two phase culture system that enables the distinction between the expansion of early and late progenitors. In the first phase (3 weeks), the cultures were supplemented with both TEPA and cytokines (FLt3, SCF, TPO, IL-6). In the second phase (additional 5-7 weeks) the cultures were supplemented with cytokines alone, in the absence of TEPA. Late progenitors (CD34+ and CFUc) as well as early progenitor cells (CD34+CD38-, CD34+Lin-(CD38, 33, 14, 15, 61, 41, 3, 4, 19), CD34+38-HLA-DR-) were measured at the termination of the treatment phase (week 3) as well as at different intervals during the long-term assay phase. After the first phase (3w), TEPA cultures contained significantly higher percentages of early progenitors (21% CD34+38- +/- 3.8; 7.0% CD34+Lin- +/-1.9, 1.5%+/-0.5 CD34+38-HLA-DR, n=9-) as compared to the control, only cytokine treated cultures (2.6% CD34+38- +/-1, 0.9% CD34+Lin- +/-0.3, 0% CD34+38-HLA-DR- n=9). At the same time, no differences were noticed in the late progenitors (TNC, CFUc and CD34+ cells) between control and TEPA cultures. Fold expansion of CD34+ cells in both control and treated cultures was in the range of 10-40 (N=6) folds. After the second phase (additional 5-7w) in the TEPA pre-treated cultures, CD34+ cells expanded by 370-1320 folds (N=6) whereas no CD34+ cells could be recovered in control cultures. CFUc expansion in TEPA pre-treated cultures was by 20 to 30 folds (n=6) higher than in the control cultures. These results indicated that the chelator enabled preferential proliferation of early progenitors during the first three weeks. At this stage, no positive or negative effect on the expansion of late progenitors and TNC was observed. These early progenitors continued to proliferate and differentiate during the TEPA free phase and supported the extensive expansion of late progenitors during the long-term cultures.The chelator based method of three weeks ex vivo expansion, which resulted in a cell graft enriched in early progenitors increased by 10 fold the engraftment in SCID mice. We plane to test the safety and efficacy of such a graft in clinical cord blood transplantation.

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THE PRESENCE CXCR4 ON CD34+ CELLS WAS ASSOCIATED WITH THE EFFECTIVENESS OF G-CSF PBPC MOBILIZATION

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The presence of CXCR4 on CD34+ cells plays a role in hematopoietic progenitors trafficking and reflects the stage of these cells differentiation. The most primitive cells are CXCR4 strongly positive than they loose this epitope and gain it again while become DR positive. Therefore, we investigated the proportions of CXCR4+ and DR+ cells among CD34+ cells in ninety G-CSF mobilized leukapheresis products (PBPC) obtained from fifty eight volunteer, healthy donors and in thirty one bone marrow harvests (BM) for allogeneic grafting. Marrow transplant materials differed from PBPC with respect to the proportions of CD34+ cells (0.91%+-0.07 vs 0.64%+-0.04, p=0.001) which were, however, to the similar proportion DR negative (14.15%+-1.81 vs 13.89%+-1.44). Looking at the co-expression of CXCR4 and DR on CD34+ cells, it was found that PBPC CD34+ cells were more frequently CXCR4-DR+ as compared to marrow (48.42%+-2.45 vs 39, 34%+-4.38, p=0.05). Marrow CD34+ cells were more frequently CXCR4+ than those in PBPC (62.47%+-4.09 vs 50.91%+-2.47, p=0.02). PBPC preparations were richer in CD34+ cells if the latter cells were in higher proportions CXCR4 negative (r=0.45, p=0.00001), in variance, marrows harvested for transplantation contained more CD34+ cells if they were CXCR4+ (r=0.34, p=0.06) It may be concluded that the presence of CXCR4 on CD34+ cells influenced the yield of transplant material in hematopoietic progenitors. The coexopression of CXCR4 on CD34+ cells made the marrow harvest richer in hematopoietic progenitors but the content of CD34+ cells in G-CSF mobilized PBPC significantly depended on the presence of CD34+ cells lacking CXCR4.

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SIBLING DONOR CORD BLOOD (SDCB) BANKING AND TRANSPLANTATION FOR CHILDREN WITH SICKLE CELL ANEMIA AND THALASSEMIA: CHILDRENS HOSPITAL OAKLAND SIBLING DONOR CORD BLOOD PROGRAM,

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Sickle cell anemia and thalassemia are two genetic diseases with a worldwide incidence. While current treatment protocols for these patients have improved their lives, hematopoetic stem cell (HSC) transplantation is the only current treatment that offers a promise of cure. The donor for a HSC transplant is most often an HLA matched sibling. Recent evidence suggests that umbilical cord blood (CB), obtained from a sibling, may provide an important source of HSCs for these transplants. We have developed a National SDCB Banking program to provide a resource to evaluate CB transplantation. Our bank has fascilitated the collection of over 200 CB samples for families who already had a child with sickle cell anemia or thalassemia.. The volume of CB (average 100 ml) collected and the nucleated cell counts were sufficient to provide at least 2.5x107 cells/kg recipient weight in 85% of the cases. Taken together with recent results reported by NETCORD, a total of 44 children with hemoglobinopathies (33 thalassemia, 11 sickle cell anemia) have been transplanted with HLA matched SDCB. 85% appear to have been cured. The remaining children failed to engraft and had a return of disease. Minimal GVHD was noted. None of the children died as a result of the transplant. While a common transplant protocol was not followed, our results suggest that modification of the conditioning regimen and providing a critical nucleated cell dose will enhance engraftment and improve outcome. We conclude that SCDB banking should be available to all families who currently have a child with sickle cell anemia or thalassemia. A prospective study to determine the optimal regime for SDCB transplantation is currently underway.

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OPTICAL IMAGING OF EARLY POST-TRANSPLANT DESTINY OF BONE MARROW PROGENITOR CELLS IN MICE

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Use of non-invasive visualization techniques to follow up the destiny of transplanted hematopoietic stem cells provide a reliable tool for investigation of the processes of transplant engraftment and reconstitution of hematopoiesis. Bone marrow, harvested from C57BL/6-Ly.1 donor mice, was negatