cells. Pts received busulfan and cytoxan, followed by re-infusion of previously cryopreserved P-ODN purged MNC. In the pilot marrow purging study 7 CP and 1 AP CML pts have been treated. 7/8 engrafted. In 4/6 evaluable CP pts, metaphases were 85-100% normal 3 months after engraftment suggesting that a significant purge had taken place in the marrow graft. 5 CP pts have demonstrated marked, sustained, hematologic improvement with essential normalization of their blood counts. Follow-up ranges from 6 months to ~ 2 years. In an attempt to further increase purging efficiency we incubated patient MNC for 72 hours in the P-ODN. Though PCR and LTCIC studies suggested a very efficient purge had occurred, engraftment in five patients was poor. In the Phase I systemic infusion study, 18 refractory leukemia pts (2 pts were treated at 2 different dose levels; 13 had AP or BC CML). Myb AS P-ODN was delivered by continuous infusion at dose levels ranging between [0.3 mg/kg/ day x 7 days] to [2.0 mg/kg/day x 7 days]. No recurrent dose related toxicity has been noted though idiosyncratic toxicities, not clearly drug related, were observed (1 transient renal insufficiency; 1 pericarditis). One BC pt survived ~14 mo with transient restoration of CP disease. These studies show that P-ODN may be administered safely to leukemic pts. Whether pts treated on either study derived clinical benefit is uncertain, but the results of these studies suggest that ODN may eventually demonstrate therapeutic utility in the treatment of human leukemias.

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GENE THERAPY FOR GAUCHER DISEASE

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Gaucher disease, is a hereditary deficiency of the enzyme glucocerebrosidase (GC) and results in accumulation of glucocerebroside within cells of the reticulcendothelial system. Gene therapy has been considered as a potential treatment. We have treated 3 Type I Gaucher patients with GC transduced, CD34 enriched peripheral blood cells in a Phase I study. Peripheral blood stem cells were mobilized with rh G-CSF for 4 days and then collected by leukapheresis on two consecutive days. Cells were enriched for CD34+ cells by the CEPRATE® SC Stem Cell Concentrator (provided by CellPro, Bothell) and transduced by 5 day incubation in a long-term marrow culture system containing PG13.LgGC vector supernatant (provided by Targeted Genetics, Seattle). The culture conditions included protamine sulfate 4 ug/ml and recombinant human IL-1, IL-3, IL-6 and SCF at 50 ng/ml. Fifty percent of the medium was replaced daily with fresh virus containing medium. The median number of CD34 cells retrieved after 2 leukaphereses was 160 x 10^6 (range 146 to 371 x 10^6), median recovery was 46% and median increase in CD34 cells was 2.5 fold (range 1 to 4.5 fold). Transduction efficiency in all three patients after culture and before infusion was between 0.1 and 0.01% as determined by polymerase chain reaction (PCR). G C enzyme activity of infused cells was increased at least two-fold after transduction in all three patients. Follow-up is now between 5 and 10 months after infusion of transduced cells. None of the patients showed evidence of transduced GC sequences in the peripheral blood by PCR and no significant changes in clinical course and GC enzyme activity was observed. We conclude that the transduction of mobilized peripheral blood stem cells with a retrovirus containing the human cDNA for glucocerebrosidase is safe and without side effects. Transduction efficiency at the time of infusion into the patients, however, was low. No engraftment of transduced cells was observed in these patients who did not receive myelosuppressive treatment before cell infusion.

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FLT-3 LIGAND PROMOTES THE DEVELOPMENT AND FUNCTION OF CD1a$^+$ CELLS GENERATED EX-VIVO FROM CD34$^+$ PERIPHERAL BLOOD PROGENITOR CELLS. S. Schiesling', L. Gruber', S. Wirths', H.J. Bühring', B. Ziegler, T. Bock, L. Kanz, and W. Brugger. Department of Hematology/Oncology, University of Tübingen, Germany.

Autologous CD1a$^+$ cells may serve as a cell population for use as professional antigen-presenting cells in adoptive immunotherapy, provided they are available in sufficient numbers. Therefore, CD34$^+$ peripheral blood progenitor cells (PBPC) from cancer patients were expanded in liquid suspension cultures to define the optimum conditions for an effective production of CD1a$^+$ cells. Positively selected CD34$^+$ PBPC were cultured at 3 x 10^4/ml for up to 35 days with weekly half-medium changes. Stimulation by SCF, IL-1, IL-6, IL-3, and EPO (S163E), a combination previously demonstrated to produce large numbers of progenitor cells for transplantation (Brugger et al., N.Engl.J.Med. 333:283-287, 1995) showed a median (range) yield of CD1a$^+$HLA-DR^{++} cells of 2.5 (1-8.5), 5 (3-13.4) and 22 (13-28.5) per CD34$^+$ cell after 14, 21, and 35 days, respectively. Addition of GM-CSF and IL-4 (S163E-GM-IL-4) led to an increase to up to 70% CD1a$^+$ cells with a maximum total yield of 53 and 51 CD1a$^+$ cells produced from 1 CD34$^+$ PBPC in serum and serum-free medium, respectively. Kinetic analysis of phenotypical changes during culture showed the appearance of two distinct CD1a^{dim} and CD1a^{bright} populations within 14 days, with the latter increasing over time. Triple color flowcytometry revealed that CD1a^{dim} cells were predominantly CD14$^+$, whereas CD1a^{bright} cells were CD14$^-$. The pattern of CD4 co-expression of CD1a^{dim} and CD1a^{bright} cells was comparable to the pattern observed for CD14. Both CD1a$^+$ populations co-expressed high levels of HLA-DR, CD33, CD80, CD86, CD40, and CD54. Omitting IL-1 from the cytokine cocktail (S36E+GM+IL-4) resulted in a considerable reduction of CD1a$^+$ cells to about 60% when compared to S163E-GM+IL-4. Omission of EPO had no significant effect. The addition of Flt-3 ligand (S163-GM+IL-4+Flt-3) resulted in a 2.2 ± 0.2 fold and 1.9 ± 0.16 fold increase of %CD1a$^+$ cells on day 14 when compared to S163E+GM+IL-4 and S163+GM+IL-4, respectively. At 21 days of culture, this effect was even more pronounced. Overall cell production was also increased by Flt-3 ligand. Functional analysis of ex-vivo generated CD1a$^+$ cells by allogeneic mixed lymphocyte reaction showed that ex-vivo generated cells were approximately 20-fold more potent when compared to control pooled PBMC. Furthermore, CD1a$^+$ cells generated in Flt-3-containing medium were about 3-fold more active than those expanded without Flt-3 ligand. Taken together, the results demonstrate that functional active CD1a$^+$ cells can be produced in large numbers from CD34$^+$ PBPC from cancer patients for a potential clinical use.

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IN VITRO AND IN VIVO EVIDENCE THAT EX VIVO CYTOKINE PRIMING OF TRANSPLANTED MARROW CELLS MAY AMELIORATE POST-TRANSPLANT THROMBOCYTOPENIA. M.Z. Ratajczak, B. Machalinski, J. Ratajczak, T. Skorski, W. Marlicz, and A.M. Gewirtz. Depts. of Pathology and Internal Medicine, Univ. of Pennsylvania School of Medicine, and Thomas Jefferson University Cancer Institute, Philadelphia, PA.

Thrombocytopenia remains a significant cause of post-transplant morbidity and mortality. We have been investigating strategies to address this problem, and have focused our efforts on CFU-Meg expansion. Towards this end, we have developed an ex vivo serum free expansion system designed to increase CFU-Meg prior to transplantation of harvested marrow, or peripheral blood "stem" cells. Human $CD34^+$ cells are isolated from marrow and then initially cultured serum free in Iscove DMEM for 7-10 days in the presence of TPO, KL, IL-1 and IL-3. Under these conditions, CFU-Meg were increased 40-80 fold over non-primed control cells using standard assay conditions. To increase the practicality of this approach, we then investigated whether shorter cytokine exposure times would also result in effective expansion of CFU-Meg. We found that when $CD34^+$ cells were exposed, serum free, to the identical cytokine cocktail for only 24-48 hours, the number of assayable CFU-Meg was still increased, though only 2-3 fold over control cells. We also found that when previously frozen primed marrow cells were subsequently cultured they gave rise to colonies more quickly than frozen cells which were not primed. The potential utility of this expansion strategy was tested in an in vivo model constructed to avoid the possibility that IL-1 exposure might delay or inhibit stem cell engraftment. Balb-C mice were lethally irradiated, and then transplanted with previously frozen syngeneic marrow mononuclear cells (10^6/mouse), approximately one tenth of which (10^5) were primed with TPO, KL, IL-1, and IL-3 under serum free conditions for 36 hours. Mice receiving the primed frozen marrow cells recovered their platelet and neutrophil counts 3-4 days earlier than mice transplanted with unprimed marrow cells. These results compare favorably with platelet recovery in animals receiving growth factors throughout the post-transplant period. These experiments therefore suggest that growth factor priming of processed marrow or peripheral blood progenitor cells prior to storage and transplantation will decrease platelet, and neutrophil, recovery times in a manner equivalent to that produced by constant administration of cytokines during the post transplant recovery period. For these reasons we hypothesize that pre-transplant priming of hematopoietic cells represents a highly cost-effective alternative to present marrow recovery regimens.

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AUTOLOGOUS TRANSPLANTATION OF RETROVIRALLY MARKED CD34+ ENRICHED PERIPHERAL BLOOD AND BONE MARROW CELLS CONTRIBUTE TO GENERATION OF ALVEOLAR MACROPHAGES D. Dauer, A.F. Manchester, L. Ramezani*, A.M. Levine, W.F. Anderson. Division of Hematology and Gene Therapy Lab, University of Southern California, Los Angeles, CA.

Clinical trials have shown that genetically marked bone marrow (BM) and mobilized peripheral blood progenitor cells (PBPC) infused after myeloablative therapy contribute to multilineage recovery of peripheral blood cells. Using a similar study design with two distinguishable retroviral vectors, we report in one patient that autologous transplantation of BM and mobilized PBPC can also generate alveolar macrophages. Four PBPC harvests were collected from a 56 year old male with B-cell lymphoma in 2nd complete remission during WBC recovery after cyclophosphamide 4.5 gm/m² and daily GM-CSF. One half of the 2nd and 3rd PBPC harvests and one third of the BM (harvested 7 days after cessation of GM-CSF) were processed by the CEPRATE® SC Stem Cell Concentration System (CellPro, Inc., Bothell,WA). The CD34+ enriched cells were preincubated with 10ng/mL, 50ng/mL & 10% autologous plasma for 42 hr, followed by 6 hr incubation with IL3+IL6 and a retroviral vector carrying Neo^r gene: G1Na vector for both PBPC and LNL6 vector for the BM (Genetic Therapy Inc.). Cell pellet samples from both PBPC and BM cells and from individually picked CFU-GM colonies of both PBPC were PCR positive for the Neo^r gene. Gene expression in CFU-GM colonies growing in 1.2mg/ml G418, was 1% (PBPC #1) and 0.9% (PBPC #2). Neo^r gene was not detected nor expressed in the BM CFU-GM. All transduced and untransduced PBPC and BM cells were reinfused after the patient received BCNU 15mg/kg, Etoposide 30 mg/kg and Melphalan 140 mg/m². WBC rose above 1000/mm³ on day 7 after transplantation. On day 43 he developed chemotherapy-related pneumonitis and bronchoscopy detected no infectious cause. Almost all cells in the bronchoalveolar lavage (BAL) were alveolar macrophages. Neo^r containing cells were detected by PCR in the BAL sample with a stronger signal for G1Na than for LNL6. At that time the blood mononuclear cells were PCR positive only for G1Na and the BM cells only for LNL6. Both vectors signal were stronger in the BAL than in the post transplantation blood or BM samples, suggesting the PCR positive cells were not derived from blood cells contaminating the BAL sample. No Neo^r gene was detected or expressed in CFU-GM colonies from the BM or blood cells. In summary 1) cells derived from BM or mobilized PBPC can contribute to early engraftment of alveolar macrophages 2) transduction efficiency and the engraftment of alveolar macrophages derived from PBPC do not appear to be lower than those derived from unmobilized BM cells. 3) CD34+ enriched cells from BM or mobilized PBPC cells can be used for retroviral gene transfer into alveolar macrophages for potential therapeutic intent at least for short term effects. this finding may imply a collective genetic and/or environmental cause for the increased frequency of APL among Latinos with AML.

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CHARACTERIZATION OF AN MDR1 MUTATION WHICH RESULTS IN THE DISSOCIATION OF RHODAMINE 123 EXCLUSION AND CELLULAR RESISTANCE TO COLCHICINE AND PACLITAXEL. E.A. Shaughnessy*, S. Chatterjee*, and K.K. Wong, Jr*. (Intr. by S.J. Forman) City of Hope National Medical Center, Duarte, CA.

Gene transfer of the multidrug resistance (MDR1) gene to hematopoietic progenitors is being intensively investigated as a means of ameliorating chemotherapy related myelosuppression. A single base deletion at nucleotide +2143 relative to the "ATG" ($MDRdel^{+2143}$) was identified during construction of vectors encoding the human MDR1 cDNA. This deletion results in a frameshift mutation which truncates the MDR1 product by 561 amino acids, removing transmembrane regions 7-12, and alters amino acids at positions 717-719 from cysteine, alanine, and isoleucine to valine, proline, and leucine. $MDRdel^{+2143}$ was inserted into an adeno-associated virus (AAV)-based expression vector under control of the Rous sarcoma virus (RSV) promoter ($CWRMDRdel^{+2143}$). In contrast to an analogous construct encoding the wild type MDR cDNA (CWRMDR), the full length 170 kDa MDR1 product was not detected by either Western blot analysis or radioimmunoprecipitations with monoclonal antibodies directed against the amino or carboxyl-termini of P-glycoprotein following transfection of NIH 3T3 cells with $CWRMDRdel^{+2143}$. Furthermore, cells transfected with CWRMDR were resistant to colchicine, whereas cells transfected with the $CWRMDRdel^{+2143}$ were not. Unselected, or 5-FU or Sca-1 selected murine marrow cells, and human $CD34^+$ bone marrow cells transduced with encapsidated $CWRMDRdel^{+2143}$ expressed P-glycoprotein as detected by flow cytometry using monoclonal antibodies directed against the amino terminus of P-glycoprotein. Furthermore, $CWRMDRdel^{+2143}$ transduced marrow cells excluded rhodamine 123, but failed to develop resistance to colchicine or paclitaxel (Taxol), known P-glycoprotein substrates. These data confirm the importance of the carboxyl-terminal region of P-glycoprotein in conferring drug resistance, and suggest that rhodamine 123 exclusion and resistance to colchicine and paclitaxel may be dissociated.

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USE OF NESTED PCR FOR DETECTION OF t(14;18)-POSITIVE CELLS FOLLOWING CD34+ SELECTION IN SAMPLES FROM NON-HODGKIN'S LYMPHOMA PATIENTS. T.J. Lavin,* A.B. Ormander,* T. Shea, H.M. Lazarus, R. Brown, J. DiPersio, A.A. Ross.* CellPro, Inc, Bothell, WA, University of North Carolina, Chapel Hill, NC, University Hospitals of Cleveland, Cleveland, OH, and Washington Univ. School of Medicine, St. Louis, MO.

Tumor cell depletion can be assessed by testing for the presence of cells containing t(14;18) in positively selected CD34+ fractions from some non-Hodgkin's lymphoma (NHL) patients. We have compared tumor contamination in CD34+ fractions from peripheral blood stem cells (PBSC) from NHL patients using two methods of sample preparation. The methods compared DNA extraction with phenol/chloroform versus whole cell lysis for nested PCR analysis. In a model system using SU-DHL-6 NHL cells seeded into a normal apheresis sample, the phenol/chloroform extraction method showed a sensitivity of $1:1x10^5$, while the whole cell lysis method showed a sensitivity of $1:1x10^6$. A total of 56 stem cell apheresis samples from 40 patients were processed for CD34+ cell selection using the CEPRATE® LC Laboratory Cell Selection System. The apheresis and CD34+ fractions were collected and either lysed and extracted for DNA by the phenol/chloroform method, or frozen and subsequently tested using whole cell PCR. There was inadequate sample available to test each specimen by both methods. A nested PCR assay was used to identify those fractions containing t(14;18)-positive cells. Of the 56 tested samples, 17 were t(14;18)-positive, and of those, 5 were positive in both the apheresis and CD34+ selected fractions. The remaining 12 positive samples were PCR-positive prior to CD34+ selection but the CD34+ selected fraction appeared depleted of t(14;18)-positive cells. All samples in which the CD34+ selected fractions were positive for t(14;18) had been tested using the whole cell PCR method. These results are summarized in the following table:

		t(14;18) PCR Results	
DNA Type	# of specimens	PBSC	CD34+
extracted	9	+	-
extracted	0	+	+
whole cell	3	+	-
whole cell	5	+	+

Although this study was not a direct comparison of the two sample preparation methods, the results indicate that a sensitive method ($>1:10^6$) may be required to assess tumor cell depletion in samples that contain a low level of contamination. This is particularly important when measuring tumor depletion by cell selection systems. It remains to be determined if contamination at these low levels is clinically significant.

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G-CSF INDUCES MOBILIZATION OF BOTH CD34 POSITIVE AND PROLIFERATING CD34 NEGATIVE CELLS IN HEALTHY DONORS.

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G-CSF mobilization kinetics of hemopoietic stem cells in normal peripheral blood is poorly understood and standardization of CD34$^+$ cell content in both blood and apheresis product for allogenic trasplantation is difficult to achieve. We report that G-CSF mobilizes not only CD34-positive cells but also a large number of CD34-negative proliferating cells and a good correlation exists between CD34-positive cells and S-G2M phase cells in peripheral blood. Our group studied the mobilization kinetics of hemapoietic precursors in 20 stem cell donors treated with rhG-CSF (16μg/kg/d x 5-7). Two to four leukaphereses were performed on each donor and the products depleted of T cells by E-rosetting with sheep red blood cells and density gradient separation followed by positive selection for CD34$^+$ cells (CEPRATE system). CD34$^+$ stem cells and S-G2M phase cells were assessed daily during G-CSF treatment by flow cytometry using anti-CD34 (HPCA-2) FICT and 50μg propidium iodide, respectively. Assays were done on daily blood samples during G-CSF treatment and on the apheresis harvest (before and after the different phases of manipulation). The proliferating cells from peripheral blood samples displayed the same behavior as the CD34$^+$ hemopoietic progenitors. There was a positive linear correlation between the total S-G2M phase cells and the CD34$^+$ cells ($r=0.68$; $p<0.0001$). The mean values of both CD34$^+$ and S-G2M phase cells reached the maximum on the 5th day of G-CSF administration (129cells/μl and 874cells/μl, respectively). The apheresis products behaved in a like manner: both CD34$^+$ and proliferating cells achieved their maximum peak on the 5th day of G-CSF stimulation (mean CD34$^+$ cells 326.6×10^6, mean S-G2M phase cells 2.1×10^9). Cell cycle analysis on E-rosete negative mononuclear cell fraction and CD34-positive cell fraction revealed that almost all S-G2M phase cells mobilized in the blood were contained in the CD34 negative fraction, whereas only 2% of CD34$^+$ cells were in S-G2M phase. It appears that in the blood of normal subjects G-CSF induces not only mobilization of early stem cells, but also mobilization of a high number of proliferating cells that have ceased to express the CD34 antigen. We conclude that cytofluorimetric DNA analysis of S-G2M phase cells may be a useful additional assay to quantify the release of stem cells in the peripheral blood and to optimize collection by leukapheresis.

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SELECTION AND TRANSPLANTATION OF AUTOLOGOUS CD34+ PERIPHERAL BLOOD STEM CELLS IN NON-HODGKIN'S LYMPHOMA USING HIGH-SPEED FLUORESCENCE-ACTIVATED CELL SORTING. H.K. Holland, W.H. Fleming, E.K. Waller, D.G. Carnaghan, R.B. Geller, J.R. Wingard, A.M. Vredezer Bone Marrow Transplantation Program and Hemapheresis Center, Emory University School of Medicine, Atlanta, GA.

We report on a pilot study to evaluate hematopoietic reconstitution in non-Hodgkin's lymphoma (NHL) patients undergoing marrow ablative chemotherapy and receiving highly purified autologous CD34+ hematopoietic stem cells (HSC) isolated from peripheral blood stem cells (PBSC) by using high-speed cell sorting with the Becton-Dickinson FACS Vantage apparatus. Eight patients with poor-risk NHL underwent mobilization of PBSC by receiving cyclophosphamide (Cy) and G-CSF. The PBSC were incubated with a biotinylated anti-CD34 monoclonal antibody (Mab) and passed over an avidin (Av) immuno-affinity column to enrich for CD34+ HSC and to deplete red blood cells. The CD34+ enriched cell fraction was reincubated with the biotinylated anti-CD34 Mab and with phycoerythrin (PE)-Av and was processed on the cell sorter at sort rates ranging from 3,000 to 20,000 cells/sec; the sorted cells were then cryopreserved. The median range of CD34+ cell viability, purity and quantity after cell sorting was 96% (88 - 97), 97% (93 - 99) and 1.7 (1.3 - 3.7) x 10^6 cells/kg, respectively. Patients received a preparative regimen of oral busulfan (4 mg/kg/d x 4 days), i.v. Cy (60 mg/kg/d x 2 days) and i.v. VP-16 (10 mg/kg/d x 3), followed by infusion of cryopreserved CD34+ cells. All patients demonstrated prompt neutrophil recovery to $\geq$ 0.5 x 10^9/L at a median of 11 days (10 - 14) and platelet recovery to $\geq$ 20 x 10^9/L at a median of 25 days (19 -164) following transplantation. One patient died of multi-organ failure on day +54. The other patients are alive with sustained hematopoietic engraftment at a follow up of +56 to +339 days. We conclude that high-speed cell sorting for the isolation of putative HSC results in post-transplant hematological reconstitution comparable to that observed after transplantation of autologous CD34+ cells positively selected by the biotin-avidin affinity column method. We are currently evaluating the clinical use of this approach to isolate CD34+ HSC bearing non-malignant immunophenotypes.

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ALLOGENEIC TRANSPLANTATION OF CD34+ SELECTED PERIPHERAL BLOOD PROGENITOR CELLS (PBPC) FROM MATCHED RELATED DONORS. A. Urbano-Ispizua*, C. Rozman, C. Martinez*, L. Briones*, P. Marin*, E. Carreras*, M.C. Viguria*, M. Rovira*, L. Sierra*, R. Mazzara*, and E. Montserrat. Department of Hematology. Hospital Clinic. University of Barcelona, Spain.

An approach to improve clinical results in T- cell depleted allogeneic transplants is to increase the number of progenitor cells infused to the patients. This can be attained by using CD34+ selected G-CSF mobilized PBPC. 14 patients, median age of 38 years (21-51) and diagnoses of CML in 1st CP (n=3), AML in 1st CR (n=4), ALL in 1st CR (n=1), CMML (n=1). MDS (n=3), histiocytosis X (n=1) and CLL (n=1) were conditioned with cyclophosphamide (120 mg/kg) and TBI (15 Gy; 4 fractions). HLA identical sibling donors received G-CSF at 10 μg/kg/day s.c. On day 5 and 6 (13 cases) and day 5, 6, 7 and 8 (1 case) donors underwent 10 L leukapheresis. PBPC were purified by positive selection of CD34+ cells using an immunoadsorption avidin-biotin method (CEPRATE SC), and were infused to the patients as the sole source of progenitor cells. No growth-factors were administered post-transplant. The mean recovery of CD34+ cells after the procedure was of 77%. The median number of CD34+ cells x 10^6/kg before and after the procedure was of 5.2 (1.8-10) and 4 (1.8-6.8), respectively. The number of CD34+ cells x 10^6/kg infused to the patients was: <2 (n=2), 2 to <4 (n=5), and ≥ 4 (n=7), and the median number of CD3+ cells administered was 0.45 x 10^6/kg. Neutrophil recovery >500 and 1,000/μL were achieved at a median of 13 days (11-17) and 15 days (11-27), respectively. Platelets recovered to >20,000 and >50,000/μL at a median of 11 days (6-14) and 16 days (12-36), respectively. GVHD prophylaxis consisted of CsA and prednisone (0.5 mg/kg days 7-14 and 1 mg/kg days 15-28). Acute GVHD was clinical grade 0 (n=10) and I (n=4). One patient presented a cutaneous GVHD at 102 days post-transplant, with an immediate and complete resolution with methylprednisolone. After a median follow-up of 8 months (range 1-16), two patients have relapsed: an ALL case that eventually died and a CML case that is again in cytogenetical remission after donor lymphocytes infusion. Thirteen of the 14 patients are alive and in good clinical condition. In conclusion, this method allows a high CD34+ cell recovery and is associated with rapid engraftment without significant GVHD.

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1707 507-III

RETROVIRAL GENE MARKING TO IDENTIFY THE ORIGIN OF RELAPSE FOLLOWING AUTOLOGOUS CD34 POSITIVE BONE MARROW (BM) AND PERIPHERAL BLOOD (PB) TRANSPLANTATION IN FOLLICULAR NON-HODGKIN'S LYMPHOMA (FNHL). C. Bastier, R. E. Giles*, D. Ellerson*, E. G. Hanania, E. Garcia-Sanchez*, T. Haley*, M. Andreeff, E. Cabanillas, R. Champlin, R. Berenson, S. Heimfeld*, and A. B. Deisseroth. South Texas Cancer Institute, San Antonio, TX, The University of Texas M.D. Anderson Cancer Center, Houston, TX, CellPro, Inc., Bothell, WA, and Yale University School of Medicine, New Haven, CT.

The t(14;18) is present in approximately 80 % of patients (pts.) with FNHL. Studies by Gribben et. al. suggest that pts. undergoing autologous bone marrow transplantation with grafts that are positive for the t(14;18) by the polymerase chain reaction (PCR) have a higher risk of relapse than PCR negative pts. We are conducting a double gene marking trial on pts. with FNHL in sensitive relapse. CD34+ selection using the CellPro Ceprate CD34+ column was performed on PB stem cells obtained after cyclophosphamide/G-CSF mobilization. The t(14;18) was determined prior to and after CD34+ selection using Southern blot analysis of DNA PCR products amplified by nested PCR using primers to the IgH consensus and bcl-2 MBR regions. A fraction of the CD34+ cells was exposed for 4 hours to the LNL5 or G1Na retroviral vector (RV) (Genetic Therapy Inc.) in the absence of growth factors or stromal monolayers. One week later, BM cells were similarly processed. Patients then received TBI (12 Gy), cyclophosphamide (120 mg/kg), and etoposide (1500 mg/kg) followed by infusion of both PB and BM CD34+ cells. Three pts. have been enrolled. Semiquantitative Southern blot analysis of DNA t(14;18) amplification products showed approximately a one log reduction in t(14;18) positive cells after CD34+ selection. Transduction efficiency was determined by BM and PB DNA PCR amplification of neomycin RV sequences and by growth of CFU-GM colonies in G418 selective media. The first patient showed evidence of engraftment with RV transduced BM and PB cells for 9 months. He relapsed one year after transplant. At the time of relapse he lost evidence of RV transduction in ficolled mononuclear BM and PB as well as in CD19/kappa flow cytometry sorted cells. The other 2 pts. are in remission and BM and PB cells from these pts. are still positive for neomycin sequences at 6 months. All 3 pts. showed engraftment of white cells and platelets and no significant transplant related toxicities. Our results demonstrate that CD34+ selection decreases tumor cell contamination by approximately one log. Engraftment of retrovirally transduced hematopoietic cells was documented for up to 9 months. Long-term engraftment of retrovirally transduced cells as well as the contribution of BM and PB contamination to relapse will require longer follow-up in the 2 remaining pts.

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RETROVIRAL TRANSDUCTION OF HUMAN HEMATOPOIETIC PROGENITOR CELLS USING A VECTOR ENCODING A CELL SURFACE MARKER (LNGFR) TO OPTIMIZE TRANSGENE EXPRESSION AND CHARACTERIZE TRANSDUCED CELL POPULATIONS

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Studies of retroviral transduction of hematopoietic progenitor cells have demonstrated significant discrepancies between levels of gene integration (as measured by DNA analysis) and expression (as determined by mRNA and protein assays). To optimize vector design and transduction protocols using protein expression as a readout, as well as to characterize the population of cells successfully transduced and expressing transgene derived protein, we developed a retroviral vector system encoding a cell surface marker, mutated p75 human low affinity Nerve Growth Factor Receptor (LNGFR). This molecule does not bind NGF and is unable to mediate signal transduction, but is recognized by some antibodies against NGFR.

Using a protocol of human peripheral blood CD34+ selection by immunomagnetic beads, 72-96 hour prestimulation in cytokines to induce cell cycling, 24 hour co-culture with viral producer cells and polybrene or protamine sulfate, then subsequent FACS analysis 72-96 hours later, we evaluated various cytokine combinations as support for transduction. Regimens substituting Flk-2 ligand for c-kit ligand (with IL-1 and IL-3 or IL-3 and IL-6) consistantly yielded higher percentages of CD34+ LNGFR- and CD45+LNGFR+ cells after the transduction protocol. Using producer cells providing viral particles with the Gibbon Ape Leukemia Virus envelope (rather than the amphotropic Murine Leukemia Virus envelope) also consistantly improved transduction rates. Up to 35.2% of CD45+ cells and 32.2% of CD34+ cells were positive for expression of the mutant LNGFR by flow cytometry after this protocol (background less than 1%). We have subsequently evaluated the phenotype of the CD34+LNGFR+ cells compared to the CD34+LNGFR- cells as isolated by FACS sorting in human peripheral blood (PB), cord blood (CB), and fetal liver (FL) progenitor CD34+ target populations. The frequency of CFU-GM per 10^3 cells plated is significantly less in the CD34+LNGFR+ fraction relative to the CD34+LNGFR- fraction in all cell types. We then placed these cells into long-term stromal cultures, and performed weekly flow cytometric analysis of suspension cells for LNGFR expression. At 2 weeks post sort (over 3 weeks ex vivo) 91.1% of FL, 82.7% of CB and 42.7% of PB derived cells were LNGFR+. At 6 weeks post sort (over 7 weeks ex vivo) 47.6% of FL, 33.3% of CB and 7.7% of PB derived cells were LNGFR+ (n=3 or more for all). Ongoing studies are evaluating the mechanisms of the progressive decline in gene expression in these cells of different developmental stage. This system, which utilizes a functionally inactive human cell surface marker that is expected to be non-immunogenic, is useful to rapidly purify transduced CD34+ cells for in vitro study and potentially for expansion/infusion in a clinical setting.

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LONG-TERM OUTCOME OF A PHASE II STUDY OF AUTOLOGOUS CD34+ PERIPHERAL BLOOD STEM CELL TRANSPLANTATION AS TREATMENT FOR MULTIPLE MYELOMA.

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High-dose chemo- radiotherapy followed by transplantation of autologous bone marrow or unpurged progenitor cells produces progression-free survival of 40-60% at 1 year, but few studies present long-term results. In this report we present results of 51 pts with advanced chemotherapy-responsive myeloma age 34-69 yrs (median 52 yrs) who were enrolled on a trial of CD34+ PBPC transplantation following high-dose chemotherapy. The median time from diagnosis to Tx was 9.1 mos. (range 4-47 mos.). At Tx, paraproteinemia ± marrow plasmacytosis and extensive bone lesions were identified in all pts. Progenitor cells were harvested 10-14 days after cyclophosphamide (2.5 gm/m^2 IV), prednisone (2 mg/kg/d x4d), and G-CSF (10 µg/kg subQ qd until last day of leukapheresis). Leukapheresis and immunoadsorption with the CEPRATE system were performed as previously described. CD34+ selected cells infused contained a median of 7.02 x10^6 cells/kg (range 1.57-35.3) and reduced tumor contamination by >2.7->4.5 logs as determined by a quantitative PCR assay using patient-specific Ig gene primers. Cells were infused one day after completing preparative conditioning with busulfan (.875 mg/kg q6h x16 doses) and cyclophosphamide (60 mg/kg/day x2). Following infusion GM-CSF (500 µg IVPB) was given daily. Patients received α-interferon and/or dexamethasone maintenance. 36 pts (71%) achieved a complete or partial response. After a median follow-up of 28 mos. (range 2-36+ mos.) 19 pts (37%) have evidence of disease progression for a 3-year actuarial risk of relapse of 63 ± 20%. Progression-free and overall survival are 34 ±15% and 57 ±16%, respectively. In conclusion, CD34+ PBPC are an effective form of purified hematopoietic support for pts with multiple myeloma undergoing myeloablative chemotherapy producing prolonged survival but no plateau on progression-free survival.

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1718 518-III

EFFICIENT GENE TRANSFER INTO PRIMITIVE HUMAN HEMATOPOIETIC PROGENITOR CELLS BY A DEFINED, HIGH TITER, NON-CONCENTRATED VECTOR CONTAINING MEDIUM PRODUCED UNDER SERUM-FREE CONDITIONS. H. Glimm,* D. Möbest,* V.M. Hofmann,* J. Henschler,* H.P. Kiem,* W. Lange,* S. Merkelsmann, C.v. Kalle.*
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Defined serum-free conditions would have great advantages for the biological safety and standardization of clinical gene transfer into hematopoietic stem cells. In the only study reported to date, Sekhar et al. achieved low serum conditions by a complex concentration procedure combining tangential flow filtration, polyethylene-glycol-precipitation and ultracentrifugation of a retroviral supernatant with 10 % FCS (HGT, 7: 33 - 38, 1996). The high cost, small volume, possible co-enrichment of serum-derived pathogens, limited recovery of vector particles and low titer of the final diluted medium restrict the clinical application of this procedure. In the present study, we can demonstrate efficient transduction of long-term culture-initiating cells (LTC-IC) and extended (E) LTC-IC with a high titer, completely serum-free medium containing PG13/LN. Serum-free PG13/LN retroviral medium was harvested from a confluent PG13/LN producer cell layer without requiring a physical enrichment procedure. On HeLa cells, an average titer of 4.8×10^5 cfu/ml was achieved under serum-free conditions as compared to 7.6×10^5 cfu/ml under standard conditions containing 10 % FCS. $CD34^+/HLA\text{-}DR^{lo}$ peripheral blood progenitor cells were obtained by FACS sorting 10% of CD34 antigen enriched cells (Ceprate LC stem cell concentrator, CellPro, Bothell, WA) with the lowest HLA-DR antigen expression. The target cells were set up in serum-free vector containing medium at 1×10^6 cells/ml in the presence of IL-3, SCF (100 ng/ml each) and flt-3 ligand (300 ng/ml). 50 % of vector medium with growth factors was exchanged daily for 7 days. Cells were transferred onto irradiated allogeneic stroma and maintained 5 weeks for the detection of LTC-IC and 9 weeks for the detection of ELTC-IC before plating into a clonogenic assay with and without 1.5 mg/ml G418. Total cell number and LTC-IC content of $CD34^+$ and $CD34^+/HLA\text{-}DR^{lo}$ cells increased during the transduction period ($CD34^+$ cells: total cell number 3.4 ± 2.2 fold expansion, LTC-IC number 3.9 ± 2.3 fold expansion; $CD34^+/HLA\text{-}DR^{lo}$ cells: total cell number 1.6 ± 0.2 fold expansion, LTC-IC number 2.7 ± 2.4 fold expansion). The average transduction efficiency in LTC-IC colonies generated from $CD34^+/HLA\text{-}DR^{lo}$ cells was 57 ± 10.3 %. In one experiment, efficient transduction (57.1 %) of ELTC-IC colonies could be demonstrated as well. The described procedure allows the transduction of very primitive hematopoietic cells. The defined high titer serum-free vector containing medium can be produced exclusively from pharmaceutical grade components, making it ideally suited for applications in clinical gene therapy.

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CO-TRANSDUCTION OF H-RAS AND ERYTHROPOIETIN RECEPTOR cDNAs INTO SINGLE ISOLATED CD34^{-} CORD BLOOD CELLS BY RETROVIRAL MEDIATED GENE TRANSFER ENHANCES PROLIFERATION OF ERYTHROID AND MULTIPOTENTIAL PROGENITORS.

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In the erythroid signal transduction pathway, erythropoietin (Epo) induces Raf-1 and p21^{ras} activation. To evaluate the link between ras and the Epo receptor (R) in primary progenitor cells, we constructed retroviral vectors containing the H-ras cDNA and evaluated if the increased numbers of BFU-E in CD34^{-} cord blood (CB) cells transduced with human EpoR (Lu et al. Blood 87:525, 1996) could be influenced by co-transduction of H-ras. Highly purified single sorted CD34^{-} cells from human CB were prestimulated with cytokines and incubated with viral supernatant containing EpoR gene and/or H-ras gene, and assayed for colony formation in the presence of steel factor, IL-3, GM-CSF and Epo. In response to stimulation by these cytokines, colony formation by BFU-E and CFU-GEMM was significantly increased in cells transduced with H-ras (5.3% and 8.4% cloning efficiency, CE) or EpoR (10.2% and 11% CE) genes, respectively, compared to mock-transduced cells (2.7% CE). Increases in CE by BFU-E was additive (14.5% CE) when both genes were introduced simultaneously into CD34^{-} cells. No further enhancement (12.2%) was seen in CFU-GEMM colony numbers after transduction of progenitors with both genes. The size of all colonies from progenitors transduced with both genes was increased and the greatest increase was obtained from cells transduced with both genes. Integration and expression of either gene as assessed by PCR and RT-PCR analysis was 60 and 52%, respectively, with approximately 31% of the cells containing and expressing both genes. These results demonstrate the interacting roles of H-ras and EpoR in the erythroid differentiation of primary progenitor cells.

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1670 470-III

DO INDIVIDUAL GRAFT PARAMETERS INFLUENCE ENGRAFTMENT OR GVHD IN PATIENTS RECEIVING ELUTRIATED/ CD34+ AUGMENTED ALLOGRAFTS? P. V. O'Donnell*, S. J. Noga, G.B. Vogelsang, A. Seber*, K. Schepers*, J.M.Davis*, and R.J. Jones. Johns Hopkins Oncology Center, Baltimore, MD.

We have previously used elutriated, lymphocyte dose modified (LDM) bone marrow (BM) grafts to support high dose chemotherapy for hematologic malignancies. Engraftment was somewhat delayed compared to unmanipulated grafts with median times to granulocyte and platelet recovery of 18-21 days (D) and 29-41 D respectively. The graft failure rate was 4%. The incidence of acute GVHD ranged from 30-65% depending on the lymphocyte dose used. It is now recognized that the lymphocyte-rich small cell fractions excluded in the initial studies contained 70% of the BM CD34+ cells and that the primitive progenitor cells may also reside here. Consequently, a phase I/II trial augmenting the elutriated graft with salvaged CD34+-selected cells from these lymphocyte-rich fractions was conducted to determine if engraftment kinetics could be improved without affecting the incidence of GVHD. A total of 104 evaluable patients (median, 42 yrs) were transplanted between 1993 and 1996. The composite engineered graft contained 4.2(1.7,S.D.)x10^7 nucleated cells/kg, 1.5(0.9)x10^5 CFU-GM/Kg, 3.2(1.1)x10^6 CD34+ cells/kg and 3.8(3.3)x10^8 lymphocytes/kg. Individual graft parameters were paired with the clinical endpoints for each patient. The median time to an ANC >500 was 16D. Three patients (3%) failed to engraft. There was no correlation of cell dose (CFU-GM: 0.2-5.1x10^5, CD34+: 1.1-7.2x10^6) to length of neutropenia. Similarly, there was no correlation with platelet independence (>50k) which was achieved by 24 days (median). Eight patients had prolonged engraftment times (>45D) that were associated with previous alloimmunization. There was no CD34+ cell dose, below which engraftment was delayed or graft failure ensued. The incidence of acute GVHD was inversely related to the duration of cyclosporine A (CSA) prophylaxis (30D vs 90D). Overall incidence was 32% (12% >clinical stage 1) comparable to previous studies of elutriated patients receiving 180 days of CSA. The incidence of GVHD was 50% (23% >stage1) with CSA: 30 days compared to 20% (4%>stage1) with CSA: 80 days. We conclude that elutriation combined with CD34+ augmentation reproducibly delivers a high quality allograft. Currently recognized parameters of graft "stemness" are tightly controlled resulting in low post-tranplant outcome complications.

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1611 411-III

TRANSPLANTATION WITH CD34+ AUTOLOGOUS PERIPHERAL BLOOD PROGENITOR CELL (PBPC) MOBILIZED WITH G-CSF ALONE IN HIGH-RISK MULTIPLE MYELOMA (MM): ONE-CENTER STUDY IN 17 PATIENTS (PTS).

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In pts with MM, PBPC are usually mobilized with high-dose cyclophosphamide plus G-CSF, especially when CD34+ selection is planned. In order to decrease this procedure-related toxicity and cost, we have tried to select CD34+ cells from PBPC mobilized with G-CSF alone. In a feasibility study, we have demonstrated that in pts with MM, G-CSF alone was effective to collect PBPC in quantity sufficient for positive CD34 selection only in newly diagnosed slightly pretreated pts (Mahé et al, Br J Haematol 1996;92:263). We here report the results of such procedure in 17 consecutive pts with high-risk newly diagnosed MM, allowing subsequent autologous stem cell transplantation (ASCT). They were 7 males and 10 females patients with a median age of 53 years (37-65). PBPC were harvested after 1 to 3 courses (median 2) of VAD chemotherapy regimen. At time of stem cell collection, 16/17 patients were in first partial response and 1 patient was refractory to VAD. Leukapheresis were performed in steady-state hematopoiesis on days 5, 6, 7 after the beginning of 10 µg/kg G-CSF daily administration. The first two leukapheresis products were subjected to positive selection of CD34+ cells (CEPRATE stem cell concentrator system, Cell Pro Inc: Bothell Wash.). A median number of $7x10^6$ CD34+ cells/kg (2.4-28.4) were harvested with two leukapheresis. After positive selection, the grafts contained a median number of $3.2x10^6$ CD34+ cells/kg (0.7-14.6).The median purity was 79% (63-90). Thus ASCT was feasible for all pts. The conditioning regimen consisted in Melphalan alone (200 mg/m^2) (n=5) or Melphalan (140 mg/m^2) in combination with a fractionated 8 Gy total body irradiation (n=12). All patients received G-CSF (5µg/kg/d), from day 7 after transplantation until granulocyte recovery. The median time to granulocyte (> 0.5 10^9/L) and platelet (> 20 10^9/L) engraftment were 12 (9-13) and 12 (5-38) days respectively. No toxic death was observed. No late graft failure occured.

Our study provides further evidance that G-CSF alone mobilizes easily sufficient peripheral blood CD34+ cells for positive selection in pts with de novo MM, and is a good alternative to cyclophosphamide + G-CSF priming. This procedure allows rapid and sustained reconstitution of hematopoiesis after myeloablative therapy.

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1571 371-III

A COMPARISON OF ESHAP + G-CSF VS CYCLOPHOSPHAMIDE 1.5g/m² + G-CSF FOR PBSC MOBILISATION IN PRE-TREATED LYMPHOMA PATIENTS: A MATCHED PAIR ANALYSIS. Watts MJ,* Leyvraz O,* Sullivan AM,* Perriver AJ,* Perry AR,* Macmillan A,* Goldstone AH, Linch DC.* Department of Haematology, University College, London.

Cyclophosphamide 1.5 g/m² and G-CSF is an effective PBSC mobilisation regimen in patients previously treated for lymphoma. In a consecutive series of 250 lymphoma patients (Hodgkins disease, low grade NHL and high grade NHL) mobilised with this regimen 91% had an adequate harvest defined as >1 x 10^6/kg CD34+ cells. However if stem cells are to be purified at least 2 x 10^6/kg CD34+ cells are required to allow for the losses associated with purification. Furthermore in 50 CD34+ cell purifications using the CEPRATE® column we have found that final purity of CD34+ cells is related to the percentage of CD34+ cells in the harvest material and a CD34+ cell purity of ≥ 1% is required to ensure good purity (median purity 71%, range 47 - 93%). Using the cyclophosphamide and G-CSF mobilisation regimen only 37% of lymphoma patients achieved both of these thresholds with a single apheresis which is desirable for maximal cost efficiency. Furthermore, cyclophosphamide 1.5 g/m² has only limited anti-lymphoma activity and is not ideal when tumour reduction as well as PBSC mobilisation is required. The mini-BEAM regimen is an effective lymphoma salvage regime but is stem cell toxic and a poor mobiliser. We have therefore evaluated the ESHAP regimen, one of the most effective conventional dose lymphoma salvage regimens for stem cell mobilisation. Thirty three lymphoma patients were mobilised with ESHAP harvesting on a rising white blood cell count, with apheresis typically beginning on day 15. The mobilisation results on the first day of harvesting were compared with 33 patients mobilised with cyclophosphamide 1.5 g/m² + G-CSF who were matched from the larger population database for type of lymphoma, whether or not they had received prior radiotherapy or prior BEAM / mini-BEAM therapy and number of previous cycles of therapy, with apheresis performed on the first day the post nadir wbc exceeded 5 x 10^9/l. The median number of MNC collected in a single apheresis was 2.1 x 10^8/kg with ESHAP mobilisation compared to 2.9 x 10^8/kg in the matched cyclophosphamide / G-CSF controls (p = 0.003). The number of CD34+ cells mobilised with ESHAP was greater than with cyclophosphamide and G-CSF at 4.7 x 10^6/kg and 2.9 x 10^6/kg respectively (p =0.07), as was the percentage of CD34+ cells in the apheresis product (2.4% vs 1.1% respectively p = 0.0003). In the matched cyclophosphamide and G-CSF group 48% of patients failed to achieve both the minimal CD34 dose and CD34% thresholds compared to 33% in the ESHAP group. ESHAP is thus a highly effective mobilisation regimen which is preferable to cyclophosphamide 1.5 g/m² + G-CSF if anti lymphoma activity is required from the mobilisation regimen or CD34+ cell purification is intended.

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1458 258-III

SUPRAPHYSIOLOGICAL CONCENTRATIONS OF ALL TRANS RETINOIC ACID STIMULATES GROWTH OF CFU-GM PROGENITORS INDUCED BY IL3 OR GM-CSF BUT NOT BY G-CSF FROM NORMAL HUMAN CD34+ CELLS D. Douer, L. Ramezani*, A.M. Preisler. Division of Hematology, University of Southern California School of Medicine, Los Angeles, California

Pharmacological concentrations of all-trans retinoic acid (TRA) induces differentiation in vitro and in vivo of acute promyelocytic leukemia cells that harbor one rearranged retinoic acid receptor (RARα) gene. Studies with normal murine cells indicate that RARα in conjunction with GM-CSF plays a role in the terminal differentiation of normal neutrophil precursors (PNAS 90:7153, 1993). We have previously shown that TRA stimulates the in vitro growth of normal human CFU-GM colonies from bone marrow (BM) cells (Exp.Cell Res 138:139,1982). To further examine the effect of pharmacological concentrations of TRA (0.3μM) on normal human BM CFU-GM, we used CD34+ cells enriched by an avidin-biotin immunoabsorption column (CellPro) induced by different cytokines. TRA continuously present in the semisolid cultures, increased the number (mean±SD) of day 14 CFU-GM colonies per 10^5 BM cells induced by 100u/ml IL3 from 68±4 (no TRA) to 203±136 (3.8 fold; p=0.002) or by 50ng/ml GM-CSF from 199±136 to 352±206 (2.2 fold p=0.03). The increase in CFU-GM by TRA with was statistically significant greater with IL3 than with GM-CSF (p=0.05). TRA had no effect on CFU-GM induced by G-CSF, 201±163 colonies (no TRA) and 215± 151 (with TRA). TRA without a growth factor or with IL-6 did not induce CFU-GM growth. TRA increased the number of IL3-induced CFU-GM from CD34+38+ cells separated by flow cytometry by 6 fold, but not from CD34+CD38- subset. In time-response experiments, TRA was added on different days (1-13) after the start of IL3 or GM-CSF induced CFU-GM cultures. The increase in CFU-GM peaked when TRA was added on day 1 and then gradually declined. TRA added after day 9 had no stimulatory effect. Time-response experiments had no effect on G-CSF induced CFU-GM. Further separation of column-enriched CD34+ cells by flow cytometry to >95% purity showed that the increment of IL3 -induced CFU-GM by TRA was higher than cells purified to a lesser degree (50-75%) by the column. In summary 1) Supraphysiological concentrations of TRA can increase the in vitro growth of normal human myeloid progenitors having normal RARα genes. 2) TRA acts directly on the CD34+ cell without accessory cells but requires IL3 or GM-CSF. 3) TRA does not increase CFU-GM induced by G-CSF or when added after 9 days to semisolid culture induced by IL3 or GM-CSF, suggesting that normal myeloid progenitors may lose their stimulatory response to TRA in the process of differentiation. 4) APL patients treated by TRA often have an early rise of the differentiating leukemic cells, followed by decline in blood counts. We hypothesize that our results with normal myeloid progenitors may have relevance in understanding this clinical reaction of the APL clone to TRA.

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Promegapoietin, an engineered chimeric growth factor for platelet producing cells. J.G. Giri,* L.E. Kahn,* P.D. Doshi, N.L. Minster,* P.R. Streeter,* L.E. Pegg,* J.W. Thomas,* A.L. Abegg,* J.P. Favara,* N.R. Staten,* M.S. Huynh,* J.B. Monahan,* D.C. Wood,* B.L. Summers,* D. Villani-Price,* A.M. Farese, T.J. MacVittie, W.G. Smith,* and J.P. McKearn.* Searle Research & Development, Monsanto Co., St. Louis, MO University of Maryland, Baltimore, MD

Promegapoietin (PMP) is a novel engineered chimeric hematopoietic growth factor designed to bind with high affinity to human IL-3 and c-mpl receptors. In a cell line TF1.2.B4 responsive to both IL-3 and c-mpl ligand. PMP activates both receptor complexes and induces signaling events such as tyrosine phosphorylation of receptors, JAKs and STATs representing a combination of the effects of both c-mpl and IL-3 receptor agonists. In cultures of human bone marrow-derived CD34+ cells, PMP stimulated multilineage expansion and a specific expansion and differentiation of cells of the megakaryocytic lineage (MK). In the presence of c-mpl ligand CD34+ enriched cells cultured in vitro differentiated to mature CD41+ megakaryocytic phenotype, but there was only very modest cell expansion. PMP by contrast, induced substantial expansion in the total number of cells as well as CD41 expressing MK compared to either the c-mpl or IL-3 receptor agonists alone. Cellular morphology, ploidy and colony assays indicated that PMP expands less mature cells of the MK lineage, and in particular, a larger number of early BFU-MK colonies were observed with PMP. These properties may have unique advantages for ex vivo expansion of MK and myeloid progenitors. In preliminary experiments with bone marrow derived CD34+ cells, a 24-fold increase in total nucleated cells was observed with PMP of which approximately 44% expressed CD41 after 12 days of culture. PMP was also shown to enhance platelet production in vivo: a 2 to 4-fold increase in platelet numbers was seen in normal primates. In a radiation induced primate myelosuppression model PMP mitigated the nadir in platelet numbers (platelets maintained above 68,000/µl compared to 5,000/µl in animals that did not receive growth factor), and also enhanced neutrophil recovery. These results suggest that PMP, due to its combined early lineage and MK growth and differentiation activities, is a promising platelet restorative factor for thrombocytopenia created by myelosuppression and will have utility for ex vivo expansion of MK progenitors for transplantation.

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GENE MARKING STUDIES INDICATE THAT EX-VIVO EXPANSION OF MOBILIZED RHESUS PERIPHERAL BLOOD CELLS RESULTS IN RAPID INITIAL ENGRAFTMENT BUT DIMINISHED LONG TERM REPOPULATING ABILITY. J.F. Tisdale, S.E. Sellers,* B. A. Agricola,* R.E. Donahue, C.E. Dunbar. Hematology Branch, NHLBI, NIH, Bethesda, MD.

The ex vivo expansion of primitive hematopoietic cells is an area of intense interest for gene therapy and transplantation applications. Expanded peripheral blood (PB) progenitors have been used clinically (NEJM 333:283,1995); however, the long term engraftment potential of expanded cells remains uncertain. To address this question, gene marking technology was used to allow tracking of both expanded and non-expanded rhesus monkey PB progenitors in the autologous transplantation setting. SCF/G-CSF-mobilized rhesus PB cells were collected by apheresis and enriched for primitive progenitors by CD34 selection. The CD34+ cells from each animal were split into 2 equal aliquots and were transduced with either LNL6 or G1Na Neomycin-resistance gene marking retroviral vectors (with equivalent titers of 5 X 10^6/ml) using a standard 4 day supernatant transduction in the presence of IL3, IL6, and SCF. At the end of transduction, one aliquot was frozen, while the other was expanded in the continued presence of IL-3, IL-6, and SCF for a total of 10-14 days. At the end of this expansion period, the second aliquot was also frozen. The animals then received 650 cGy of total body irradiation x2. The following day, both expanded and non-expanded transduced cells were reinfused. In the first two animals, there was a 40 fold expansion of total nucleated cells over 10 days, and engraftment (ANC>500/mm^3) occurred at day 5 (compared to day 12 for historical controls transplanted with transduced but non-expanded G-CSF/SCF mobilized peripheral blood CD34+ cells). Semiquantitative PCR for the Neo gene demonstrated an equal contribution towards short term engraftment (weeks 1 and 2) by both the G1Na (nonexpanded) and the LNL6 (expanded) marked cells; however, by week 6, the signal from the expanded cells had fallen to below the limit of detection (<0.01%) with a stable signal persisting from only the nonexpanded population at >12 weeks. Two subsequent animals were transplanted with a 14-day expanded cell population marked with G1Na and a non-expanded population marked with LNL6. In one animal, at 2 weeks there was equal contribution of expanded and non-expanded cells, but by week 3 only the non-expanded signal was detectable. The other animal mobilized poorly and received a very low dose of the non-expanded CD34+ cells. Despite an initial rise in neutrophils marked by G1Na (expanded cells) at week 2, the animal was euthanized at day 38 for graft failure. These results suggest that ex-vivo expansion of peripheral blood progenitors leads to prompt initial engraftment but impaired long term repopulating ability.

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FLT3 DEPENDENT HUMAN PERIPHERAL BLOOD (PB) LONG TERM CULTURE INITIATING CELL (LTCIC) EXPANSION LEADS TO RETROVIRAL TRANSDUCTION WITH MUTANT MGMT AND RESISTANCE TO O6-BENZYLGUANINE & BCNU. Q.N. Kan, J.S.Reese*, E.M. Szekely*, K.M. Lee*, S.L. Gerson. Department of Medicine and CWRU Ireland Cancer Center, Univ. Hosp. of Cleveland and Case Western Reserve University, Cleveland OH.

Human hematopoietic stem cells express low levels of the DNA repair protein O6-alkylguanine-DNA alkyltransferase (AGT) and are expected to be markedly sensitized to BCNU following AGT inhibition by O6-benzylguanine (BG). BG reverses BCNU resistance of cancer cells in pre-clinical studies but cumulative and prolonged myelosuppression is expected to be dose limiting in current phase I trials. We have shown that MFG retroviral transduction of human committed progenitors by mutant MGMT containing a glycine to alanine mutation at position 156 (ΔMGMT) results in significant resistance to BG/BCNU (Reese *et.al.*Proc. Natl. Acad. Sci. USA, In Press 1996). In this study we evaluated ΔMGMT gene transfer into LTCICs which appear to be the target for BCNU toxicity resulting in prolonged myelosupression. Recent studies have shown that cytokine combinations containing FLT3 expand LTCICs *in vitro*, which should facilitate gene transfer into these cells. Therefore we used G-CSF mobilized human PB derived CD34 cells [Ceprate LC. CellPro] containing 0.1-0.2% LTCICs (by limiting dilution) and cultured them in the presence of FLT3 (50ng/ml) [Immunex], hIL3 (100U/ml), hIL6 (50U/ml) [Sandoz] and hSCF (100ng/ml) [Amgen], which resulted in 3.3-5.2 fold LTCIC expansion on day 3 and 120-380 fold LTCIC expansion on day 10. CD34 cells were cocultured on Am12-ΔMGMT-MFG retroviral producers for the first 4 days and exposed to 10μM BG followed by 0-10 μM BCNU for 2h and cultured on irradiated allogeneic human marrow stroma for 5 weeks. Individual secondary CFUs analyzed for ΔMGMT gene transfer showed that all colonies (23/23) from BCNU treated cultures and 58% of the colonies from untreated cultures contained provirus, suggesting that LTCICs are susceptible to retroviral infection under these culture conditions. Furthermore, 3.6% of ΔMGMT transduced LTCICs survived BG/BCNU compared to none of the lacZ transduced LTCICs. When compared to committed progenitors, LTCICs appear to be more sensitive to BG/BCNU (30% vs 3.3% survival at 10μM/10μM) which might reflect the effects of partially repaired DNA damage on stem cell decisions regarding quiescence, apoptosis or commitment over the 5 week culture period. This study demonstrates that human PB LTCICs are highly sensitive to BG/BCNU, providing further evidence for anticipated clinical toxicity of prolonged myelosupression. In summary, FLT3 dependent culture expansion of human PB CD34 cells leads to retroviral ΔMGMT transduction of LTCICs, resulting in resistance against BG/BCNU, a strategy which may have clinical utility in protection from BG/BCNU induced myelosupression.

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HUMAN CD34 STEM CELLS EXHIBIT POTENT VETO ACTIVITY IN VITRO: RELEVANCE TO 'MEGA DOSE' STEM CELL TRANSPLANTS IN MISMATCHED LEUKEMIA PATIENTS

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Graft-versus-host disease (GVHD) is uniformly lethal in recipients of HLA-mismatched marrow. In SCID patients, this major obstacle can be overcome by rigorous T-cell depletion prior to transplantation. In leukemia patients, however,the benefit of preventing GVHD is offset by graft rejection or graft failure. Recently this problem was overcome by supplementing T cell-depleted bone marrow transplants with mega-doses of peripheral blood stem cells collected by leukapheresis after mobilization of the donor stem cells with granulocyte colony-stimulating factor (G-CSF). Based on the above study we further investigated and demonstrated in a mouse model (C57BL/6->C3H/HeJ) that escalation of bone marrow doses by 4-5 fold leads to full donor type chimerism in sublethally irradiated (6.5 Gy) recipients. The marked mixed chimerism found within the spleen T cell compartment of long term chimera was associated with specific tolerance for donor type skin grafts and with a profound elimination of CTL-P against donor but not against third party antigens, indicating that the substantial number of host type T cells present in these mice must be specifically tolerized towards donor type antigens. The ability of the mega dose transplants to overcome the marked resistance of the host immunity surviving the preparative protocols could be mediated by veto cells present in transplant innoculum . However, more recently we have found that further purification of the mega dose T cell depleted transplants by positive selection of CD34 cells did not reduce engraftment rate in mismatched leukemia patients (23/24) , thus indicating that PBMC CD34 cells might posses veto activity. In the present study, we demonstrate by limit dilution of CTL-P in human PBL that indeed the addition of purified human CD34 cells to the primary mixed lymphocyte culture leads to marked abrogation of CTL-P frequency against irradiated PBL stimulators when the later were collected from the stem cell donor, but not when they were of a third party origin. Irradiated (30 Gy), or membrane separated (transwell culture system) CD34 stem cells did not exhibit inhibitory effect. FACS analysis of the purified CD34 cells showed that these cells are highly positive for HLA DR and negative for the costimulatory molecule B7. It is possible, therefore, that CD34 cells in the 'megadose' transplants - perhaps by their inability to provide costimularity molecules - are actively reducing the frequency of CTL-P directed against their antigens and thereby help to overcome allogeneic rejection, and enhance their own engraftment.

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GENETIC MODIFICATION OF CELLS USED FOR TRANSPLANT FOLLOWING INTENSIVE THERAPY FOR OVARIAN CANCER AND BREAST CANCER. I. Kavanagh,* E. Hanania,* R. Giles,* S.O. Fu,* Z. Zu,* D. Ellerson,* T. Wang,* D. Claxton, Z. Rahman, R. Berenson,* S. Heimfeld,* R. Cote,* T. Holzmayer,* E. Mechetner,* A. Dayne,* M. Andreeff, R. Champlin, and A.B. Deisseroth. U.T. M.D. Anderson Cancer Center, Houston, TX. CellPro, Inc., Bothell, WA, Systemix, Inc., Palo Alto, CA, Kenneth Norris Jr. Cancer Hospital, Los Angeles, CA, Ingenex, Inc., Menlo Park, CA, and Yale University School of Medicine, New Haven, CT.

Peripheral blood or marrow cells were collected from patients with advanced carcinomas of the breast or ovary, and then selected with a CD34 monoclonal antibody column. We then incubated the CD34+ cells with a MDR-1 vector under two conditions: 1. suspension in retroviral supernatant for 4 hours, and 2. inoculation on stromal monolayers for 2 days in the presence of IL3 and IL6. The goal of the MDR-1 modification was to make safe the post transplant administration of taxol chemotherapy. Eight of the 20 patients reached a complete response after transplant and nine of the twenty reached a partial response after transplant. The median progression-free interval was 10 months, and the complete responses have lasted 16.5+, 15.8+, 15.8+, 11.0, 10.5+, 10.5+, 10.0+, 10.0, and 8.5 months. Following transplant, 0/10 of the patients transplanted with suspension transduced cells had MDR-1 modified cells post transplant, whereas 5/8 of the evaluable patients transplanted with the stromally transduced cells had MDR-1 vector positive cells post transplant. In situ PCR showed that up to 3-7 percent of the cells were positive for the vector MDR-1 transgene following the transplant. Solution PCR analysis prior to the transplant showed that the transduction frequency, which ranged from 1-20%, was not different for the CFUGM derived from the solution and the stromally transduced cells. This data indicate that it is possible to modify sufficient numbers of the CD34 selected cells to repopulate lethally-irradiated human recipients with the MDR-1 genetically-modified cells, when the stromal but not solution transduction method is used to introduce the MDR-1 vector transcription unit into the CD34 selected cells.

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PERSISTENCE OF ENGRAFTMENT AND DOCUMENTATION OF DONOR DERIVED CD34+ CD38- CELLS IN THE RECIPIENT BONE MARROW AFTER IN UTERO TRANSPLANTATION FOR X-LINKED SEVERE COMBINED IMMUNODEFICIENCY. A.W. Flake*, M.G. Roncarolo, J.M. Puck, G. Almeida-Porada and E.D. Zanjani. Dept. Of Surgery, Children's Hospital of Philadelphia, Philadelphia, PA; DNAX, Palo Alto, CA; Natl. Center for Human Genome Research/NIH, Bethesda, MD; VA Medical Center, Univ. Nevada, Reno, NV.

We recently reported the successful treatment of a fetus with X-SCID by in utero transplantation of CD34 enriched paternal BM. Analysis after birth revealed "split" chimerism with all of the patients T-cells being donor in origin and all other lineages host in origin. The peripheral expression of only T-cells could represent persistence of committed donor lymphoid progenitors, or selective expression of engrafted donor multipotent hematopoietic stem cells (HSC). The former circumstance would raise concern about the permanence of engraftment, as well as the applicability of in utero HSC transplantation to hematopoietic diseases lacking the selective advantage for normal lymphoid progenitors present in X-SCID. We report here the clinical status of the patient at 13 months of age (18 months after transplantation), as well as evidence for the engraftment and persistence of a donor derived HSC candidate population in patient's BM. The fetus had received a series of three ultrasound guided IP transplants (1.14×10^6, 8.9×10^6, and 6.2×10^6 cells/kg) of CD34-enriched paternal BM at 16, 17.5, and 18.5 weeks gestation. Postnatal analysis revealed a pattern of "split" chimerism with essentially all of the patients T-cells being of donor origin, and all B-cells, monocytes, and NK cells being of host origin. The patient is now 13 months old, clinically healthy with normal growth and development and no significant infections. The pattern of "split" chimerism has persisted. His cell counts and lymphocyte subsets are completely normal. T-cell function as assessed by non-specific mitogen response as well as response to IL2 and anti-CD3 antibody is normal. He has evidence of immunoglobulin class switching and has measurable titers of IgG to tetanus, diptheria, and hemophilus after three vaccinations. Assessment of tolerance by MLR shows unequivocal evidence of donor specific tolerance. To assess the engraftment of a donor derived "HSC candidate" population, BM was obtained at 3 months of age. Analysis by flow cytometry revealed 3% of CD34+ and 17% of CD34+/CD38- BM cells to be donor in origin. This strongly supports the engraftment and persistence of donor HSC and suggests that there are unique features of the X-SCID hematopoietic environment which regulate and restrict differentiation and peripheral expression of donor cells. We conclude that the in utero transplantation of enriched adult BM can successfully treat X-SCID and that selective peripheral expression of T-cells is secondary to regulatory events in the X-SCID hematopoietic environment. Although X-SCID offers a selective advantage for normal versus γc deficient lymphocyte progenitors, not present in most hematologic diseases, the engraftment of a donor derived CD34+/CD38- population supports the cautious application of in utero transplantation to other hematopoietic diseases.

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1010 453-II

QUALITY OF IL-3 AND G-CSF MOBILIZED STEM CELLS IN PATIENTS WITH EARLY CHRONIC PHASE CML. M.Heinzinger,* C.Waller*, S.Scheid,* R.Mertelsmann, W.Lange. Department of Medicine, Hematology/Oncology, University Medical Center Freiburg and Department of Biology, University Freiburg, Germany

Chronic myelogenous leukemia (CML) is a clonal myeloproliferative disorder of the hematopoietic stem cell. The occurrence of the Philadelphia chromosome (Ph^+), t(9; 22), is characteristic in more than 95% of CML patients. As most of the CD34 positive, HLA-DR negative cells are Ph^+ negative, it should be possible to separate mainly Ph^+ negative hematopoetic stem cells by the lack of HLA-DR antigen expression. Leukocyte count and CD34 expression of eight patients treated with IL-3 and G-CSF were analyzed at different time points during mobilization after modified ICE chemotherapy. Collection of peripheral blood progenitor cells (PBPC) started when CD34+ cells ≥1% and leukocyte counts ≥500/µl were reached. HLA-DR negative and CD34 positive cells were purified by immunoaffinity and magnetic bead separation. Successful expansion of progenitor cells could be demonstrated in some of the patiens using IMDM medium supplemented with IL-1, IL-3, IL-6, stem cell factor (SCF) and erythropoietin. The quality of cells after CD34 positive and HLA-DR negative separation techniques and subsequent expansion was evaluated by fluorescence in situ hybridisation (FISH) and RT-PCR. Before mobilization chemotherapy peripheral blood leukocytes of all patients was Ph^+ positive as analyzed by FISH (range 6-60%) and RT-PCR. Leukapheresis products and selected progenitor cells after a first (usually CD34+) and a second (usually HLA-DR-) column purification and after 14 days of expansion were below 5% Ph^+ positive cells, our laboratory's threshold of detection for FISH analyses. A substantial number of samples also tested negative for bcr:abl rearrangement by RT-PCR. Our data show, that it is possible to mobilize Ph^+ negative PBPC during the early phase of hematopoetic recovery after ICE chemotherapy and priming with simultaneous IL-3 and G-CSF.

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T CELL DEPLETION OF G-CSF MOBILIZED PERIPHERAL BLOOD PROGENITOR CELLS FROM NORMAL DONORS. D. Mavroudis, S.J. Read, F. Viens*, O. Chau*, C. Cirrone*, S. Leitman, J. Molldrem*, M. Emde*, R. Bernstein*, G. Risdon*, K. Anderson-Hargreaves*, A.J. Barrett. BMT Unit, Hematology Branch, NHLBI and Dept of Transfusion Medicine, NIH, Bethesda, MD and CellPro, Inc., Bothell, WA.

The higher T cell content of peripheral blood progenitor cell (PBPC) allografts compared to bone marrow makes T cell depletion of PBPC a technical challenge. To evaluate a new 2 step T cell depletion system, 8 normal donors received G-CSF 10 μg/kg/d SC for 5 days. On day 5, circulating PBPC were collected by a 15 liter apheresis using the Baxter CS3000Plus cell separator. In an attempt to reduce lymphocyte content of the collected product, dexamethasone 8 mg po was given to 4 of the 8 donors 6hr before apheresis. PBPC collections were processed using an immunoaffinity column (CellPro) for positive selection of CD34 antibody(Ab)-sensitized cells, followed by T cell Ab sensitization and absorption in a second smaller column. CD2 Ab alone was used for 4 donors and CD2+CD4+CD8 were used for the other 4 donors. Average lab processing time was 4 hours. Day 5 circulating CD34+ cells ranged from 28-197/μl (mean 102/μl). Assuming a recipient body weight of 70 kg, a single 15L apheresis followed by the 2 step depletion procedure resulted in a final product containing a mean of 3.6 $x10^6$/kg CD34+ cells (range 0.4-6.3) and 0.34 $x10^5$/kg CD3+ cells (range 0.13-1.2). The mean CD34+ cell yield of the 2 step procedure was 43.6% of the original product (range 32-58%) with mean CD34 purity of 72% (range 28-91%). The mean CD3+ T cell depletion achieved was 3.75 logs (range 3.4-4.2). The table shows the cell content of the final product calculated for a 70 kg recipient.

Donor	T cell depletion	Dexame-thasone	CD34 $x10^6$/kg	CD34 yield %	CD3 $x10^5$/kg	CD3 log depletion
1	CD2	-	4.4	45	1.2	3.4
2	CD2	-	2.9	52	0.17	3.9
3	CD2	+	0.4	38	0.14	3.7
4	CD2	+	2.7	58	0.5	3.4
5	CD2+4+8	-	3.7	38	0.18	3.8
6	CD2+4+8	-	6.3	40	0.25	4.2
7	CD2+4+8	+	2.5	32	0.14	3.7
8	CD2+4+8	+	5.5	46	0.13	3.9

There was no definite effect of dexamethasone on CD3+ cells in circulating blood, apheresis product, or final T-depleted product. Split product studies in the last 3 donors showed no differences between depletion with CD2 alone vs CD2+CD4+CD8. This 2 step procedure results in a product with CD34+ and CD3+ cell doses suitable for a T cell depleted transplant. Since the CD3+ cell dose is low, a further increase in the CD34+ cell dose could be accomplished with a 2nd apheresis procedure. This approach may be especially useful in PBPC transplantation from unrelated and mismatched donors.

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ISOLATION AND TRANSPLANTATION OF HIGHLY PURIFIED AUTOLOGOUS AND ALLOGENEIC CD34+ PROGENITORS.
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A method for the isolation of highly purified CD34+ progenitors from mobilized peripheral blood and from bone marrow for the clinical use in autologous and allogeneic transplantation was investigated. The method consists of a combination of an immunoaffinity column (CellPro) followed by magnetic-activated cell sorting (MACS) (6 patients) or the use of the large-scale MACS system (Supermacs, Miltenyi Biotec) followed by a lab scale MACS system (Variomacs) (14 patients). Using this method, autologous peripheral CD34+ cells were isolated in 14 pediatric patients (12 neuroblastoma, 1 large anaplastic lymphoma, 1 B-cell leukemia) and in 6 allogeneic donors (4 haploidentical, 1 unrelated donor with 1 mismatch and 1 sibling with 1 mismatch). In 3 of the allogeneic donors, CD34+ cells from bone marrow were additionally isolated to augment the stem cell dosis. The mean purity of the CD34+ cells in the 20 preparations was 99% (range 98 - 99.9%) with a recovery the CD34+ progenitors of 90% (range 80-100%). The percentage of contaminating T- and B- cells was 0.1% (range 0-1.2%) and 0.2% (range 0.1%-1.5%), respectively. Up to now, 7 patients were reconstituted with the autologous peripheral CD34+ cells after myeloablative therapy. The mean number of infused CD34+ was 2.5 x 10^6/kg BW (range 1.0 -20 x 10^6/kg). The median time to reach 1x 10^9/L granulocytes was 10 days (range 8-14 days). In all 7 patients, a complete hematopoietic reconstitution was observed with the longest follow-up of > 1 year. The isolated allogeneic CD34+ progenitors were used in all 6 patients (3 ALL, 1 WAS, 1 SCID, 1 Osteopetrosis). The mean number of reinfused CD34+ cells was17x10^6/kg (range 9.8 -30 x10^6/kg). The mean number of reinfused T- cells was 6x10^4/kg (range 1.5-20 x 10^4/kg). In 2 patients, contaminating T-cells were not detected by FACS analysis. A rapid and complete hematopoietic reconstitution was observed in 5 patients after myeloablative therapy with a mean time to reach >1x10^9/L granulocytes of 12 days (range 9-17 days). In the SCID patient, peripheral haploidentical CD34+ cells were infused without conditioning regimen. In none of the patients, a significant GvHD (grade 2-4) was observed. In conclusion, the described method offers a highly effective purging efficiency in CD34-negative malignancies in the autologous situation and might also be used in the therapy of autoimmune diseases for autologous T- and B-cell depletion. In the allogeneic setting, the method is very effective in the prevention of GvHD in the mismatch or haploidentical situation and offers an increase of the donor pool.

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CD34$^+$HLA-DR$^+$ PROGENITOR CELLS IN CHRONIC PHASE CML, BUT NOT IN MORE ADVANCED PHASE CML, ARE POLYCLONAL.

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Chronic myelogenous leukemia (CML) is a malignant disease of the hematopoietic stem cell characterized by the Philadelphia chromosome and bcr-abl gene rearrangement. We have previously shown by RT-PCR, cytogenetics and FISH that CD34$^+$HLA-DR$^+$ (DR$^+$) cells, but not CD34$^+$HLA-DR$^-$ (DR$^-$) cells, present in steady-state bone marrow of some CML patients are highly enriched for bcr-abl mRNA$^+$ and Ph negative (Ph$^-$) cells. Although this suggests that these DR$^+$ cells may be benign, the possibility that this population is part of the clonal disorder has not yet been ruled out. To determine the clonal origin of DR$^+$ and DR$^-$ cells, we compared the expression of bcr-abl mRNA with X-chromosome inactivation (XCI) patterns in DR$^+$ and DR$^-$ fractions in peripheral blood and marrow, obtained in steady-state and after mobilization, from 6 female CML patients (3 in chronic phase (CP), and 3 in accelerated phase (AP)). Bcr-abl expression was measured by RT-PCR using β-actin as an internal control, and XCI patterns were determined on the same cell fractions using the methylation-based HUMARA assay. 1,000 to 5,000 DR$^+$ cells from steady-state BM of 3/3 CP patients were bcr-abl mRNA$^+$ and polyclonal. The same polyclonal pattern was also found in the progeny of long term culture-initiating cells (LTC-IC) derived from these DR$^+$ fractions. However, a small subpopulation of these DR$^+$ derived LTC-IC was bcr-abl mRNA$^-$ and clonal. In contrast to the DR$^+$ cells, DR$^+$cells from steady-state BM of 3/3 CP patients were bcr-abl mRNA$^+$, but still polyclonal based on XCI patterns. In 1 patient, DR$^+$ derived LTC-IC all originated from the same clone. In the 3 AP patients, all analyzed progenitor fractions were bcr-abl mRNA$^+$ and clonal, and no polyclonal fraction could be found in blood or marrow after in vivo mobilization. These studies demonstrate that : (1) DR$^+$ cells in chronic phase CML are highly enriched for bcr-abl mRNA$^+$, Ph$^+$ cells which are polyclonal. This indicates that the majority of these DR$^+$ cells are derived from the residual polyclonal, benign stem cell population. (2) Contamination with Ph$^+$, bcr-abl mRNA$^+$ monoclonal LTC-IC, is higher in the DR$^+$ population of CP CML marrow. Since the bulk population is however still polyclonal and FISH studies demonstrate that only 40-50% of DR$^+$ cells in CML are Ph$^+$ (Verfaillie et al., Blood 87 4770, 1996), but the majority of DR$^+$ derived LTC-IC is monoclonal, the DR$^+$ population must contain Ph$^+$, DR$^+$ derived progenitors and precursors. (3) Once the disease progresses, few, if any bcr-abl mRNA$^+$, Ph$^+$ polyclonal cells remain in either blood or marrow which underlies the clinical observation that high dose mobilization regimens usually not lead to Ph$^+$ collections in these patients. We conclude that polyclonal, bcr-abl negative, CD34$^+$HLA-DR$^+$ cells present in CP CML marrow may serve as a source of benign stem cells for autografting in CML.

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IN VITRO DIFFERENTIATION OF CD34+ HEMATOPOIETIC PROGENITOR CELLS TOWARDS DISTINCT DENDRITIC CELL SUBSETS OF THE MIIC-POSITIVE LANGERHANS CELL- AND THE INTERDIGITATING DENDRITIC CELL TYPE.

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The effective generation of antigen (AG)-specific T cell responses is based on an early AG uptake at the epithelial borders of the organism and subsequent presentation of these AG's at centers of T cell traffic and activation, i.e. lymph nodes (LN) and spleen. Langerhans cells (LC) in the skin and their counterparts in other epithelial tissues take up AG by micro- and macropinocytosis and may be by phagocytosis. Concomitantly they exhibit distinct functional changes, starting with their migration to the draining LN where they finally home to the T cell areas as interdigitating dendritic cells (iDC) in order to present those AG's taken up in the periphery to induce naive or primed T cells. - We have demonstrated recently that Birbeck granule-positive Langerhans cells can be derived from CD34+ peripheral blood progenitor cells (PBPC) in the presence of a 7-cytokine cocktail (CC7-7). Here we show that the sequential use of early acting hematopoietic growth factors, SCF, IL-3 and IL-6, followed on day 8 by differentiation in the two factor combination IL-4 + GM-CSF (CC4GM) is about 5x more efficient generating $9x10^6$ LC from $2x10^5$ CD34+ PBPC. Furthermore it allows to arrest the cells in the LC-stage for more than a week while continuous maturation occurs in CC7-7. Maturation of LC to interdigitating dendritic cells (IDC) could specifically be induced within 60 hours by addition of TNF-α (20 ng/ml) or LPS (100 ng/ml). While molecules and functions involved in antigen (AG)-uptake and processing were highly expressed in LC, those involved in antigen presentation were at maximum in iDC. LC were CD1a++ DR++, CD23+, CD36+, CD80-, CD86-, CD25-, while iDC were CD1a+/- DR+++, CD23-, CD36-, CD80+, CD86++, CD25+. Macropinocytosis of FITC-dextran was dominant in LC as were multilamellar MHC-class II compartments (MIICs) which were detected by electron microscopy. The functional dichotomy of these cell types was finally supported by testing the APC-function for tecanus toxoid to primed autologous T cell lines, which was optimal when cells were loaded with AG as LC and subsequently induced to become iDC.

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A PHASE I/II TRIAL OF TOTAL MARROW IRRADIATION, BUSULFAN AND CYCLOPHOSPHAMIDE FOLLOWED BY PERIPHERAL BLOOD STEM CELL TRANSPLANTATION IN PATIENTS WITH ADVANCED MULTIPLE MYELOMA.

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Patients with multiple myeloma (MM) in advanced stage have a poor prognosis. Recently the "Intergroupe francais du myelome" has finished a prospecive randomized study demonstrating a survival advantage of patients with stage II/III multiple myeloma undergoung high-dose chemotherapy and autologous stem cell support compared to patients receiving conventional chemotherapy. Moreover, in a multivariate analysis, response (CR + VGPR) to high dose chemotherapy was found to significantly improve survival. Thus, the attainment of a higher CR rate is one of the major objectives in high dose therapy for patients with multiple myeloma. We designed a new conditioning regimen to increase the response rate in these patients. In a phase I/II study from March 1995 to July 1996 34 patients (median age of 52 years, range 32 - 60 years) underwent high dose chemoradiotherapy. Inclusion criteria were stage II or III MM at diagnosis, age less than 60 years. 32 patients included in the study had stage III, 2 stage II MM. The isotype of the monoclonal component was IgG in 19 cases, IgA in 11, only Bence-Jones Protein in 4. Twenty-two patients had received previous chemotherapy, 10 of them less than 6 cycles, 8 6-10 and 4 more than 10 cycles of chemotherapy. 13 patients were considered as nonresponsive to alkylating agents, 4 were progressive under melphalan and VAD. All patients had received 2-4 courses of VAD prior to stem cell harvest. Mobilization of PBPCs was performed after high-dose cyclophosphamide (4 g/m²) and filgrastim (10 µg/kg s.c.) administration. Patients were only eligible for high dose chemoradiotherapy when at least $2x10^6$ CD34+-cells were collected as achieved in 34 of 39 patients by a median of 3 (range 1 - 7) leukaphereses. Conditioning therapy consisted of total marrow irradiation (9 Gy applied in 3 fractions over 3 days with shielding of the lung and liver), oral busulfan given at a dose of 1 mg/kg every 6 hours days -6 to -3 (total dose 12 mg/kg) and cyclophosphamide 120 mg/kg. No fatal or life-threatening complications were observed in these patients. None of the patients developed clinical or biochemical signs of VOD. Inspite of rapid neutrophil recovery (> 500/µl at day 10) and platelet recovery (day +12) mucositis grade III/IV (19/34) and febrile episodes (23/34) were observed in the majority of patients. Among 17 patients eligible for response assessment 5 achieved a CR lasting for 4 - 14 months, 9 a VGPR (reduction of the paraprotein by > 75%) with 2 relapsing after 11 and 12 months. Two with a PR relapsed after 2 and 4 months, one patient did not respond after PBSCT. Further patients and follow up data will be presented.

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PREVENTION OF GRAFT-VERSUS-HOST-DISEASE BY TRANSPLANTATION OF ALLOGENEIC CD34$^+$ BLOOD CELLS ADDITIONALLY T-CELL DEPLETED WITH CAMPATH-1H. B. Herrenstein,* L. Artemiev,* I. Novotny,* K. Barmer,* A. Sucdi,* I. Südmeier,* I. Zeune,* I.G. Kadar,* G. Hale,* H. Waldmann,* and A. Ganser. Dept. Hematology and Dept. of Transf. Med., Hannover Medical School, Germany and Dept. Pathology, University of Oxford, UK

The transplantation of immunoselected of allogeneic CD34$^+$ blood cells provides rapid and stable hematopoietic recovery, is, however, alone not sufficient as effective GvHD-prophylaxis. We evaluated whether an additional T-cell depletion of the immunoselected CD34$^+$ blood cell grafts could further reduce acute GvHD and eliminate the need for post-transplant immunosuppressive treatment. Five patients (3 CML, 1 AML, 1 NHL, median age 29 years, range 25-38, 1 male, 4 female) received G-CSF mobilized (5µg/kg s.c. bid) peripheral blood progenitor cells (PBPC) from HLA-identical sibling donors. CD34$^+$ cells were selected by immunoadsorption (Ceprate® SC, CellPro, Bothell, WA) and frozen. The nonselected T cells were portioned and stored separately. The conditioning regimen consisted of TBI (12Gy) and Cy (120mg/kg). According to the results from T-cell depleted bone marrow transplantation Campath-1H was given i.v. prior to conditioning for prophylaxis of graft rejection (20mg/d, d -11 to -7). At transplantation the CD34$^+$ cells were thawed and Campath-1H was added (10mg/150ml). Campath-1H labelled > 99% of the residual T-cells. The graft was transfused without further ex vivo manipulation within 30-40 min. Median transplanted cell numbers were: 3.3 CD34$^+$, 0.21 CD3$^+$ and 0.31 CD52$^+$ Campath-1H labelled cells x10^6/kg. The patients received G-CSF (5µg kg/d, s.c.) post-transplant and no further GvHD prophylaxis was given. All patients engrafted. No graft failure or rejection were observed so far (follow up 30-85 days). Median recovery time of neutrophils to reach 500 and 1,000/µl was 11 and 13 days, respectively. Median recovery time of platelets to reach 50,000/µl was 24 days. The last platelet (median 44 units/patient) and RBC (median 6 units/patient) transfusions were required on median days 10 and 11, respectively. One patient with active CMV infection and antiviral treatment did not achieve 50,000/µl platelets up to day 30. None of the patients developed acute GvHD. Lymphocytes began to recover after day 35 with an inverted CD4/CD8 ratio of 1:4 and greater proportions of CD56$^+$ cells (3-24%). All three patients seropositive for CMV developed CMV antigenemia (day -1, 14 and 43). Preemptive ganciclovir or foscarnet treatment was given and no progress to CMV-disease occurred. One CML patient received donor T lymphocytes at day +80 because a switch to bcr-abl PCR-positivity was demonstrated. The transplantation of T-cell depleted allogeneic CD34$^+$cells prevented effectively acute GvHD while preserving the rapid hematopoietic reconstitution seen with PBPC grafts. Since no immunosuppressive treatment is used post-transplant, this approach provides appropriate conditions for induction of GvL with donor lymphocyte transfusions. Whether this will translate in improvement of disease free survival and whether the high rate of active CMV infections will cause clinical problems remains to be determined.

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CROSS-REACTIONS OF ANTI-CD34 AND OTHER HAEMATOPOIETIC MONOCLONAL ANTIBODIES TO NEUROBLASTOMA CELLS. A. Voigt,* R. Hifer,* and E. Ziegler* (Intr. by P.D. Wickramanayake) University of Jena, Department of Pediatrics, Jena, Germany.

Peripheral blood stem cells (PBSC) are being used with increasing frequency as a progenitor cell source after myeloablative irradiation or high-dose chemotherapy for neuroblastoma patients. But more and more evidences of circulating tumour cells in blood are found so that PBSC harvests may contain viable, high clonogenic tumour stem cells carrying the risk of being reinfused into the patient To reduce the potential risk of tumour contamination harvested mononuclear cells can be purged with a selection of anti-CD34 monoclonal antibodies (moAbs). In the present study we have examined the surface membrane antigens of six freshly obtained neuroblastoma tumour specimens and eight permanent six primary, and four SCID-mouse passaged cell lines with the use of a panel of moAbs developed against haematopoietic cells including the CD34 moAbs BirmaK3 (DAKO), ICH3 (MEDAC), Qbend10 (SEROTEC), and 12.8 (CellPro) respectively, and moAbs primarily developed against neuroblastoma cells by flow-cytometric analysis. We separated the non-adherent, small round-shaped clonogenic neuroblastoma cells from the adherent cells of permanent and SCID-mouse passaged neuroblastoma cell lines comparing this in vitro modell system with the circulating tumour cells in patients and analysed the cell surface expression of CD34 antigen. As a result the majority of neuroblastoma cell lines shared haematopoietic-associated antigens with B (10-92%) and T (0-75%) cells, myeloid cells and monocytes (0-100%) as well as with megakaryocytes (0-80%). In literature, there is reported that the CD34 antigen may be involved in cell adhesion processes and in „homing" interactions between stem cells and the stroma of bone marrow. In fact, we found high reactions of ICH3 (90%) and BirmaK3 (75%) in the lower differentiated non-adherent neuroblastoma cells characterized by a diminished adhesion capability in comparison with the adherent cells (36% and 29%, respectively). Similar results are produced with SCID-mouse passaged high clonogenic neuroblastoma cells immediately after tumour resection in contrast to further cultivated and differentiated cells. Results of the considerable specific cross-reactions of anti-CD34 moAbs to neuroblastoma cells were to be found in an experiment of stem cell selection of a bone marrow morphologically free of neuroblastoma cells. After preparing bone marrow with the biotinylated 12.8 moAb and its passage through a column of avidin-coated polyacrylamide beads we observed a neuroblastoma cell clone growing up from the selected stem cells at the 21st day of in vitro culture. In conclusion, these cross reactions found with haematopoietic moAbs, especially the CD34 moAbs ICH3 and 12.8, indicate that there is a potential risk of accumulation of just the circulating neuroblastoma cells during selection of CD34+ cells from eventually tumour cell contaminated bone marrow or peripheral blood. Therefore, the stem cell selection with CD34 moAbs should be performed only with tested moAbs before and with precaution in neuroblastoma patients.

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3648

ENHANCED DETECTION OF BREAST TUMOR CELLS BY IMMUNOCYTOCHEMISTRY FOLLOWING ENRICHMENT USING AN AVIDIN AFFINITY COLUMN. T.J. Lavon,* A.B. Ormerod,* M.L. Kennedy, E.J. Shpall, R.B. Jones, L. Hami,* A.A. Ross.* CellPro, Inc., Bothell, WA, Johns Hopkins Oncology Center, Baltimore, MD, and Univ. of Colorado Health Sciences Center, Denver, CO.

The ability to detect very low levels of tumor cells in blood or bone marrow is useful for detection of micrometastatic disease and appears to have prognostic significance. The presence of micrometastases in primary breast cancer patients at the time of surgery is significantly associated with poor prognosis and early relapse (Cote, et al. *J Clin Oncol* 9:1749, 1991). The presence of micrometastases has also been shown to be associated with reduced disease-free and overall survival in the high-dose chemotherapy, autologous hematopoietic stem cell transplantation setting (Vredenburgh, et al. *Proc Amer Soc Clin Oncol* 14:316, 1995). Current detection methods (Immunocytochemistry, PCR) lack the sensitivity to detect beyond $1:1x10^6$, while tumor cells may be contaminants at levels lower than this. The sensitivity of current methods of detection may be increased significantly if combined with a tumor enrichment method that allows for the selective capture of micrometastatic tumor cells. We have developed a system to enrich for tumor cells from bone marrow, apheresis product, and peripheral blood that can isolate one tumor cell in $1x10^8$ hematopoietic cells. Using this laboratory-scale avidin-affinity column system we have shown between 2 and 3 logs of enrichment of seeded tumor cells. The isolated tumor cells were capable of *in vitro* growth. A monoclonal antibody that is specific for a membrane-associated antigen, PAN-05 (NeoRx Corp, Seattle, WA), is used for positive selection of the tumor cells. Post-enrichment detection of tumor cells is accomplished by means of a sensitive immunocytochemical (ICC) assay using a cocktail of anti-cytokeratin antibodies. In addition, 18 stage II and 6 stage IV breast cancer patient apheresis samples have been processed on this system and analyzed by ICC. Of the 6 stage IV samples, only one showed an ICC-positive cell prior to enrichment, but this same sample showed 7 tumor cells post-enrichment. Two of the 6 samples (33%) converted from ICC-negative to ICC-positive following enrichment for tumor cells. Of the 18 stage II patient apheresis specimens, 3 (17%) have shown ICC-positive cells following enrichment. However, suboptimal morphology of these cells resulted in histopathologically inconclusive results. None of these samples was ICC-positive prior to enrichment. One sample had a single ICC-positive cell prior to enrichment, but was ICC-negative following enrichment. We are continuing to test samples from stage II and stage IV breast cancer patients. This tumor cell enrichment system permits a rapid, sensitive, and specific method for the enrichment of extremely low numbers of tumor cells in hematopoietic products.

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IMMUNOCHEMICAL CHARACTERIZATION OF THE PROTEIN SEQUENCE OF HUMAN CD34 USING AN IMMOBILIZED PEPTIDE ARRAY. H.M. Jones,* B.L. Focarty,* M.S. Lodge,* R.W. Seminger,* and S.J. Tamowski,* (intr. by S Rowley) CellPro Inc, Bothell, Washington, USA.

The precise role of the CD34 molecule present on hematopoietic stem cells and progenitors remains unclear, as a ligand for the human CD34 molecule has not yet been definitely identified. CD34 may simply play a role in adhesive interactions with other cell types within the hematopoietic microenvironment or alternatively, directly modulate hematopoietic cells through cellular signaling by binding of a specific ligand. Antibodies against CD34 in addition to being valuable reagents for the isolation of stem cells are useful tools with which to examine conformationally distinct regions of this cell surface molecule. In particular, mapping of the different peptide regions recognized by antibodies to CD34 may provide clues to the identity of a ligand for the CD34 molecule. Using a published amino acid (AA) sequence (Nakamura *et al.*, Exp. Hematol. 21, 236-42) we produced an array of 125 different overlapping peptides scanning residues AA 29 - 291 of the extracellular domain of human CD34. For the initial series of experiments each peptide was generated as a 13 mer anchored onto a derivatized cellulose support with individual peptides overlapping the previous sequence of residues by two AA. Individual test antibodies were applied to the membrane and incubated for 3 hours at room temperature. This was followed by washing and subsequent detection of any antibody bound to the membrane with a β-galactosidase conjugated anti-mouse IgG antibody and a chromogenic substrate. With this system we have been able to examine a variety of CD34 monoclonals (Moabs) and determine which Moabs recognize discrete epitopes not wholly dependent on the presence of carbohydrate moieties. For example, the Moab QBEND10 recognized 4 peptides spanning AA 37 - 55 and the ICH3 Moab recognized 4 peptides spanning AA 49 - 67. Studies are ongoing to determine the precise length and boundary residues of the regions recognized by reacting Moabs. Screening by this method has allowed the further delineation and classification of the various antibodies within the CD34 monoclonal cluster. Additionally, the short, linear, peptide sequences identified using this technique provide an alternative form of immunogen for the generation of new CD34 Moabs. Furthermore, we conclude that this is a rapid and informative means of discovering and characterizing peptide epitopes. Finally, we believe that the characterization of these peptide sequences may provide both tools and insights enabling the identification of potential ligands for the CD34 molecule.

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MANAGEMENT OF HEMATOLOGIC RELAPSE WITH DONOR LEUKOCYTE INFUSIONS: EVALUATION OF THE CEPRATE SYSTEM FOR POSITIVE SELECTION OF LYMPHOCYTE SUBSETS. G. Risdon,* M. Emde,* I. Petersons,* N. Saund,* and K. Auditore-Hargreaves.* CellPro, Inc., Bothell, WA USA. Introduction by T. Keenan.

Durable remissions can be achieved in patients with relapsed chronic myelogenous leukemia (CML) by infusion of donor allogeneic blood lymphocytes. Such infusions however are complicated by acute and chronic GVHD and marrow aplasia. Optimally, the treatment of relapsed CML would involve the identification and infusion of the lymphocyte population(s) responsible for the anti-leukemia effect (GVL) in the absence of GVHD. To this end, we have evaluated the CellPro CEPRATE separation system for the isolation of large numbers of CD4, CD8 and CD56-positive cells from peripheral blood leukapheresis. This system permits the rapid isolation of as many as $5x10^9$ cells with a purity typically greater than 90%. These isolated CD4 and CD8 T cells maintain proliferative responses to PHA, anti-CD3 and alloantigens and cytotoxic activity against allogeneic targets. Strong cytolytic activity against K562 targets is maintained in CD56 selected cells. IL-2 induced LAK activity against HL-60 targets is observed with all populations following short-term culture. The clinical-scale isolation of functional T and NK cells is the first step in developing effective salvage and prophylactic immunotherapy against leukemic relapse. These positively selected cells are also candidates for ex-vivo expansion, activation and genetic modification prior to infusion.

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IDENTIFICATION OF NOVEL HEMATOPOIETIC GROWTH FACTORS THAT PROMOTE THE EX-VIVO EXPANSION OF HEMATOPOIETIC PROGENITOR CELLS. N.J.Minster,* L.E.Kahn,* S.L.Ellison,* A.M.Donnelly,* J.G.Giri,* J.P.McKearn,* and P.R.Streeter*. (Intr. by P.D.Doshi). Searle Discovery Research, St. Louis, MO

Autologous stem cell transplantation following high dose chemotherapy results in an 8 - 12 day window before patient neutrophils and platelets recover to safe levels. Ex-vivo expansion and subsequent transplantation of autologous hematopoietic progenitor cells offers the potential of dramatically reducing or eliminating these cellular deficiencies by hastening patient recovery. We engineered and characterized a series of synthetic cytokines with distinct growth and differentiation promoting activities including Daniplestim (Syntheikine), Myelopoietin and Promegapoietin which were designed to drive proliferation and differentiation of neutrophil and megakaryocyte progenitors. The goal of this study was to define optimal conditions for multilineage ex-vivo cell expansion. The ex-vivo cell expansion potentials of these cytokines were evaluated using immunoaffinity purified normal bone marrow-derived CD34+ progenitor cells. The isolated CD34+ enriched cells were cultured with novel hematopoietic growth factors for a period of 10 - 12 days in X-Vivo 10 media supplemented with 1% HSA. Cultured cells were analyzed for total cellular expansion and expansion of neutrophil (CD11b,CD15) megakaryocyte (CD41a) lineage committed cells. For Daniplestim (Syntheikine), Myelopoietin and Promegapoietin alone a mean of 40%, 55%, and 29% of the expanded cells expressed CD15 respectively, while the combination yielded 43% of these cells expressing CD15. Similarly, for Daniplestim (Syntheikine), Myelopoietin and Promegapoietin alone a mean of 14%, 26%, and 44% of the expanded cells expressed CD41a respectively, while the combination yielded 36% of these cells expressing CD41a. Our results demonstrated that optimal concentrations of cytokines tested alone, or in combination, resulted in significant cellular expansion and that exposure to Promegapoietin or Myelopoietin yielded a selective expansion of megakaryocyte or myeloid lineage committed cells. This study demonstrated that these novel hematopoietic growth factors can significantly expand progenitors as well or better than other published ex vivo expansion protocols. Transplantation of these ex-vivo cultured, expanded and differentiated CD34+ enriched progenitor cells may shorten chemotherapy induced patient neutropenia and thrombocytopenia.

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IMMUNOPHENOTYPIC ANALYSIS OF UMBILICAL CORD BLOOD. IN VITRO EXPANSION OF THE CD34+ ENRICHED FRACTION. A POTENTIAL SOURCE OF TRANSPLANTABLE HEMATOPOIETIC STEM CELLS.

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Bone marrow transplantation is limited by the paucity of HLA-matched donors and the frequent occurrence of GvHD. Hematopoietic transplants using CB cells are being increasingly used in pediatric patients. Early estimates suggest that there may not be enough hematopoietic progenitor cells in an average cord blood sample to reconstitute adult patients. We investigated the in vitro proliferative potential of CB CD34+ selected cells in order to increase the progenitor pool from CB. We studied the phenotypic characteristics of UCB mononuclear cells (n=25) by one and two color flow cytometry. CD34+ cells were isolated using a Ceprate LC-34 Biotin kit. In clonogenic assays CFU-GEMM, CFU-GM and BFU-E were quantified by methylcellulose culture in both mononuclear cells and CD34+ enriched fractions after the addition of different combinations of Epo, IL3, GM-CSF, G-CSF and SCF. The CB cells were characterised by a low protection of CD3+ T cells, increased CD4/CD8 ratio, minimal expression of HLA-DR and increased proportion of CD5/CD19 double positive B cells. All these probably reflect the immaturity of CB lymphocytes contributing to decreased GvHD. The number of CFU-GM, CFU-GEMM, BFU-E in cultures of CD34+ enriched cells had increased 34-65-225 fold respectively over the cell cultures of mononuclear cells. The addition of SCF expanded the CFU-GM and CFU-GEMM colonies 3 fold in the CD34+ enriched fraction whereas it had no influence on the mononuclear cells fraction. The influence of SCF was minimal on the BFU-E formation in both CD34+ enriched and mononuclear cell fractions when added to GM-CSF, IL3 and Epo. We noticed though that the combination of Epo and SCF increased by 3,7 the BFU-E numbers in the CD34+ enriched fraction. These data demonstrate that CD34+ enriched UBC progenitor cells have greater clonogenic capacity compared to UCB mononuclear cells and they are more sensitive to the addition of SCF. We therefore suggest that cord blood can be a valuable alternative to bone marrow or peripheral blood as a source of pluripotent hemopoietic stem cells and additional investigation is necessary to establish whether ex vivo expanded cord blood progenitors will engraft adult patients as efficiently.

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QUANTITATION OF CD34$^+$DR$^-$/CD34$^+$DR$^+$ CELLS BEFORE AND AFTER IMMUNOSELECTION OF CD34$^+$ CELLS FROM G-CSF MOBILIZED PERIPHERAL BLOOD PROGENITOR CELLS (PBPC).

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Positive selection techniques to isolate CD34$^+$ cells are increasingly used for hematopoietic progenitor cell enrichment. However, between 30% to 70% of progenitor cells may be lost by using these methods. To analyse whether this loss affects predominantly CD34$^+$DR$^-$ or CD34$^+$DR$^+$ subsets, we studied the phenotypic characteristics of CD34$^+$ cells from G-CSF-mobilized PBPC before and after positive selection using the immunoadsorption avidin-biotin method (CEPRATE SC). Twelve healthy subjets, median age 34 years (21-61), received G-CSF 10μg/kg/d s.c. x 5 d. On day 5 and 6 donors underwent 10 L leukapheresis using a CS3C0 plus cell separator The sum of the two apheresis products was incubated with a biotinylated anti-CD34McAb (12.3, CellPro) and then passed through a column of avidin-coated polyacrylamide beads. Bound cells were removed from the column by agitation and rinsing and were collected. Flow cytometry (FC; FACScan, Becton Dickinson) was performed on the original PBPC and on the immunoselected product bag. McAb used for FC included CD3, CD34, HLA-DR, and a isotype control. Quantitation of CD34$^+$ subpopulations was performed by analysis of a total of 300,000 (PBPC product) or 50,000 cellular events (selected product). Recovery and purity of CD34$^+$ cells after positive selection were of 64% and 57.6%, respectively. Results of FC analysis of CD34$^+$ cell subsets before and after the selection procedure are shown in the table. A 62% of the CD34$^+$DR$^+$ cells presents in the original PBPC products was recovered after immunoselection, as compared to 24% of the CD34$^+$DR$^-$ cells.

Cell fraction	Pre-column	Post-column
CD34$^+$ cells (%)	0.70 (0.37-1.70)	57.6 (37.0-82.4)
CD34$^+$ cells x 10^6/kg*	6.5 (2.8-13.4)	4.25 (2-8.63)
CD34$^+$HLA-DR$^+$ x 10^6/kg*	6.54 (2.78-12.78)	4.06 (1.96-8.44)
CD34$^+$HLA-DR$^-$ x 10^6/kg*	0.21 (0.02-0.61)	0.05 (0.01-0.43)

Values are expressed as median and ranges. * kg of receptor.

In conclusion, this method allows a high enrichment of CD34$^+$ cells and is associated to a predominant recovery of CD34$^+$ DR$^+$ cells.

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RAPID ENGRAFTMENT WITH MINIMAL GVHD AFTER HLA-MISMATCHED DOUBLY SELECTED T-CELL DEPLETED BLOOD CELL PRECURSOR TRANSPLANTS (BCPT). H. Atkins*, C. Bredeson*, I. Bence Bruckler*, S. McDiarmid*, H. Hopkins*, A. Guilfoi, L. Huscsch*. BMT program, Ottawa General Hospital, Ottawa, Ontario.

Three pts with ALL (2-post BMT relapses, 1-1st relapse) received Cy (120 mg/kg), rTBI (200 cGy x 6) and rabbit ATG (1.25 mg/kg x 4) (1 pt) or Bu (16 mg/kg), Cy (200 mg/kg) and rATG (1.25 mg/kg x 4) (2 pts). BCPT followed using a graft from the patient's father (2 donors - 2 antigen HLA mismatch, 1 donor - 3 antigen HLA mismatch). Patients received G-CSF (300 ug/d sc until ANC>750) and Cyclosporin post BCPT. Donors received 7 days of G-CSF (~16 ug/kg/d). Leukopheresis was done on the 4th to 6th day and bone marrow harvest was done on the 7th day. Moderate thrombocytopenia (38 K, 65 K and 128 K) occurred in all donors. Because of thrombocytopenia, the marrow harvest was deferred in 1 donor. Purified T cell depleted BCPs were isolated using Cellpro CD34(+) selection and Baxter Maxsep CD2(-)/CD8(-) depletion. Procedure parameters are given below:

	CD34 enrichment	CD34 recovery	Log T cell depletion
CD34(+) selection	114-319 x		2.58 - 3.31
T cell depletion	1.06 - 1.15 x	47 - 87 %	1.25 - 1.78
Overall	190 - 353 x		3.92 - 4.93

Grafts contained 252 to 406x10^6 CD34(-) cells and 0.9 to 3.5x10^6 CD3(+) cells. All patients had ANC > 1000 by d13. An untransfused platelet counts of 20x10^9/L for 3 days was achieved by d22 and d42. One pt did not become independent of platelet transfusions. ABO switching was documented at d35 and d49 in patients with ABO mismatched BCPT. The maximum ALC ranged between 0.1 to 0.68x10^9/L. Major toxicities were transient pulmonary edema that required intubation following rATG infusion (1 pt), acyclovir resistant HSV mucositis and esophagitis (1 pt), CMV reactivation (1 pt) and CMV pneumonia (1 pt) and severe hemorrhagic cystitis (1 pt). Grade II acute skin GvHD that rapidly responded to 1 mg/kg/d prednisone occurred in 2 pts. There was no acute GvHD of other organs. VOD and renal insufficiency did not occur. One pt relapsed at d84 and died at d144. One pt died of CMV pneumonia at d51. The 3rd pt died of progressive cachexia and hemorrhagic cystitis at d132. This report demonstrates: 1.) Sufficient purified blood cell precursors are present following a positive selection and negative depletion to allow alloengraftment across HLA disparities, while significantly depleting T cells. 2.) 4 to 5 log T cell depletion can be achieved to minimize the risk of GvHD in HLA mismatched allogeneic BCPT recipients. 3.) Life-threatening viral infections occur commonly in these patients and their treatment needs to be addressed in future studies.

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A COMPARISON OF RETROVIRAL TRANSDUCTION PROTOCOLS FOR GENE TRANSFER INTO RHESUS CD34+ BONE MARROW CELLS.

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Retroviral gene transfer into primate and human long term repopulating hematopoietic stem cells (HSC) is inefficient when compared to murine HSC. Utilizing existing methodologies employed in these murine studies, we have evaluated four gene transfer protocols for the transduction of HSC in a rhesus transplant model. They are the following: 1) co-culture with retroviral producers, 2) retroviral supernatant infection, 3) *in vivo* priming with cytokines, and 4) immunoselection of transduced cells prior to infusion. The transferred marker gene, CD4z, is a chimeric immune receptor composed of the human CD4 extracellular domain and the zeta (z)-chain of the CD3 T-cell receptor. Cell surface expression of this chimeric protein facilitates rapid determination of transduction efficiency by FACS analysis. A transient retroviral transduction system, *kat*, was used which yields vector titers of 1-2 x 10^7/ml as assayed on NIH 3T3 cells. Eleven monkeys have been transplanted using total body irradiation (650 cGy x 2) as the conditioning regimen. In 4 animals, autologous CD34-enriched bone marrow (BM) cells were prestimulated with SCF and IL-6 for 48 hours and then co-cultured with retroviral producers for 48 hours in the presence of SCF, IL-6, and polybrene. Despite *in vitro* transduction efficiencies of 25-40% as measured by FACS analysis, detectable CD4z expression in peripheral blood (PB) or BM was absent when analyzed by FACS or PCR post transplant. In 4 subsequent animals, CD34-enriched BM was transduced with retroviral supernatant for 96 hours on plates coated with anti CD34 mAb. *In vitro* transduction efficiencies were 10-45%. In 3 of the 4 primates, 1-3% of PB cells expressed CD4z at 1 month post transplant. By day 90 however, CD4z could be detected in only 1 of the 4 animals(0.5% by PCR). These results might represent transduction of short-lived hematopoietic progenitors. An additional 2 animals underwent *in vivo* priming with SCF and G-CSF for 5 days prior to bone marrow harvest in an attempt to improve stem cell transduction efficiency. Transduced CD4z-expressing cells were subsequently purified by cell sorting. In this protocol the sorted populations (>95% $CD4^+$, 2-4 x10^6 cells/animal) were re-infused. This represented 5-70 fold fewer cells than the unsorted inocculums used in earlier transplants. Engraftment was delayed in both animals. In the first animal, CD4z was detected in 2% of PB cells at day 30 and 0.5% at day 75 . The second animal was negative by PCR. The delayed engraftment observed with unmarked cells in these animals is consistent with late endogenous hematopoietic reconstitution despite what was presumed to be a myeloablative dose of irradiation. Furthermore, this conditioning regimen is associated with a high transplant-related mortality (7/11 primates died within 3 months of transplant). Based on these studies, we conclude that 1) the CD4z chimeric receptor is efficiently expressed in hematopoietic cells at early time points following reconstitution; 2) supernatant transduction of rhesus $CD34^+$ cells appears to be superior to co-cultivation with retroviral producers, and 3) re-infusion of sorted, transduced cells does not enhance gene transfer efficiency *in vivo*.

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IN VITRO EXPANSION OF CD34+DR-/+ CELLS FROM BONE MARROW OF ACUTE MYELOID LEUKEMIA (AML) IN FIRST REMISSION. Ph. Lemman[1], A. Ferrant[1], H. Van Den Berghe[2], J.L. Michaux[1], N. Straetmans[1]. [1]Haematology CPT, UCL, Brussels [2]Centrum voor Menselijke Erfelijkheid KUL, Leuven, Belgium

Selection of a residual normal stem cell population and expansion of this population *in vitro* could theoretically provide a graft devoid of malignant cells able to promptly reconstitute hematopoiesis after autologous transplant for AML in CR1. We hypothesized that the CD34+DR-/+ fraction could be suitable to this approach. Aliquots of BM harvest from patients (pts) with AML in CR1 (n=9) were enriched in CD34+ cells by immunoaffinity column and cryopreserved until use. After thawing, CD34+ cells were sorted by FACS into CD34+DR+ and CD34+DR-/+ fractions. Cell fractions were thereafter cultured in medium conditioned by an allogeneic irradiated normal human stroma supplemented with IL3, IL6, IL11, SCF ± G-CSF for 21 days. Cultures were weekly cell-counted and refed. CFU-GM, HPP-CFC and BFU-E content was assessed initially and at weekly intervals. LTC-IC were semi-quantified initially and after 2 weeks of liquid culture. For pts with an abnormal karyotype at diagnosis, karyotype was assessed initially and after 2 weeks of liquid culture. These results were compared to those obtained with normal (nl) BM taken from allogeneic donors (n=15). Cellularity of BM harvests from pts was low compared to nl and their percentage of CD34+DR-/+ cells was decreased, resulting in the recovery of small numbers of CD34+DR-/+ cells after sorting. Initially, the number of CFU-GM /10⁴ CD34+DR-/+ cells was strongly decreased compared to nl (70±97 *vs* 2038±1[illegible], p=0.003) whereas it was not different from nl for the CD34+DR+ fraction (643±446 *vs* 723±334, p=0.5). The number of HPP-CFC produced by the 2 subpopulations was lower than nl (DR-/+: 122±141 *vs* 403±282, p=0.005; DR+ 203±152 *vs* 380±211, p=0.05). Initial numbers of LTC-IC/10⁴ cells were decreased compared to nl (DR-/+ : 60±81 *vs* 397±288, p= 0.0008; DR+: 56±33 *vs* 149±90, p=0.003). For AML BM cells, CD34+DR+ and DR-/+ subpopulations generated equivalent numbers of LTC-IC, whereas in nl the CD34+DR-/+ fraction was enriched in LTC-IC compared to the CD34+DR+ fraction. LTC-IC generated by CD34+DR-/+ cells were almost equivalently distributed between the supernatant and the adherent layer when nl LTC-IC were preferentially located in the adherent layer. CFU-GM expansion from pts CD34+DR-/+ cells was poor and decreased after d14 of culture (maximal CFU-GM expansion for pts: x10±8 at d14 ; for nl: x55±24 at d21). Therefore, given their low initial cloning efficiency, pts CD34+DR-/+ cells generated 10 times less CFU-GM than nl *in vitro* (i.e. at d14, 948 vs 10,570 CFU-GM/10⁴ cells cultured). In almost all instances, no HPP-CFC expansion could be observed for pts CD34+DR-/+ cells. Nearly no LTC-IC could be found after 14 days of liquid culture (median 0, range 0-4% compared to initial input). Karyotype was evaluable for 4 pts. Very few mitoses could be observed directly after sorting. After 14 days expansion, all the cell fractions analysed so far were karyotypically normal (12-50 mitoses analysed). In conclusion, although possibly devoid of leukemic cells, the BM CD34+DR-/+ cell fraction from pts with AML in CR1 show profound hemopoietic defect *in vitro*, is not enriched in LTC-IC and does not give rise to satisfactory expansion. Further understanding of the mechanisms of this defective hematopoiesis is required to make expansion feasible.

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CELL CYCLE STATUS AND KINETICS OF CD34$^+$ SUBPOPULATIONS FROM UMBILICAL CORD BLOOD, BONE MARROW AND MOBILIZED PERIPHERAL BLOOD. De Bruyn C.*, Delforge A.*, Bron D., Bernier M.*, Stryckmans P. Institut J. Bordet, Brussels, Belgium.

We have compared the cell cycle status of CD34$^+$CD38$^-$ and CD34$^+$CD38$^+$ cells from umbilical cord blood (UCB) (n=12), bone marrow (BM) (n=9) and peripheral blood progenitor cells mobilized by G-CSF after chemotherapy (PBPC) (n=7). The mononucleated cell fraction was enriched in CD34$^+$ cells using Ceprate LC (CellPro) systems. Cell cycle status of CD34$^+$CD38$^-$ and CD34$^+$CD38$^+$ cells was assessed using a flow-cytometer by double-labelling analysis using propidium iodide for DNA content and FITC-conjugated monoclonal antibody for CD38 expression. The results were expressed as the percentage of CD34$^+$, CD34$^+$CD38$^-$ and CD34$^+$CD38$^+$ cells in proliferative phase of the cell cycle (S+G2M), and summarized in the table below:

	CD34$^+$	CD34$^+$CD38$^-$	CD34$^+$CD38$^-$	CD34$^+$CD38$^+$	p
UCB	9.4±1.2	46.9±10.9	4.7±0.7	21.2±2.4	-
BM	14.3±1.5	25.3±2.9	4.8±1.1	8.1±1.2	0.006
PBPC	6.6±1.3	30.5±9.4	2.9±0.7	11.2±4.1	ND

* cells at day 0 without any stimulation * cells cultured for 48 hours with SCF, IL-3 and IL-6
p significant difference between UCB and BM DNA content after 48 hours culture.

Cell cycle analysis indicated that (a) the large majority of CD34$^+$ cells from CB as from BM and PBSC were quiescent, (b) compared to CD34$^+$CD38$^-$ fraction, significantly more CD34$^+$CD38$^+$ cells were in active phase of the cell cycle , (c) there was no significant difference when the DNA content of UCB, BM and PBPC was compared, and that was true for each progenitor fraction (CD34$^+$, CD34$^+$CD38$^+$ or CD34$^+$CD38$^-$ fraction). After 48 hours incubation of CD34$^+$ cells in Iscove's modified Dulbecco's medium containing 10% of fetal calf serum and stimulated by a combination of SCF(10 ng/ml), IL-3 (100 U/ml) and IL-6 (100 U/ml), BM and PBSC CD34$^+$CD38$^-$ cells remained essentially quiescent with only 8.1±1.2 % (n=4) and 11.2±4.1% (n=3) of the cells in DNA synthesis, in comparison to 21.2 ± 2.4 % (n=4) for CB CD34$^+$CD38$^-$ cells. In conclusions, assuming a similar duration of DNA synthesis in the various fractions, our results show that (1) there was no significant difference between the cell cycle status of the three sources of hematopoietic cells as well as for the different subpopulations of CD34$^+$ cells studied (2) the proportion of cells in active cell cycle in the CD34$^+$CD38$^-$ and the CD34$^+$CD38$^+$ subpopulations is significantly different, the first one being more quiescent that the second one, this observation has been confirmed for CB, BM and PBSC (3) although we did not observe any significant difference between cell cycle status of CB, BM and PBSC CD34$^+$CD38$^-$ fractions before in vitro culture, the CB CD34$^+$CD38$^-$ population showed greater proliferative response to stimulation by CSFs.

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CYCLING STATUS OF CD 34+ CELLS MOBILIZED INTO PERIPHERAL BLOOD OF HEALTHY DONORS BY RECOMBINANT HUMAN GRANULOCYTE COLONY-STIMULATING FACTOR (G-CSF). Roberto M. Lemoli, Agostino Tafuri, Alessandra Fortuni* Maria Teresa Petrucci, Maria Rosaria Ricciardi* Luana Catani* Damiano Rondelli* Miriam Foschi* Giuliana Lemoli* Cristina Amola* Sante Tura.
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In this study we assessed the functional and kinetic characteristics of highly purified hematopoietic CD 34+ cells from the apheresis products and the bone marrow (BM) of 16 normal donors undergoing G-CSF treatment for peripheral blood stem cells (PBSC) mobilization and transplantation in allogeneic recipients. Mobilized and BM CD 34+ cells were evaluated for their colony-forming capacity and multilineage proliferative response to selected recombinant human-CSF in-vitro, and the content of very primitive long-term culture initiating cells (LTC-IC). In addition, the cycling status of circulating CD 34+ cells, including committed clonogenic progenitor cells and the more immature LTC-IC, was determined by the cytosine arabinoside (ARA-C) suicide test and the acridine orange (A.O.) flow cytometric technique. Clonogenic assays in methylcellulose showed the same frequency of CFU-C when PB primed-CD 34+ cells and BM cells were stimulated with the conditioned medium PHA-LCM. However, mobilized CD 34+ cells were significantly more responsive than their steady-state BM counterparts to IL-3 and SCF combined with G-CSF or IL-3 in presence of Epo. In cultures added with SCF, IL-3 and Epo we found a mean of 1.5 ±1 SEM -fold increase of PB CFU-GM and BFU-E as compared to BM CD 34+ cells ($p < 0.05$). After 5 weeks of liquid culture supported by the engineered murine stromal cell line M2-10B4 to produce G-CSF and IL-3, we reported 48.2 ± 35 SEM and 62.5 ± 54 SEM LTC-IC per 10^4 CD 34+ cells in PB and steady-state BM, respectively ($p = NS$). The ARA-C suicide assay demonstrated that 4 ± 5% SD of committed precursors and 1 ± 3% SEM of LTC-IC in PB are in S-phase as compared to 25.5 ± 12% SD and 21 ± 8% SEM of baseline BM, respectively ($p < 0.001$). However, longer incubation with ARA-C (16-18 hours), in presence of SCF, IL-3 and G-CSF or IL-6, demonstrated that greater than 60% of LTC-IC are actually cycling with no difference with BM cells. Furthermore, studies of cell-cycle distribution on PB and BM CD 34+ cells confirmed the low number of circulating progenitor cells in S- and G_2M-phase whereas simultaneous DNA/RNA analysis demonstrated that the majority of PB CD 34+ cells are not quiescent (i.e. G_0-phase) being in G_1-phase with a significant difference with baseline and G-CSF treated BM (80 ± 5% SEM versus 61.9 ± 6% SEM and 48 ± 4% SEM, respectively. $P < 0.05$). Moreover, G-CSF administration prevented a little but significant proportion of mobilized CD 34+ cells from apoptosis. In summary, our results indicate that mobilized and BM CD 34+ cells can be considered equivalent for the frequency of both committed and more immature hematopoietic progenitor cells although they show different kinetic and functional profiles. Moreover, in contrast with previous reports, we found that PB CD 34+ cells, including very primitive LTC-IC, are recruited in cell-cycle.

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COMPARISON OF IMMUNE RECONSTITUTION AND GVHD AFTER ALLOGENEIC PERIPHERAL BLOOD STEM CELL TRANSPLANTATION WITH OR WITHOUT CD34+ SELECTION

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32 patients (mean age 35 years, 19-54) with advanced hematologic malignancies were transplanted from HLA-identical related donors using filgrastim-mobilized peripheral grafts only. In 15 patients CD34+ selected grafts were transplanted containing a median of 4.3x10⁶/kg body weight CD34+ cells with a median purity of 55%, and 0.45x10⁶/kg CD3+ cells (group I). 17 patients were transplanted with unselected grafts containing 5.5x10⁶/kg CD34+ cells and 142x10⁶/kg CD3+ cells (group II). Patients were conditioned with Bu/Cy120 or TBI/VP16/Cy. GvHD prophylaxis was Cyclosporin A only in group I and additional prednisolone was used in group II. All patients received filgrastim 5ug/kg body weight post transplant. Engraftment was equivalent in both groups with neutrophils >500/μl after a median time of 10 days (range 9-15) and platelets >20 000 after 14.5 days (10-20) in group I and 15.5 days (10-70) in group II. Transfusion requirements were similar in both groups with 8 units of packed red cells and 72 units of platelets. Acute GVHD >°I occured in 5 patients all presenting with grade II GVHD which resolved with appropriate treatment. Half of the patients are alive with a median follow up of 265 days (72-668) and chronic GVHD was not seen in group I patients and in 1 patient only in group II. Lymphocyte subsets were analyzed monthly post Tx. In both groups NK cell numbers normalized within 2 months and B cells within 6 months. CD4+ counts reached >200/μl after half a year and CD8+ cells normalized by 8 months. Allogeneic PBSCT results in rapid reconstitution of lymphocyte subsets without significant acute or chronic GVHD. Transplantation of peripheral blood derived CD34+ selected cells is safe and results in stable long term engraftment without compromising immune reconstitution. The >300fold reduction of CD3+ cells in the CD34+ selected graft may allow reduction of post transplant immunosuppression.

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2369 430-IV

MDR-1 VECTOR MARKING SHOWS THAT CFUGMS DO NOT CONTRIBUTE TO ENGRAFTMENT IN PATIENTS FOLLOWING INTENSIVE SYSTEMIC COMBINATION CHEMOTHERAPY. E. Hanania,* R. Giles,* S.O. Fu,* Z. Zu,* R. Cote,* A. Dayn,* T. Wang,* D. Ellerson,* J. Kavanagh, T. Holzmayer,* E. Mechetner,* R. Berenson,* S. Heimfeld,* Z. Rahman, M. Andreeff, R. Champlin, and A.B. Deisseroth. U.T. M.D. Anderson Cancer Center, Houston, TX, Systemix, Inc., Palo Alto, CA, CellPro, Inc., Bothell, WA, Microbiological Associates, Rockville, MD, Ingenex, Inc., Menlo Park, CA, Kenneth Norris Jr. Cancer Hospital, Los Angeles, CA, and Yale University School of Medicine, New Haven, CT.

The total nucleated cell count/kg, the number of CFUGM/kg, and the number of CD34+ cells/kg have all been used as independent predictors of the reconstituting cell content of peripheral blood or marrow cells of hematopoietic stem cells. These data have suggested that if the dose of CD34+cells/kg is greater than 2 x 10^6/kg, prompt and complete recovery will occur. It is not clear whether cells belonging to later stages of maturation will contribute to hematopoietic reconstitution following intensive systemic therapy. In order to test if the CFUGM stage of maturation contains reconstituting cells, we analyzed the transduction frequency of CFUGM using two different methods for introduction of the retroviral vector containing the MDR-1 cDNA into CD34 cells: 1) The suspension method, which consists of suspending the cells collected soon after chemotherapy and CD34 selection in retroviral supernatants for 4 hours), and 2) the stromal transduction method, which consists of inoculating the cells on stromal monolayers, in the presence of IL3 and IL6 and retroviral supernatants for 48 hours, and found that the transduction frequency was equal in methods 1 and 2. Post transplant cells of 5/8 evaluable patients were positive for vector MDR-1 in the patients transplanted with the cells transduced by the stromal transduction method, whereas the cells of 0/10 of the patients transplanted with cells transduced by the suspension method were positive for the vector MDR-1 cells. These results suggest that subsets of CFUGM exist which do not reconstitute patients following intensive chemotherapy.

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TRANSPLANTATION OF POSITIVELY SELECTED ALLOGENEIC BLOOD CD34+ CELLS. W. Brugger, S. Schering,* M. Subklewe,* C. Faul,* S. Halene,* A. Brandes,* A. Wiesmann,* B. Weil,* S. Heimfeld,# H. Einsele, and L. Kanz. Department of Internal Medicine, Division of Hematology and Oncology, University of Tübingen, Germany, and #CellPro, Bothell, WA, USA.

In allogeneic transplantation, G-CSF mobilized peripheral blood progenitor cells (PBPCs) are now being used with increasing frequency as an alternative for bone marrow transplantation (BMT). However, there is concern about the greater number of immunocompetent T-cells in an unmanipulated PBPC allograft as compared to a conventional BM graft which might lead to an increased risk of severe acute and/or chronic graft-versus-host disease (GVHD). In order to potentially decrease the risk of GVHD, we positively selected CD34+ blood cells using the Ceprate SC® device (CellPro, Bothell, WA) which was shown to results in a 1-3 log depletion of T-cells (Brugger et al., Blood 84: 1421, 1994). Here, we report on the transplantation of CD34+ PBPCs from 15 allogeneic sibling donors in patients with MDS AML [n=12] as well as in patients with high-risk or relapsed acute lymphoblastic leukemia [n=3]. The median age of the patient population was 42 years (24-53). The donors were HLA-matched in 13 cases, while 2 donors had 1 antigen mismatch. All donors received G-CSF (Neupogen®; 2x12 µg/kg s.c.) for up to 7 days for mobilization of PBPCs. A median of 3.5 (range 3-5) aphereses were performed to collect sufficient numbers of PBPC for subsequent selection of CD34+ cells. Because of insufficient yield after CD34+ cell separation, 5 patients received unseparated PBPC in addition to CD34 selected PB cells (group A). The remaining 10 patients were transplanted with CD34+ selected cells only (group B). A median of $3.8x10^6$ CD34+ cells/kg (range 3.5-4.9) and $3.6x10^6$ CD34+ cells/kg (range 3.2-6.5) were transplanted in group A and B, respectively. The yield of CD34+ cells after CD34+ cell selection was 45% (23-61) with a purity of 76% (range 51-83). The number of CD3+ cells transplanted were $165x10^6$/kg (range 60-344) in group A, and $0.53x10^6$/kg (range 0.28-0.95) in group B. Conditioning consisted of BuCy or TBI/Cy. Cyclosporin A (CSA) and MTX were given for GVHD prophylaxis in 12 patients, while 3 patients received CSA only. Time to neutrophil recovery > $0.5x10^9$/l was 16 days (range 13-19) in group A, and 14 days (range 10-15) in group B. Time to platelet transfusion independency > 25.000/µl occured at day 16 (12-29) in group A, and day 19 (15-24) in group B. No graft failure was observed. One patient developed fatal infectious complications at day +3. In patients transplanted with CD34+ selected cells alone (group B), we only observed grade 0-II acute GVHD, while 3/5 patients treated with unselected PBPC (group A) developed grade III-IV GVHD, and 2 of them subsequently died at day +25 and +124, respectively. At a median follow up of 240 days (28-358), 8 patient are alive and well. These data suggest that positively selected allogeneic PB CD34+ cells induce a rapid and stable engraftment of hematopoiesis with a possibly reduced incidence and severity of acute GVHD.

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MOBILIZATION OF PERIPHERAL BLOOD PROGENITOR CELLS USING G-CSF IN HIV-1 INFECTED PERSONS. I.Zaia,* P. Yam,* S. Li,* H. Payne,* S. Herman,* L. Spiegelinski, B. Tegtmeier,* S. Forman, J. Ito.* City of Hope National Medical Center, Duarte CA and *Roche Molecular Systems, Somerville NJ.

G-CSF-based mobilization of peripheral blood progenitor cells (PBPC) has been associated with transient reduction in CD4 cell counts in normal donors (M. Korbling *et al.*, BMT in press, 1996). The purpose of this study was to determine the safety and effectiveness of granulocyte-colony stimulating factor (G-CSF) for mobilization of PBPC in HIV-1 infected persons. Seven HIV-1 infected persons with >200 CD4 cells and no evidence of AIDS were treated with G-CSF for 5 days (10 ug/kg day). Sixteen hours after the fourth dose of G-CSF, mononuclear cells (MNC) were harvested during a 12 L apheresis using a Fenwall CS-3000 cell separator, and CD34+ cells were enriched using an Ceprate® SC Stem Cell Collection System (CellPro Inc. Bothell WA). Clinical status, CD4 counts, plasma HIV RNA using RT-PCR (Roche), and HIV-1 infectivity assays of plasma and MNC were monitored for 6 months. Six subjects completed the apheresis without significant problems, and one failed to complete the apheresis because of inadequate venous access. During the 4 days of G-CSF treatment, there was a prompt mobilization of total leukocytes with a peak WBC range of 23-61 x 10^9/L and peak CD34+ percent of MNC of 0.5-2.3%, both at a median time of 4 days post-G-CSF. The apheresis products yielded a mean 3.6 x 10^9 MNC/kg (range 2.3-5.7 x 10^9/kg). CD34+ cell selection yielded a mean 2.8 x 10^6 cells/kg (range 1.1-5.4 x 10^6/kg). BFU-E and CFU-GM were within normal limits, but CFU-GEMM were reduced. Baseline CD4 counts (mean = 450) were reduced by a mean of 32% at one month post-apheresis ($p = 0.015$) and returned to levels not significantly different from baseline after 2 months. There was an increase in mean plasma HIV-1 RNA levels from baseline 6,310 genome copies per ml (gc/ml) to a peak of 12,600 gc/ml at 4 days post G-CSF ($p = 0.1$). At 3 months and 6 months after G-CSF, the plasma HIV RNA decreased to a mean of 4,000 gc/ml and 1,600 gc/ml, respectively ($p \geq 0.35$). Leukocyte and plasma infectivity assays were positive for HIV-1 before and after apheresis in all subjects and decreased during the time of cell mobilization. There were no changes in clinical status of the subjects during the 6 month period of observation. PBPC can be mobilized from HIV-1 infected persons using G-CSF, and functional CD34+ cells can be efficiently selected. CD4 counts are transiently depressed following apheresis without significant increase in plasma HIV RNA levels or change in clinical status.

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INHIBITION OF HIV-1 REPLICATION IN ANTI HIV-1 GENE EXPRESSING LONG TERM BONE MARROW CULTURES ESTABLISHED FROM CD34+ CELLS OF HIV-1 INFECTED DONORS G. Bauer,* S.F.Wen,* I.Banner,* K.Kaams,* P.Valdez,* J.Zaia,* and D.B. Kohn. Childrens Hospital Los Angeles, CA. City of Hope Medical Center, Duarte, CA.

Long term bone marrow cultures established from CD34+ cells isolated from cord blood or bone marrow of HIV-1 negative donors transduced with several retroviral vectors containing anti HIV-1 genes strongly inhibit HIV-1 replication after challenge with the macrophage tropic isolate HIV-1 JR-FL. To determine the feasibility of gene therapy for AIDS in individuals already infected with HIV-1, G-CSF mobilized peripheral blood CD34+ cells were isolated from HIV-1 infected individuals, and transduced with retroviral vectors containing three different anti HIV-genes: An RNA decoy vector overexpressing the rev binding domain of the Rev Responsive Element, L-RRE-neo, a double hammerhead ribozyme vector targeted to the tat and rev transcript, L-TR/TAT-neo, and a vector containing the transdominant mutant of M10 in the construct L-M10-SN. As a control, a vector mediating only neomycin resistance, LN, was used. After three days of transduction on allogeneic stroma in the presence of SCF, IL-6 and IL-3, the cultures were G418 selected, and challenged with HIV-1 JR-FL and a primary HIV-1 isolate.

Results: Compared to the control, the L-RRE-neo, L-TR/TAT-neo and L-M10-SN transduced cultures displayed up to 1000 fold inhibition of HIV-1 replication after challenge with HIV-1 JR-FL and a primary HIV-1 isolate. This preliminary study suggests that anti HIV-genes can be introduced into CD34+ cells from individuals already infected with HIV-1, and strongly inhibit HIV-1 replication in primary monocytes derived from CD34+ progenitors. As the presence of bone marrow stroma during retroviral transduction enhances gene transfer into CD34+ cells, and long term engraftment in recipients of transduced CD34+ cells, we evaluated the feasibility of using stroma from HIV-1 infected individuals to support transduction of CD34+ cells. A comparison between the growth rates of cultured stroma from HIV-1 negative and HIV-1 positive donors showed nearly identical proliferative capacity, and gene transfer into CD34+ cells from HIV-1 negative and HIV-1 positive donors was supported equally well by stroma from HIV-1 negative and HIV-1 positive individuals.

Conclusions: In all, these data support the feasibility of applying retroviral - mediated transduction of CD34+ cells from HIV-1 infected individuals for gene therapy.

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TRANSPLANTATION OF POSITIVELY SELECTED CD34+ BONE MARROW AND MOBILIZED PERIPHERAL BLOOD CELLS FROM HAPLOIDENTICAL RELATED DONORS FOR HIGH-RISK HEMATOLOGIC MALIGNANCIES. A.M. Yeager, C. Arasteh, T. Chauncey, J.E. DiPersio, H.Y. Holland, C.F. Lemaistre, W. Rubin, C.A. Jacobs,* and M.C. Bensinger. Emory University, Atlanta, GA, Fred Hutchinson Cancer Research Center, Seattle, WA; Veterans Administration Medical Center, Seattle, WA, Washington University, St. Louis, MO; South Texas Cancer Institute, San Antonio, TX, University of Pittsburgh, Pittsburgh, PA; and CellPro, Inc., Bothell, WA.

The lack of histocompatible related or unrelated donors limits the application of allogeneic bone marrow transplantation (BMT) for treatment of high-risk hematologic malignancies. For patients (pts) who lack HLA-matched donors, transplantation of selected CD34+ hematopoietic progenitor cells from haploidentical relatives provides an alternative source of stem cells with reduced numbers of T cells and might be associated with reduced risks of severe graft-versus-host disease (GVHD). To test this hypothesis, we evaluated transplantation of CD34+ cells selected with an avidin-biotin immunoadsorption column (CEPRATE SC System) from both G-CSF-mobilized peripheral blood cell (PBC) leukapheresis products and bone marrow collections from HLA-haploidentical related donors in 13 pediatric pts (median age, 6 yr; range, 1-13) and 11 adult pts (median age, 36 yr; range, 22-44) with high-risk acute lymphocytic leukemia (n=13), acute myelocytic leukemia (n=4), chronic myelocytic leukemia (n=4), myelodysplastic syndrome (n=2), or non-Hodgkin lymphoma (n=1). All pts received pre-transplant conditioning with fractionated total body irradiation (12-14 Gy), cyclophosphamide (60 mg/kg/d x 2 d), and anti-thymocyte globulin (30 mg/kg/d x 3 d) and post-transplant cyclosporine and short-course methotrexate. The median dose of CD34+ cells (x 10^6/kg) was 14.6 (range, 8.4-75.6) in pediatric pts and 10.4 (range, 3.7-15.6) in adult pts; the median dose of CD3+ cells (x 10^6/kg) was 2.2 (range, 0.2-6.3) in pediatric pts and 0.8 (range, 0.1-1.7) in adult pts. Twelve of 13 pediatric pts (92%) and 8 of 11 adult pts (73%) had donor neutrophil engraftment; median time to attain absolute neutrophil count >0.5 x 10^9/l was 12 d (range, 10-21) in pediatric pts and 20 d (range, 12-27) in adult pts. Two adult pts died without engraftment at 9 and 21 d, respectively, after transplant, and two pts (1 adult, 1 pediatric) had graft failure followed by autologous hematopoietic recovery at 33 and 36 d, respectively, after transplant. Eight of 12 evaluable pediatric pts (67%) and 2 of 8 evaluable adult pts (25%) had Grade 0-II acute GVHD; 4 pediatric pts (33%) and 6 adult pts (75%) had Grade III-IV acute GVHD. Chronic GVHD developed in 2 of the 7 pediatric pts (29%) and 2 of the 4 adult pts (50%) who survived >100 d after transplant. Deaths occurred in 7 pediatric pts (4 GVHD, 1 VOD, 2 progressive disease) and 10 adult pts (6 GVHD, 2 regimen-related toxicity, 2 sepsis). Six pediatric pts, including 1 with autologous recovery, are alive at a median of 51+ wk (range, 14+-66+) after transplant, and 1 adult (also with autologous recovery) is alive at 52+ wk after transplant. The probability of survival at 1 yr is 45% for pediatric pts and 10% for adult pts. We conclude that transplantation of positively selected CD34+ cells from haploidentical relatives is feasible and associated with prompt engraftment in the majority of recipients; however, risks of severe acute GVHD and of chronic GVHD remain. A study has been initiated in children with hematologic malignancies to evaluate additional T cell depletion and transplantation of CD34+ PBC products from haploidentical related donors.

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AUTOLOGOUS PERIPHERAL BLOOD SELECTED CD3+ CELL TRANSPLANTATION FOR THE TREATMENT OF SEVERE PROGRESSIVE SYSTEMIC SCLEROSIS

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Autologous bone marrow transplantation has been recently proposed as potentially curative treatment for severe or poor-prognosis autoimmune diseases. In view of results obtained in animal models, we decided to give an autologous peripheral blood stem cell (PBSC) transplant to an 11-year-old girl affected by systemic sclerosis with progressive lung fibrosis, but without pulmonary hypertension. After having obtained approval of the local Ethical Committee and written informed consent of the parents, the child received a mobilizing chemotherapy consisting of cyclophosphamide (CY) at a dose of 4 gr/m^2, followed by the administration of G-CSF at a dose of 10 μg/Kg/day. Two leukapheresis procedures were performed on day +12 and day +13, respectively. The number of PBSC collected was 4.3 x 10^6/Kg. A three-log T-cell depletion was performed as CD34+ cell positive selection by means of Ceprate SC, and purified CD34+ cells (percentage recovery 70%) were subsequently cryopreserved. Pre-transplant conditioning regimen consisted of CY at a dose of 50 mg/Kg from day -5 to day -2 and the monoclonal antibody Campath-1G at a dose of 10 mg/day for 2 days. After thawing, the total infused CD34+ cell count was 3.5 x 10^6/Kg; the residual T lymphocyte dose was 3 x 10^4/Kg. G-CSF was administered after transplant at a dose of 5 μg/kg/day for 12 days. The early post-transplant period was uneventful; neutrophil and platelet engraftment (PMN > 0.5 x 10^9/L and PLT > 50 x 10^9/L) was achieved on day +11 and day +14, respectively. The patient was discharged on day +24 and she is alive and well, with a normal blood count 70 days after the transplant procedure. The immunological evaluation performed 2 and 4 weeks after transplantation showed a moderate reduction of mature T lymphocytes (patient's CD3+ cells = 50%; normal controls = 65-85%) and profound impairment of the proliferative response to phytohaemoagglutinin, concanavalin-A and anti-CD3 monoclonal antibody (< 5% of normal control subjects). This pattern of immunological recovery is similar to that normally observed after unmanipulated autologous BMT. Our experience demonstrates the feasibility and safety of this procedure in children affected by severe autoimmune diseases. A longer follow-up and careful monitoring of signs and symptoms of the original disease will be necessary in order to evaluate the efficacy of the treatment.

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CROSS-REACTIONS OF ANTI-CD34 AND OTHER HAEMATOPOIETIC MONOCLONAL ANTIBODIES TO NEUROBLASTOMA CELLS. A. Voigt,* R. Hafer,* and E. Ziegler* (Intr. by P.D. Wickramanayake) University of Jena, Department of Pediatrics, Jena, Germany.

Peripheral blood stem cells (PBSC) are being used with increasing frequency as a progenitor cell source after myeloablative irradiation or high-dose chemotherapy for neuroblastoma patients. But more and more evidences of circulating tumour cells in blood are found so that PBSC harvests may contain viable, high clonogenic tumour stem cells carrying the risk of being reinfused into the patient. To reduce the potential risk of tumour contamination harvested mononuclear cells can be purged with a selection of anti-CD34 monoclonal antibodies (moAbs). In the present study we have examined the surface membrane antigens of six freshly obtained neuroblastoma tumour specimens and eight permanent, six primary, and four SCID-mouse passaged cell lines with the use of a panel of moAbs developed against haematopoietic cells including the CD34 moAbs BirmaK3 (DAKO), ICH3 (MEDAC), Qbend10 (SEROTEC), and 12.8 (CellPro) respectively, and moAbs primarily developed against neuroblastoma cells by flow-cytometric analysis. We separated the non-adherent, small round-shaped clonogenic neuroblastoma cells from the adherent cells of permanent and SCID-mouse passaged neuroblastoma cell lines comparing this in vitro modell system with the circulating tumour cells in patients and analysed the cell surface expression of CD34 antigen. As a result the majority of neuroblastoma cell lines shared haematopoietic-associated antigens with B (10-92%) and T (0-75%) cells, myeloid cells and monocytes (0-100%) as well as with megakaryocytes (0-80%). In literature, there is reported that the CD34 antigen may be involved in cell adhesion processes and in „homing" interactions between stem cells and the stroma of bone marrow. In fact, we found high reactions of ICH3 (90%) and BirmaK3 (75%) in the lower differentiated non-adherent neuroblastoma cells characterized by a diminished adhesion capability in compariton with the adherent cells (35% and 29%, respectively). Similar results are produced with SCID-mouse passaged high clonogenic neuroblastoma cells immediately after tumour resection in contrast to further cultivated and differentiated cells. Results of the considerable specific cross-reactions of anti-CD34 moAbs to neuroblastoma cells were to be found in an experiment of stem cell selection of a bone marrow morphologically free of neuroblastoma cells. After preparing bone marrow with the biotinylated 12.8 moAb and its passage through a column of avidin-coated polyacrylamide beads we observed a neuroblastoma cell clone growing up from the selected stem cells at the 21st day of in vitro culture. In conclusion, these cross reactions found with haematopoietic moAbs, especially the CD34 moAbs ICH3 and 12.8, indicate that there is a potential risk of accumulation of just the circulating neuroblastoma cells during selection of CD34+ cells from eventually tumour cell contaminated bone marrow or peripheral blood. Therefore, the stem cell selection with CD34 moAbs should be performed only with tested moAbs before and with precaution in neuroblastoma patients.

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PREVENTION OF GRAFT-VERSUS-HOST-DISEASE BY TRANSPLANTATION OF ALLOGENEIC CD34$^+$ BLOOD CELLS ADDITIONALLY T-CELL DEPLETED WITH CAMPATH-1H. B. Hermanstein,* L. Arseniev,* I. Novotny,* K. Barmer,* A. Stucki,* I. Stückmann,* I. Bunjes,* J.G. Kadar,* G. Hale,* H. Waldmann,* and A. Ganser. Dept. Hematology and Dept. of Transf. Med., Hannover Medical School, Germany and Dept. Pathology, University of Oxford, UK

The transplantation of immunoselected of allogeneic CD34$^+$ blood cells provides rapid and stable hematopoietic recovery, is, however, alone not sufficient as effective GvHD-prophylaxis. We evaluated whether an additional T-cell depletion of the immunoselected CD34$^+$ blood cell grafts could further reduce acute GvHD and eliminate the need for post-transplant immunosuppressive treatment. Five patients (3 CML, 1 AML, 1 NHL, median age 29 years, range 25-38, 1 male, 4 female) received G-CSF mobilized (5µg/kg s.c. bid) peripheral blood progenitor cells (PBPC) from HLA-identical sibling donors. CD34$^+$ cells were selected by immunoadsorption (Ceprate® SC, CellPro, Bothell, WA) and frozen. The nonselected T cells were poricined and stored separately. The conditioning regimen consisted of TBI (12Gy) and Cy (120mg/kg). According to the results from T-cell depleted bone marrow transplantation Campath-1H was given i.v. prior to conditioning for prophylaxis of graft rejection (20mg/d, d -11 to -7). At transplantation the CD34$^+$ cells were thawed and Campath-1H was added (10mg/150ml). Campath-1H labelled > 99% of the residual T-cells. The graft was transfused without further ex vivo manipulation within 30-40 min. Median transplanted cell numbers were: 3.3 CD34$^+$, 0.21 CD3$^+$ and 0.31 CD52$^+$ Campath-1H labelled cells x10^6/kg. The patients received G-CSF (5µg/kg/d, s.c.) post-transplant and no further GvHD prophylaxis was given. All patients engrafted. No graft failure or rejection were observed so far (follow up 30-85 days). Median recovery time of neutrophils to reach 500 and 1,000/µl was 11 and 13 days, respectively. Median recovery time of platelets to reach 50,000/µl was 24 days. The last platelet (median 4 units/patient) and RBC (median 6 units/patient) transfusions were required on median days 10 and 11, respectively. One patient with active CMV infection and antiviral treatment did not achieve 50,000/µl platelets up to day 30. None of the patients developed acute GvHD. Lymphocytes began to recover after day 35 with an inverted CD4/CD8 ratio of 1:4 and greater proportions of CD56$^+$ cells (3-24%). All three patients seropositive for CMV developed CMV antigenemia (day -1, 14 and 43). Preemptive ganciclovir or foscarnet treatment was given and no progress to CMV-disease occurred. One CML patient received donor T lymphocytes at day +80 because a switch to bcr-abl PCR-positivity was demonstrated. The transplantation of T-cell depleted allogeneic CD34$^+$cells prevented effectively acute GvHD while preserving the rapid hematopoietic reconstitution seen with PBPC grafts. Since no immunosuppressive treatment is used post-transplant, this approach provides appropriate conditions for induction of GvL with donor lymphocyte transfusions. Whether this will translate in improvement of disease free survival and whether the high rate of active CMV infections will cause clinical problems remains to be determined.

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ENHANCED DETECTION OF BREAST TUMOR CELLS BY IMMUNOCYTOCHEMISTRY FOLLOWING ENRICHMENT USING AN AVIDIN AFFINITY COLUMN. T.J. Layton,* A.B. Ormander,* M.L. Kennedy, E.J. Shpall, R.B. Jones, L. Hami,* A.A. Ross.* CellPro, Inc., Bothell, WA, Johns Hopkins Oncology Center, Baltimore, MD, and Univ. of Colorado Health Sciences Center, Denver, CO.

The ability to detect very low levels of tumor cells in blood or bone marrow is useful for detection of micrometastatic disease and appears to have prognostic significance. The presence of micrometastases in primary breast cancer patients at the time of surgery is significantly associated with poor prognosis and early relapse (Cote, et al. *J Clin Oncol* 9:1749, 1991). The presence of micrometastases has also been shown to be associated with reduced disease-free and overall survival in the high-dose chemotherapy, autologous hematopoietic stem cell transplantation setting (Vredenburgh, et al. *Proc Amer Soc Clin Oncol* 14:316, 1995). Current detection methods (Immunocytochemistry, PCR) lack the sensitivity to detect beyond $1:1x10^6$, while tumor cells may be contaminants at levels lower than this. The sensitivity of current methods of detection may be increased significantly if combined with a tumor enrichment method that allows for the selective capture of micrometastatic tumor cells. We have developed a system to enrich for tumor cells from bone marrow, apheresis product, and peripheral blood that can isolate one tumor cell in $1x10^6$ hematopoietic cells. Using this laboratory-scale avidin-affinity column system we have shown between 2 and 3 logs of enrichment of seeded tumor cells. The isolated tumor cells were capable of in vitro growth. A monoclonal antibody that is specific for a membrane-associated antigen, PAN-05 (NeoRx Corp, Seattle, WA), is used for positive selection of the tumor cells. Post-enrichment detection of tumor cells is accomplished by means of a sensitive immunocytochemical (ICC) assay using a cocktail of anti-cytokeratin antibodies. In addition, 18 stage II and 6 stage IV breast cancer patient apheresis samples have been processed on this system and analyzed by ICC. Of the 6 stage IV samples, only one showed an ICC-positive cell prior to enrichment, but this same sample showed 7 tumor cells post-enrichment. Two of the 5 samples (33%) converted from ICC-negative to ICC-positive following enrichment for tumor cells. Of the 18 stage II patient apheresis specimens, 3 (17%) have shown ICC-positive cells following enrichment. However, suboptimal morphology of these cells resulted in histopathologically inconclusive results. None of these samples was ICC-positive prior to enrichment. One sample had a single ICC-positive cell prior to enrichment, but was ICC-negative following enrichment. We are continuing to test samples from stage II and stage IV breast cancer patients. This tumor cell enrichment system permits a rapid, sensitive, and specific method for the enrichment of extremely low numbers of tumor cells in hematopoietic products.

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IMMUNOCHEMICAL CHARACTERIZATION OF THE PROTEIN SEQUENCE OF HUMAN CD34 USING AN IMMOBILIZED PEPTIDE ARRAY. H.M. Jones,* B.L. Fogarty,* M.S. Lodge,* R.W. Seminger,* and S.J. Tamowski,* (intr. by S Rowley) CellPro Inc. Bothell, Washington, USA.

The precise role of the CD34 molecule present on hematopoietic stem cells and progenitors remains unclear, as a ligand for the human CD34 molecule has not yet been definitely identified. CD34 may simply play a role in adhesive interactions with other cell types within the hematopoietic microenvironment or alternatively, directly modulate hematopoietic cells through cellular signaling by binding of a specific ligand. Antibodies against CD34 in addition to being valuable reagents for the isolation of stem cells are useful tools with which to examine conformationally distinct regions of this cell surface molecule. In particular, mapping of the different peptide regions recognized by antibodies to CD34 may provide clues to the identity of a ligand for the CD34 molecule. Using a published amino acid (AA) sequence (Nakamura *et al.*, Exp. Hematol. 21, 236-42) we produced an array of 125 different overlapping peptides scanning residues AA 29 - 291 of the extracellular domain of human CD34. For the initial series of experiments each peptide was generated as a 13 mer anchored onto a derivatized cellulose support with individual peptides overlapping the previous sequence of residues by two AA. Individual test antibodies were applied to the membrane and incubated for 3 hours at room temperature. This was followed by washing and subsequent detection of any antibody bound to the membrane with a β-galactosidase conjugated anti-mouse IgG antibody and a chromogenic substrate. With this system we have been able to examine a variety of CD34 monoclonals (Mcabs) and determine which Moabs recognize discrete epitopes not wholly dependent on the presence of carbohydrate moieties. For example, the Moab QBEND10 recognized 4 peptides spanning AA 37 - 55 and the ICH3 Moab recognized 4 peptides spanning AA 49 - 67. Studies are ongoing to determine the precise length and boundary residues of the regions recognized by reacting Moabs. Screening by this method has allowed the further delineation and classification of the various antibodies within the CD34 monoclonal cluster. Additionally, the short, linear, peptide sequences identified using this technique provide an alternative form of immunogen for the generation of new CD34 Moabs. Furthermore, we conclude that this is a rapid and informative means of discovering and characterizing peptide epitopes. Finally, we believe that the characterization of these peptide sequences may provide both tools and insights enabling the identification of potential ligands for the CD34 molecule.

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MANAGEMENT OF HEMATOLOGIC RELAPSE WITH DONOR LEUKOCYTE INFUSIONS: EVALUATION OF THE CEPRATE SYSTEM FOR POSITIVE SELECTION OF LYMPHOCYTE SUBSETS. G. Risdon,* M. Emde,* J. Petersons,* N. Saund,* and K. Auditore-Hargreaves.* CellPro, Inc., Bothell, WA USA. Introduction by T. Keenan.

Durable remissions can be achieved in patients with relapsed chronic myelogenous leukemia (CML) by infusion of donor allogeneic blood lymphocytes. Such infusions however are complicated by acute and chronic GVHD and marrow aplasia. Optimally, the treatment of relapsed CML would involve the identification and infusion of the lymphocyte population(s) responsible for the anti-leukemia effect (GVL) in the absence of GVHD. To this end, we have evaluated the CellPro CEPRATE separation system for the isolation of large numbers of CD4, CD8 and CD56-positive cells from peripheral blood leukapheresis. This system permits the rapid isolation of as many as $5x10^9$ cells with a purity typically greater than 90%. These isolated CD4 and CD8 T cells maintain proliferative responses to PHA, anti-CD3 and alloantigens and cytotoxic activity against allogeneic targets. Strong cytolytic activity against K562 targets is maintained in CD56 selected cells. IL-2 induced LAK activity against HL-60 targets is observed with all populations following short-term culture. The clinical-scale isolation of functional T and NK cells is the first step in developing effective salvage and prophylactic immunotherapy against leukemic relapse. These positively selected cells are also candidates for ex-vivo expansion, activation and genetic modification prior to infusion.

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IDENTIFICATION OF NOVEL HEMATOPOIETIC GROWTH FACTORS THAT PROMOTE THE EX-VIVO EXPANSION OF HEMATOPOIETIC PROGENITOR CELLS. N.J.Minster,* L.S.Kaiza,* S.L.Ellison,* A.M.Donnelly,* J.G.Gist,* J.P.McKearn,* and P.R.Streeter*. (Intr. by P.D.Dosik). Searle Discovery Research, St. Louis, MO

Autologous stem cell transplantation following high dose chemotherapy results in an 8 - 12 day window before patient neutrophils and platelets recover to safe levels. Ex-vivo expansion and subsequent transplantation of autologous hematopoietic progenitor cells offers the potential of dramatically reducing or eliminating these cellular deficiencies by hastening patient recovery. We engineered and characterized a series of synthetic cytokines with distinct growth and differentiation promoting activities including Daniplestim (Syntheikine), Myelopoietin and Promegapoietin which were designed to drive proliferation and differentiation of neutrophil and megakaryocyte progenitors. The goal of this study was to define optimal conditions for multilineage ex-vivo cell expansion. The ex-vivo cell expansion potentials of these cytokines were evaluated using immunoaffinity purified normal bone marrow-derived CD34+ progenitor cells. The isolated CD34+ enriched cells were cultured with novel hematopoietic growth factors for a period of 10 - 12 days in X-Vivo 10 media supplemented with 1% HSA. Cultured cells were analyzed for total cellular expansion and expansion of neutrophil (CD11b/CD15) megakaryocyte (CD41a) lineage committed cells. For Daniplestim (Syntheikine), Myelopoietin and Promegapoietin alone a mean of 40%, 55%, and 29% of the expanded cells expressed CD15 respectively, while the combination yielded 43% of these cells expressing CD15. Similarly, for Daniplestim (Syntheikine), Myelopoietin and Promegapoietin alone a mean of 14%, 26%, and 44% of the expanded cells expressed CD41a respectively, while the combination yielded 36% of these cells expressing CD41a. Our results demonstrated that optimal concentrations of cytokines tested alone, or in combination, resulted in significant cellular expansion and that exposure to Promegapoietin or Myelopoietin yielded a selective expansion of megakaryocyte or myeloid lineage committed cells. This study demonstrated that these novel hematopoietic growth factors can significantly expand progenitors as well or better than other published ex vivo expansion protocols. Transplantation of these ex-vivo cultured, expanded and differentiated CD34+ enriched progenitor cells may shorten chemotherapy induced patients neutropenia and thrombocytopenia.

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IMMUNOPHENOTYPIC ANALYSIS OF UMBILICAL CORD BLOOD. IN VITRO EXPANSION OF THE CD34+ ENRICHED FRACTION. A POTENTIAL SOURCE OF TRANSPLANTABLE HEMATOPOIETIC STEM CELLS.

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Bone marrow transplantation is limited by the paucity of HLA-matched donors and the frequent occurrence of GvHD. Hematopoietic transplants using CB cells are being increasingly used in pediatric patients. Early estimates suggest that there may not be enough hematopoietic progenitor cells in an average cord blood sample to reconstitute adult patients. We investigated the in vitro proliferative potential of CB CD34+ selected cells in order to increase the progenitor pool from CB. We studied the phenotypic characteristics of UCB mononuclear cells (n=25) by one and two color flow cytometry. CD34+ cells were isolated using a Ceprate LC-34 Biotin kit. In clonogenic assays CFU-GEMM, CFU-GM and BFU-E were quantified by methylcellulose culture in both mononuclear cells and CD34+ enriched fractions after the addition of different combinations of Epo, IL3, GM-CSF, G-CSF and SCF. The CB cells were characterised by a low protection of CD3+ T cells, increased CD4/CD8 ratio, minimal expression of HLA-DR and increased proportion of CD5/CD19 double possitive B cells. All these probably reflect the immaturity of CB lymphocytes contributing to decreased GvHD. The number of CFU-GM, CFU-GEMM, BFU-E in cultures of CD34+ enriched cells had increased 34-65-225 fold respectively over the cell cultures of mononuclear cells. The addition of SCF expanded the CFU-GM and CFU-GEMM colonies 3 fold in the CD34+ enriched fraction whereas it had no influence on the mononuclear cells fraction. The influence of SCF was minimal on the BFU-E formation in both CD34+ enriched and mononuclear cell fractions when added to GM-CSF, IL3 and Epo. We noticed though that the combination of Epo and SCF increased by 3,7 the BFU-E numbers in the CD34+ enriched fraction. These data demostrate that CD34+ enriched UBC progenitor cells have greater clonogenic capacity compared to UCB mononuclear cells and they are more sensitive to the addition of SCF. We therefore suggest that cord blood can be a valuable alternative to bone marrow or peripheral blood as a source of pluripotent hemopoietic stem cells and additional investigation is necessary to establish whether ex vivo expanded cord blood progenitors will engraft adult patients as efficiently.

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3719

QUANTITATION OF CD34+DR+/CD34+DR- CELLS BEFORE AND AFTER IMMUNOSELECTION OF CD34+ CELLS FROM G-CSF MOBILIZED PERIPHERAL BLOOD PROGENITOR CELLS (PBPC).

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Positive selection techniques to isolate CD34+ cells are increasingly used for hematopoietic progenitor cell enrichment. However, between 30% to 70% of progenitor cells may be lost by using these methods. To analyse whether this loss affects predominantly CD34+DR+ or CD34+DR- subsets, we studied the phenotypic characteristics of CD34+ cells from G-CSF-mobilized PBPC before and after positive selection using the immunoadsorption avidin-biotin method (CEPRATE SC). Twelve healthy subjets, median age 34 years (21-61), received G-CSF 10μg/kg/d s.c. x 5 d. On day 5 and 6 donors underwent 10 L leukapheresis using a CS3C0 plus cell separator The sum of the two apheresis products was incubated with a biotinylated anti-CD34McAb (12.3; CellPro) and then passed through a column of avidin-coated polyacrylamide beads. Bound cells were removed from the column by agitation and rinsing and were collected. Flow cytometry (FC; FACScan, Becton Dickinson) was performed on the original PBPC and on the immunoselected product bag. McAb used for FC included CD3, CD34, HLA-DR, and a isotype control. Quantitation of CD34+ subpopulations was performed by analysis of a total of 300,000 (PBPC product) or 50,000 cellular events (selected product). Recovery and purity of CD34+ cells after positive selection were of 64% and 57.6%, respectively. Results of FC analysis of CD34+ cell subsets before and after the selection procedure are shown in the table. A 62% of the CD34+DR+ cells presents in the original PBPC products was recovered after immunoselection, as compared to 24% of the CD34+DR- cells.

Cell fraction	Pre-column	Post-column
CD34+ cells (%)	0.70 (0.37-1.70)	57.6 (37.0-82.4)
CD34+ cells x 10⁶/kg*	6.5 (2.8-13.4)	4.25 (2-8.63)
CD34+HLA-DR+ x 10⁶/kg*	6.54 (2.78-12.78)	4.06 (1.96-8.44)
CD34+HLA-DR- x 10⁶/kg*	0.21 (0.02-0.61)	0.05 (0.01-0.43)

Values are expressed as median and ranges. * kg of receptor.

In conclusion, this method allows a high enrichment of CD34+ cells and is associated to a predominant recovery of CD34+ DR+ cells.

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RAPID ENGRAFTMENT WITH MINIMAL GVHD AFTER HLA-MISMATCHED DOUBLY SELECTED T-CELL DEPLETED BLOOD CELL PRECURSOR TRANSPLANTS (BCPT). H. Atkins*, C. Bredeson*, I. Bence Bruckler*, S. McDiarmid*, H. Hoggins*, A. Guilfyl, L. Huebsch*. BMT program, Ottawa General Hospital, Ottawa, Ontario.

Three pts with ALL (2-post BMT relapses, 1-1st relapse) received Cy (120 mg/kg), fTBI (200 cGy x 5) and rabbit ATG (1.25 mg/kg x 4) (1 pt) or Bu (16 mg/kg), Cy (200 mg/kg) and rATG (1.25 mg/kg x 4) (2 pts). BCPT followed using a graft from the patient's father (2 donors - 2 antigen HLA mismatch, 1 donor - 3 antigen HLA mismatch). Patients received G-CSF (300 ug/d sc until ANC>750) and Cyclosporin post BCPT. Donors received 7 days of G-CSF (~16 ug/kg/d). Leukopheresis was done on the 4th to 6th day and bone marrow harvest was done on the 7th day. Moderate thrombocytopenia (38 K, 65 K and 128 K) occurred in all donors. Because of thrombocytopenia, the marrow harvest was deferred in 1 donor. Purified,T cell depleted BCPs were isolated using Cellpro CD34(+) selection and Baxter Maxsep CD2(-)/CD8(-) depletion. Procedure parameters are given below:

	CD34 enrichment	CD34 recovery	Log T cell depletion
CD34(+) selection	114-319 x		2.58 - 3.31
T cell depletion	1.06 - 1.15 x	47 - 87 %	1.25 - 1.78
Overall	190 - 353 x		3.92 - 4.93

Grafts contained 252 to 406x10^6 CD34(-) cells and 0.9 to 3.5x10^6 CD3(+) cells. All patients had ANC > 1000 by d13. An untransfused platelet counts of 20x10^9/L for 3 days was achieved by d22 and d42. One pt did not become independent of platelet transfusions. ABO switching was documented at d35 and d49 in patients with ABO mismatched BCPT. The maximum ALC ranged between 0.1 to 0.68x10^9/L. Major toxicities were transient pulmonary edema that required intubation following rATG infusion (1 pt), acyclovir resistant HSV mucositis and esophagitis (1 pt), CMV reactivation (1 pt) and CMV pneumonia (1 pt) and severe hemorrhagic cystitis (1 pt). Grade II acute skin GvHD that rapidly responded to 1 mg/kg/d prednisone occurred in 2 pts. There was no acute GvHD of other organs. VOD and renal insufficiency did not occur. One pt relapsed at d84 and died at d144. One pt died of CMV pneumonia at d51. The 3rd pt died of progressive cachexia and hemorrhagic cystitis at d132. This report demonstrates: 1.) Sufficient purified blood cell precursors are present following a positive selection and negative depletion to allow alloengraftment across HLA disparities, while significantly depleting T cells. 2.) 4 to 5 log T cell depletion can be achieved to minimize the risk of GvHD in HLA mismatched allogeneic BCPT recipients. 3.) Life-threatening viral infections occur commonly in these patients and their treatment needs to be addressed in future studies.

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A COMPARISON OF RETROVIRAL TRANSDUCTION PROTOCOLS FOR GENE TRANSFER INTO RHESUS CD34+ BONE MARROW CELLS.

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Retroviral gene transfer into primate and human long term repopulating hematopoietic stem cells (HSC) is inefficient when compared to murine HSC. Utilizing existing methodologies employed in these murine studies, we have evaluated four gene transfer protocols for the transduction of HSC in a rhesus transplant model. They are the following: 1) co-culture with retroviral producers, 2) retroviral supernatant infection, 3) *in vivo* priming with cytokines, and 4) immunoselection of transduced cells prior to infusion. The transferred marker gene, CD4z, is a chimeric immune receptor composed of the human CD4 extracellular domain and the zeta (z)-chain of the CD3 T-cell receptor. Cell surface expression of this chimeric protein facilitates rapid determination of transduction efficiency by FACS analysis. A transient retroviral transduction system, *kat*, was used which yields vector titers of 1-2 x 10^7/ml as assayed on NIH 3T3 cells. Eleven monkeys have been transplanted using total body irradiation (650 cGy x 2) as the conditioning regimen. In 4 animals, autologous CD34-enriched bone marrow (BM) cells were prestimulated with SCF and IL-6 for 48 hours and then co-cultured with retroviral producers for 48 hours in the presence of SCF, IL-6, and polybrene. Despite *in vitro* transduction efficiencies of 25-40% as measured by FACS analysis, detectable CD4z expression in peripheral blood (PB) or BM was absent when analyzed by FACS or PCR post transplant. In 4 subsequent animals, CD34-enriched BM was transduced with retroviral supernatant for 96 hours on plates coated with anti CD34 mAb. *In vitro* transduction efficiencies were 10-45%. In 3 of the 4 primates, 1-3% of PB cells expressed CD4z at 1 month post transplant. By day 90 however, CD4z could be detected in only 1 of the 4 animals(0.5% by PCR). These results might represent transduction of short-lived hematopoietic progenitors. An additional 2 animals underwent *in vivo* priming with SCF and G-CSF for 5 days prior to bone marrow harvest in an attempt to improve stem cell transduction efficiency. Transduced CD4z-expressing cells were subsequently purified by cell sorting. In this protocol the sorted populations (>95% CD4+, 2-4 x10^6 cells/animal) were re-infused. This represented 5-70 fold fewer cells than the unsorted inoculums used in earlier transplants. Engraftment was delayed in both animals. In the first animal, CD4z was detected in 2% of PB cells at day 30 and 0.5% at day 75 . The second animal was negative by PCR. The delayed engraftment observed with unmarked cells in these animals is consistent with late endogenous hematopoietic reconstitution despite what was presumed to be a myeloablative dose of irradiation. Furthermore, this conditioning regimen is associated with a high transplant-related mortality (7/11 primates died within 3 months of transplant). Based on these studies, we conclude that 1) the CD4z chimeric receptor is efficiently expressed in hematopoietic cells at early time points following reconstitution; 2) supernatant transduction of rhesus CD34+ cells appears to be superior to co-cultivation with retroviral producers, and 3) re-infusion of sorted, transduced cells does not enhance gene transfer efficiency *in vivo*.

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3133

IN VITRO EXPANSION OF CD34+DR+/- CELLS FROM BONE MARROW OF ACUTE MYELOID LEUKEMIA (AML) IN FIRST REMISSION. Ph. Herman[1], A. Ferrant[1], H. Van Den Berghe[2], J.L. Michaux[1], N. Straetmans[1]. [1]Haematology CPT, UCL, Brussels [2]Centrum voor Menselijke Erfelijkheid KUL Leuven, Belgium

Selection of a residual normal stem cell population and expansion of this population *in vitro* could theoretically provide a graft devoid of malignant cells able to promptly reconstitute hematopoiesis after autologous transplant for AML in CR1. We hypothesized that the CD34+DR+/- fraction could be suitable to this approach. Aliquots of BM harvest from patients (pts) with AML in CR1 (n=9) were enriched in CD34+ cells by immunoaffinity column and cryopreserved until use. After thawing, CD34+ cells were sorted by FACS into CD34+DR+ and CD34+DR+/- fractions. Cell fractions were thereafter cultured in medium conditioned by an allogeneic irradiated normal human stroma supplemented with IL3, IL6, IL11, SCF ± G-CSF for 21 days. Cultures were weekly cell-depopulated and refed. CFU-GM, HPP-CFC and BFU-E content was assessed initially and at weekly intervals. LTC-IC were semi-quantified initially and after 2 weeks of liquid culture. For pts with an abnormal karyotype at diagnosis, karyotype was assessed initially and after 2 weeks of liquid culture. These results were compared to those obtained with normal (nl) BM taken from allogeneic donors (n=15). Cellularity of BM harvests from pts was low compared to nl and their percentage of CD34+DR+/- cells was decreased, resulting in the recovery of small numbers of CD34+DR+/- cells after sorting. Initially, the number of CFU-GM/ 10^4 CD34+DR+/- cells was strongly decreased compared to nl (70±37 *vs* 208±120, p=0.003) whereas it was not different from nl for the CD34+DR+ fraction (643±448 *vs* 723±334, p=0.5). The number of HPP-CFC produced by the 2 subpopulations was lower than nl (DR+/-: 122±141 *vs* 403±282, p=0.005; DR+ 203±152 *vs* 380±211, p=0.05). Initial numbers of LTC-IC/10^4 cells were decreased compared to nl (DR+/-: 60±81 *vs* 397±283, p= 0.0008; DR+: 55±33 *vs* 149±90, p=0.003). For AML BM cells, CD34+DR+ and DR+/- subpopulations generated equivalent numbers of LTC-IC, whereas in nl the CD34+DR+/- fraction was enriched in LTC-IC compared to the CD34+DR+ fraction. LTC-IC generated by CD34+DR+/- cells were almost equivalently distributed between the supernatant and the adherent layer when nl LTC-IC were preferentially located in the adherent layer. CFU-GM expansion from pts CD34+DR+/- cells was poor and decreased after d14 of culture (maximal CFU-GM expansion for pts: x10±8 at d14 ; for nl: x65±24 at d21). Therefore, given their low initial cloning efficiency, pts CD34+DR+/- cells generated 10 times less CFU-GM than nl *in vitro* (i.e. at d14, 948 *vs* 10,870 CFU-GM/10^4 cells cultured). In almost all instances, no HPP-CFC expansion could be observed for pts CD34+DR+/- cells. Nearly no LTC-IC could be found after 14 days of liquid culture (median 0, range 0-4% compared to initial input). Karyotype was evaluable for 4 pts. Very few mitoses could be observed directly after sorting. After 14 days expansion, all the cell fractions analysed so far were karyotypically normal (12-50 mitoses analysed). In conclusion, although possibly devoid of leukemic cells, the BM CD34+DR+/- cell fraction from pts with AML in CR1 show profound hemopoietic defect *in vitro*, is not enriched in LTC-IC and does not give rise to satisfactory expansion. Further understanding of the mechanisms of this defective hematopoiesis is required to make expansion feasible.

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CELL CYCLE STATUS AND KINETICS OF CD34$^+$ SUBPOPULATIONS FROM UMBILICAL CORD BLOOD, BONE MARROW AND MOBILIZED PERIPHERAL BLOOD. De Bruyn C.*,Delforge A.*, Bron D., Bernier M.*, Stryckmans P. Institut J. Bordet, Brussels, Belgium.

We have compared the cell cycle status of CD34$^+$CD38$^-$ and CD34$^+$CD38$^+$ cells from umbilical cord blood (UCB) (n=12), bone marrow (BM) (n=3) and peripheral blood progenitor cells mobilized by G-CSF after chemotherapy (PBPC) (n=7). The mononucleated cell fraction was enriched in CD34$^+$ cells using Ceprate LC (CellPro) systems. Cell cycle status of CD34$^+$CD38$^-$ and CD34$^+$CD38$^+$ cells was assessed using a flow-cytometer by double-labelling analysis using propidium iodide for DNA content and FITC-conjugated monoclonal antibody for CD38 expression. The results were expressed as the percentage of CD34$^+$, CD34$^+$CD38$^-$ and CD34$^+$CD38$^+$ cells in proliferative phase of the cell cycle (S+G2M), and summarized in the table below:

	CD34$^+$	CD34$^+$CD38$^-$	CD34$^+$CD38$^-$	CD34$^+$CD38$^+$	p
UCB	9.4±1.2	46.9±10.9	4.7±0.7	21.2±2.4	-
BM	14.3±1.5	25.3±2.5	4.8±1.1	8.1±1.2	0.006
PBPC	6.6±1.3	30.5±8.4	2.8±0.7	11.2±4.1	ND

* cells at day 0 without any stimulation * cells cultured for 48 hours with SCF, IL-3 and IL-6
p significant difference between UCB and BM DNA content after 48 hours culture.

Cell cycle analysis indicated that (a) the large majority of CD34$^+$ cells from CB as from BM and PBSC were quiescent, (b) compared to CD34$^+$CD38$^-$ fraction, significantly more CD34$^+$CD38$^+$ cells were in active phase of the cell cycle , (c) there was no significant difference when the DNA content of UCB, BM and PBPC was compared, and that was true for each progenitor fraction (CD34$^+$, CD34$^+$CD38$^+$ or CD34$^+$CD38$^-$ fraction). After 48 hours incubation of CD34$^+$ cells in Iscove's modified Dulbecco's medium containing 10% of fetal calf serum and stimulated by a combination of SCF(10 ng/ml), IL-3 (100 U/ml) and IL-6 (100 U/ml), BM and PBSC CD34$^+$CD38$^-$ cells remained essentially quiescent with only 8.1±1.2 % (n=4) and 11.2±4.1% (n=3) of the cells in DNA synthesis, in comparison to 21.2 ± 2.4 % (n=4) for CB CD34$^+$CD38$^-$ cells. In conclusions, assuming a similar duration of DNA synthesis in the various fractions, our results show that (1) there was no significant difference between the cell cycle status of the three sources of hematopoietic cells as well as for the different subpopulations of CD34$^+$ cells studied (2) the proportion of cells in active cell cycle in the CD34$^+$CD38$^+$ and the CD34$^+$CD38$^-$ subpopulations is significantly different, the first one being more quiescent that the second one, this observation has been confirmed for CB, BM and PBSC (3) although we did not observe any significant difference between cell cycle status of CB, BM and PBSC CD34$^+$CD38$^-$ fractions before in vitro culture, the CB CD34$^+$CD38$^-$ population showed greater proliferative response to stimulation by CSFs.

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CYCLING STATUS OF CD 34+ CELLS MOBILIZED INTO PERIPHERAL BLOOD OF HEALTHY DONORS BY RECOMBINANT HUMAN GRANULOCYTE COLONY-STIMULATING FACTOR (G-CSF). Roberto M. Lemoli, Agostino Tafuri, Alessandra Fortuna*, Maria Teresa Petrucci, Maria Rosaria Ricciardi*, Luisa Catani*, Damiano Rondelli*, Miriam Foglì*, Giuliana Lemoli*, Cristina Amabile*, Sante Tura.
Institute of Hematology* "L. & A. Seràgnoli", and Institute of Radiotherapy**, University of Bologna, Bologna; Institute of Hematology*, Department of Human Biopathology, University "La Sapienza" of Rome, Rome.

In this study we assessed the functional and kinetic characteristics of highly purified hematopoietic CD 34+ cells from the apheresis products and the bone marrow (BM) of 16 normal donors undergoing G-CSF treatment for peripheral blood stem cells (PBSC) mobilization and transplantation in allogeneic recipients. Mobilized and BM CD 34+ cells were evaluated for their colony-forming capacity and trilineage proliferative response to selected recombinant human-CSF in-vitro, and the content of very primitive long-term culture initiating cells (LTC-IC). In addition, the cycling status of circulating CD 34+ cells, including committed clonogenic progenitor cells and the more immature LTC-IC, was determined by the cytosine arabinoside (ARA-C) suicide test and the acridine orange (A.O.) flow cytometric technique. Clonogenic assays in methylcellulose showed the same frequency of CFU-C when PB primed-CD 34+ cells and BM cells were stimulated with the conditioned medium PHA-LCM. However, mobilized CD 34+ cells were significantly more responsive than their steady-state BM counterparts to IL-3 and SCF combined with G-CSF or IL-3 in presence of Epo. In cultures added with SCF, IL-3 and Epo we found a mean of 1.5 ±1 SEM -fold increase of PB CFU-GM and BFU-E as compared to BM CD 34+ cells ($p < 0.05$). After 5 weeks of liquid culture supported by the engineered murine stromal cell line M2-10B4 to produce G-CSF and IL-3, we reported 48.2 ± 35 SEM and 52.5 ± 54 SEM LTC-IC per 10^4 CD 34+ cells in PB and steady-state BM, respectively (p = NS). The ARA-C suicide assay demonstrated that 4 ± 5% SD of committed precursors and 1 ± 3% SEM of LTC-IC in PB are in S-phase as compared to 25.5 ± 12% SD and 21 ± 8% SEM of baseline BM, respectively ($p < 0.001$). However, longer incubation with ARA-C (16-18 hours), in presence of SCF, IL-3 and G-CSF or IL-6, demonstrated that greater than 60% of LTC-IC are actually cycling with no difference with BM cells. Furthermore, studies of cell-cycle distribution on PB and BM CD 34+ cells confirmed the low number of circulating progenitor cells in S- and G_2M-phase whereas simultaneous *DNA/RNA analysis demonstrated* that the *majority of PB CD 34+ cells are not quiescent (i.e.* G_0-phase) being in G_1-phase with a significant difference with baseline and G-CSF treated BM (80 ± 5% SEM versus 61.9 ± 6% SEM and 48 ± 4% SEM, respectively. $P < 0.05$). Moreover, G-CSF administration prevented a little but significant proportion of mobilized CD 34+ cells from apoptosis. In summary, our results indicate that mobilized and BM CD 34+ cells can be considered equivalent for the frequency of both committed and more immature hematopoietic progenitor cells although they show different kinetic and functional profiles. Moreover, in contrast with previous reports, we found that PB CD 34+ cells, including very primitive LTC-IC, are recruited in cell-cycle.

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COMPARISON OF IMMUNE RECONSTITUTION AND GVHD AFTER ALLOGENEIC PERIPHERAL BLOOD STEM CELL TRANSPLANTATION WITH OR WITHOUT CD34+ SELECTION J.Finke, D. Behringer*, H. Bertz*, C. Berzer*, K. Potthoff*, J. Winkler*, M. Hardung*, and R. Mertelsmann. Dept. Hematology & Oncology, Albert-Ludwigs University Medical Center, D-79106 Freiburg, Germany

32 patients (mean age 35 years, 19-54) with advanced hematologic malignancies were transplanted from HLA-identical related donors using filgrastim-mobilized peripheral grafts only. In 15 patients CD34+ selected grafts were transplanted containing a median of 4.3x10⁶/kg body weight CD34+ cells with a median purity of 55%, and 0.45x10⁶/kg CD3+ cells (group I). 17 patients were transplanted with unselected grafts containing 5.5x10⁶/kg CD34+ cells and 142x10⁶/kg CD3+ cells (group II). Patients were conditioned with Bu/Cy120 or TBI/VP16/Cy. GvHD prophylaxis was Cyclosporin A only in group I and additional prednisolone was used in group II. All patients received filgrastim 5ug/kg body weight post transplant. Engraftment was equivalent in both groups with neutrophils >500/μl after a median time of 10 days (range 9-15) and platelets >20 000 after 14.5 days (10-20) in group I and 15.5 days (10-70) in group II. Transfusion requirements were similar in both groups with 8 units of packed red cells and 72 units of platelets. Acute GVHD >°1 occured in 5 patients all presenting with grade II GVHD which resolved with appropriate treatment. Half of the patients are alive with a median follow up of 265 days (72-668) and chronic GVHD was not seen in group I patients and in 1 patient only in group II. Lymphocyte subsets were analyzed monthly post Tx. In both groups NK cell numbers normalized within 2 months and B cells within 6 months. CD4+ counts reached >200/μl after half a year and CD8+ cells normalized by 8 months. Allogeneic PBSCT results in rapid reconstitution of lymphocyte subsets without significant acute or chronic GVHD. Transplantation of peripheral blood derived CD34+ selected cells is safe and results in stable long term engraftment without compromising immune reconstitution. The >300fold reduction of CD3+ cells in the CD34+ selected graft may allow reduction of post transplant immunosuppression.

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2369 430-IV

MDR-1 VECTOR MARKING SHOWS THAT CFUGMS DO NOT CONTRIBUTE TO ENGRAFTMENT IN PATIENTS FOLLOWING INTENSIVE SYSTEMIC COMBINATION CHEMOTHERAPY. E. Hanania,* R. Giles,* S.O. Eu,* Z. Zu,* R. Cote,* A. Dayn,* T. Wang,* D. Ellerson,* J. Kavanagh, T. Holzmayer,* E. Mechetner,* R. Berenson,* S. Heimfeld,* Z. Rahman, M. Andreeff, R. Champlin, and A.B. Deisseroth. U.T. M.D. Anderson Cancer Center, Houston, TX, Systemix, Inc., Palo Alto, CA, CellPro, Inc., Bothell, WA, Microbiological Associates, Rockville, MD, Ingenex, Inc., Menlo Park, CA, Kenneth Norris Jr. Cancer Hospital, Los Angeles, CA, and Yale University School of Medicine, New Haven, CT.

The total nucleated cell count/kg, the number of CFUGM/kg, and the number of CD34+ cells/kg have all been used as independent predictors of the reconstituting cell content of peripheral blood or marrow cells of hematopoietic stem cells. These data have suggested that if the dose of CD34+cells/kg is greater than 2 x 10^6/kg, prompt and complete recovery will occur. It is not clear whether cells belonging to later stages of maturation will contribute to hematopoietic reconstitution following intensive systemic therapy. In order to test if the CFUGM stage of maturation contains reconstituting cells, we analyzed the transduction frequency of CFUGM using two different methods for introduction of the retroviral vector containing the MDR-1 cDNA into CD34 cells: 1) The suspension method, which consists of suspending the cells collected soon after chemotherapy and CD34 selection in retroviral supernatants for 4 hours), and 2) the stromal transduction method, which consists of inoculating the cells on stromal monolayers, in the presence of IL3 and IL6 and retroviral supernatants for 48 hours, and found that the transduction frequency was equal in methods 1 and 2. Post transplant cells of 5/8 evaluable patients were positive for vector MDR-1 in the patients transplanted with the cells transduced by the stromal transduction method, whereas the cells of 0/10 of the patients transplanted with cells transduced by the suspension method were positive for the vector MDR-1 cells. These results suggest that subsets of CFUGM exist which do not reconstitute patients following intensive chemotherapy.

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2383 444-IV

TRANSPLANTATION OF POSITIVELY SELECTED ALLOGENEIC BLOOD CD34+ CELLS. W. Brugger, S. Scheding, M. Subklewe, C. Faul, S. Halene, A. Brandes, A. Wiesmann, B. Weil, S. Heimfeld, H. Einsele, and L. Kanz. Department of Internal Medicine, Division of Hematology and Oncology, University of Tübingen, Germany, and *CellPro, Bothell, WA, USA.

In allogeneic transplantation, G-CSF mobilized peripheral blood progenitor cells (PBPCs) are now being used with increasing frequency as an alternative for bone marrow transplantation (BMT). However, there is concern about the greater number of immunocompetent T-cells in an unmanipulated PBPC allograft as compared to a conventional BM graft which might lead to an increased risk of severe acute and/or chronic graft-versus-host disease (GVHD). In order to potentially decrease the risk of GVHD, we positively selected CD34+ blood cells using the Ceprate SC® device (CellPro, Bothell, WA) which was shown to results in a 1-3 log depletion of T-cells (Brugger et al., Blood 84: 1421, 1994). Here, we report on the transplantation of CD34+ PBPCs from 15 allogeneic sibling donors in patients with MDS/AML [n=12] as well as in patients with high-risk or relapsed acute lymphoblastic leukemia [n=3]. The median age of the patient population was 42 years (24-53). The donors were HLA-matched in 13 cases, while 2 donors had 1 antigen mismatch. All donors received G-CSF (Neupogen®; 2x12 µg/kg s.c.) for up to 7 days for mobilization of PBPCs. A median of 3.5 (range 3-5) aphereses were performed to collect sufficient numbers of PBPC for subsequent selection of CD34+ cells. Because of insufficient yield after CD34+ cell separation, 5 patients received unseparated PBPC in addition to CD34 selected PB cells (group A). The remaining 10 patients were transplanted with CD34+ selected cells only (group B). A median of $3.8x10^6$ CD34+ cells/kg (range 3.5-4.9) and $3.6x10^6$ CD34+ cells/kg (range 3.2-6.5) were transplanted in group A and B, respectively. The yield of CD34+ cells after CD34+ cell selection was 43% (23-61) with a purity of 76% (range 51-83). The number of CD3+ cells transplanted were $165x10^6$/kg (range 60-344) in group A, and $0.53x10^6$/kg (range 0.28-0.95) in group B. Conditioning consisted of BuCy or TBI/Cy. Cyclosporin A (CSA) and MTX were given for GVHD prophylaxis in 12 patients, while 3 patients received CSA only. Time to neutrophil recovery > $0.5x10^9$/l was 16 days (range 13-19) in group A, and 14 days (range 10-15) in group B. Time to platelet transfusion independency > 25.000/µl occured at day 16 (12-29) in group A, and day 19 (15-24) in group B. No graft failure was observed. One patient developed fatal infectious complications at day +3. In patients transplanted with CD34+ selected cells alone (group B), we only observed grade 0-II acute GVHD, while 3/5 patients treated with unselected PBPC (group A) developed grade III-IV GVHD, and 2 of them subsequently died at day +25 and +124, respectively. At a median follow up of 240 days (28-353), 8 patients are alive and well. These data suggest that positively selected allogeneic PB CD34+ cells induce a rapid and stable engraftment of hematopoiesis with a possibly reduced incidence and severity of acute GVHD.

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1985 46-IV

MOBILIZATION OF PERIPHERAL BLOOD PROGENITOR CELLS USING G-CSF IN HIV-1 INFECTED PERSONS. J.Zain,* P. Yam,* S. Li,* H. Bayne,* S. Herman,* L. Snieciński, B. Tegtmeier,* S. Forman, J. Ito.* City of Hope National Medical Center, Duarte CA and *Roche Molecular Systems, Somerville NJ.

G-CSF-based mobilization of peripheral blood progenitor cells (PBPC) has been associated with transient reduction in CD4 cell counts in normal donors (M. Korbling *et al.*, BMT in press, 1996). The purpose of this study was to determine the safety and effectiveness of granulocyte-colony stimulating factor (G-CSF) for mobilization of PBPC in HIV-1 infected persons. Seven HIV-1 infected persons with >200 CD4 cells and no evidence of AIDS were treated with G-CSF for 5 days (10 ug/kg day). Sixteen hours after the fourth dose of G-CSF, mononuclear cells (MNC) were harvested during a 12 L apheresis using a Fenwall CS-3000 cell separator, and $CD34^+$ cells were enriched using an Ceprate® SC Stem Cell Collection System (CellPro Inc. Bothell WA). Clinical status, CD4 counts, plasma HIV RNA using RT-PCR (Roche), and HIV-1 infectivity assays of plasma and MNC were monitored for 6 months. Six subjects completed the apheresis without significant problems, and one failed to complete the apheresis because of inadequate venous access. During the 4 days of G-CSF treatment, there was a prompt mobilization of total leukocytes with a peak WBC range of 23-61 x 10^9/L and peak $CD34^+$ percent of MNC of 0.5-2.3%, both at a median time of 4 days post-G-CSF. The apheresis products yielded a mean 3.6 x 10^9 MNC/kg (range 2.3-5.7 x 10^9/kg). $CD34^+$ cell selection yielded a mean 2.8 x 10^8 cells/kg (range 1.1-5.4 x 10^8/kg). BFU-E and CFU-GM were within normal limits, but CFU-GEMM were reduced. Baseline CD4 counts (mean = 450) were reduced by a mean of 32% at one month post-apheresis ($p = 0.015$) and returned to levels not significantly different from baseline after 2 months. There was an increase in mean plasma HIV-1 RNA levels from baseline 6,310 genome copies per ml (gc/ml) to a peak of 12,600 gc/ml at 4 days post G-CSF ($p = 0.1$). At 3 months and 6 months after G-CSF, the plasma HIV RNA decreased to a mean of 4,000 gc/ml and 1,600 gc/ml, respectively ($p \geq 0.35$). Leukocyte and plasma infectivity assays were positive for HIV-1 before and after apheresis in all subjects and decreased during the time of cell mobilization. There were no changes in clinical status of the subjects during the 6 month period of observation. PBPC can be mobilized from HIV-1 infected persons using G-CSF, and functional $CD34^+$ cells can be efficiently selected. CD4 counts are transiently depressed following apheresis without significant increase in plasma HIV RNA levels or change in clinical status.

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INHIBITION OF HIV-1 REPLICATION IN ANTI HIV-1 GENE EXPRESSING LONG TERM BONE MARROW CULTURES ESTABLISHED FROM CD34+ CELLS OF HIV-1 INFECTED DONORS G. Bauer,* S.F. Wen,* I. Benner,* K. Kearns,* P. Valdez,* J. Zaia,* and D.B. Kohn. Childrens Hospital Los Angeles, CA, City of Hope Medical Center, Duarte, CA.

Long term bone marrow cultures established from CD34+ cells isolated from cord blood or bone marrow of HIV-1 negative donors transduced with several retroviral vectors containing anti HIV-1 genes strongly inhibit HIV-1 replication after challenge with the macrophage tropic isolate HIV-1 JR-FL. To determine the feasibility of gene therapy for AIDS in individuals already infected with HIV-1, G-CSF mobilized peripheral blood CD34+ cells were isolated from HIV-1 infected individuals, and transduced with retroviral vectors containing three different anti HIV-genes: An RNA decoy vector overexpressing the rev binding domain of the Rev Responsive Element, L-RRE-neo, a double hammerhead ribozyme vector targeted to the tat and rev transcript, L-TR/TAT-neo, and a vector containing the transdominant mutant of M10 in the construct L-M10-SN. As a control, a vector mediating only neomycin resistance, LN, was used. After three days of transduction on allogeneic stroma in the presence of SCF, IL-6 and IL-3, the cultures were G418 selected, and challenged with HIV-1 JR-FL and a primary HIV-1 isolate.

Results: Compared to the control, the L-RRE-neo, L-TR/TAT-neo and L-M10-SN transduced cultures displayed up to 1000 fold inhibition of HIV-1 replication after challenge with HIV-1 JR-FL and a primary HIV-1 isolate. This preliminary study suggests that anti HIV-genes can be introduced into CD34+ cells from individuals already infected with HIV-1, and strongly inhibit HIV-1 replication in primary monocytes derived from CD34+ progenitors. As the presence of bone marrow stroma during retroviral transduction enhances gene transfer into CD34+ cells, and long term engraftment in recipients of transduced CD34+ cells, we evaluated the feasibility of using stroma from HIV-1 infected individuals to support transduction of CD34+ cells. A comparison between the growth rates of cultured stroma from HIV-1 negative and HIV-1 positive donors showed nearly identical proliferative capacity, and gene transfer into CD34+ cells from HIV-1 negative and HIV-1 positive donors was supported equally well by stroma from HIV-1 negative and HIV-1 positive individuals.

Conclusions: In all, these data support the feasibility of applying retroviral - mediated transduction of CD34+ cells from HIV-1 infected individuals for gene therapy.

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TRANSPLANTATION OF POSITIVELY SELECTED CD34+ BONE MARROW AND MOBILIZED PERIPHERAL BLOOD CELLS FROM HAPLOIDENTICAL RELATED DONORS FOR HIGH-RISK HEMATOLOGIC MALIGNANCIES. A.M. Yeager, C. Arasteh, T. Chauncey, J.E. DiPersio, H.K. Holland, C.E. Lemaistre, W. Rubka, C.A. Jacobs, and M.C. Simmons. Emory University, Atlanta, GA, Fred Hutchinson Cancer Research Center, Seattle, WA; Veterans Administration Medical Center, Seattle, WA, Washington University, St. Louis, MO; South Texas Cancer Institute, San Antonio, TX, University of Pittsburgh, Pittsburgh, PA; and CellPro, Inc., Bothell, WA.

The lack of histocompatible related or unrelated donors limits the application of allogeneic bone marrow transplantation (BMT) for treatment of high-risk hematologic malignancies. For patients (pts) who lack HLA-matched donors, transplantation of selected CD34+ hematopoietic progenitor cells from haploidentical relatives provides an alternative source of stem cells with reduced numbers of T cells and might be associated with reduced risks of severe graft-versus-host disease (GVHD). To test this hypothesis, we evaluated transplantation of CD34+ cells selected with an avidin-biotin immunoadsorption column (CEPRATE SC System) from both G-CSF-mobilized peripheral blood cell (PBC) leukapheresis products and bone marrow collections from HLA-haploidentical related donors in 13 pediatric pts (median age, 6 yr; range, 1-13) and 11 adult pts (median age, 36 yr; range, 22-44) with high-risk acute lymphocytic leukemia (n=13), acute myelocytic leukemia (n=4), chronic myelocytic leukemia (n=4), myelodysplastic syndrome (n=2), or non-Hodgkin lymphoma (n=1). All pts received pre-transplant conditioning with fractionated total body irradiation (12-14 Gy), cyclophosphamide (60 mg/kg/d x 2 d), and anti-thymocyte globulin (30 mg/kg/d x 3 d) and post-transplant cyclosporine and short-course methotrexate. The median dose of CD34+ cells (x 10^6/kg) was 14.6 (range, 5.4-75.6) in pediatric pts and 10.4 (range, 3.7-15.6) in adult pts; the median dose of CD3+ cells (x 10^6/kg) was 2.2 (range, 0.2-6.3) in pediatric pts and 0.8 (range, 0.3-1.7) in adult pts. Twelve of 13 pediatric pts (92%) and 8 of 11 adult pts (73%) had donor neutrophil engraftment; median time to attain absolute neutrophil count >0.5 x 10^9/l was 12 d (range, 10-21) in pediatric pts and 20 d (range, 12-27) in adult pts. Two adult pts died without engraftment at 9 and 21 d, respectively, after transplant, and two pts (1 adult, 1 pediatric) had graft failure followed by autologous hematopoietic recovery at 33 and 36 d, respectively, after transplant. Eight of 12 evaluable pediatric pts (67%) and 2 of 8 evaluable adult pts (25%) had Grade 0-II acute GVHD; 4 pediatric pts (33%) and 6 adult pts (75%) had Grade III-IV acute GVHD. Chronic GVHD developed in 2 of the 7 pediatric pts (29%) and 2 of the 4 adult pts (50%) who survived >100 d after transplant. Deaths occurred in 7 pediatric pts (4 GVHD, 1 VOD, 2 progressive disease) and 10 adult pts (6 GVHD, 2 regimen-related toxicity, 2 sepsis). Six pediatric pts, including 1 with autologous recovery, are alive at a median of 51+ wk (range, 14+-66+) after transplant, and 1 adult (also with autologous recovery) is alive at 52+ wk after transplant. The probability of survival at 1 yr is 45% for pediatric pts and 10% for adult pts. We conclude that transplantation of positively selected CD34+ cells from haploidentical relatives is feasible and associated with prompt engraftment in the majority of recipients; however, risks of severe acute GVHD and of chronic GVHD remain. A study has been initiated in children with hematologic malignancies to evaluate additional T cell depletion and transplantation of CD34+ PBC products from haploidentical related donors.

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AUTOLOGOUS PERIPHERAL BLOOD SELECTED CD34+ CELL TRANSPLANTATION FOR THE TREATMENT OF SEVERE PROGRESSIVE SYSTEMIC SCLEROSIS

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Autologous bone marrow transplantation has been recently proposed as potentially curative treatment for severe or poor-prognosis autoimmune diseases. In view of results obtained in animal models, we decided to give an autologous peripheral blood stem cell (PBSC) transplant to an 11-year-old girl affected by systemic sclerosis with progressive lung fibrosis, but without pulmonary hypertension. After having obtained approval of the local Ethical Committee and written informed consent of the parents, the child received a mobilizing chemotherapy consisting of cyclophosphamide (CY) at a dose of 4 gr/m^2, followed by the administration of G-CSF at a dose of 10 μg/Kg/day. Two leukapheresis procedures were performed on day +12 and day +13, respectively. The number of PBSC collected was 4.3 x 10^6/Kg. A three-log T-cell depletion was performed as CD34+ cell positive selection by means of Ceprate SC and purified CD34+ cells (percentage recovery 70%) were subsequently cryopreserved. Pre-transplant conditioning regimen consisted of CY at a dose of 50 mg/Kg from day -5 to day -2 and the monoclonal antibody Campath-1G at a dose of 10 mg/day for 2 days. After thawing, the total infused CD34+ cell count was 3.5 x 10^6/Kg; the residual T lymphocyte dose was 3 x 10^4/Kg. G-CSF was administered after transplant at a dose of 5 μg/kg/day for 12 days. The early post-transplant period was uneventful; neutrophil and platelet engraftment (PMN > 0.5 x 10^9/L and PLT > 50 x 10^9/L) was achieved on day +11 and day +14, respectively. The patient was discharged on day +24 and she is alive and well, with a normal blood count 70 days after the transplant procedure. The immunological evaluation performed 2 and 4 weeks after transplantation showed a moderate reduction of mature T lymphocytes (patient's CD3+ cells = 50%; normal controls = 65-85%) and profound impairment of the proliferative response to phytohaemoagglutinin, concanavalin-A and anti-CD3 monoclonal antibody (< 5% of normal control subjects). This pattern of immunological recovery is similar to that normally observed after unmanipulated autologous BMT. Our experience demonstrates the feasibility and safety of this procedure in children affected by severe autoimmune diseases. A longer follow-up and careful monitoring of signs and symptoms of the original disease will be necessary in order to evaluate the efficacy of the treatment.

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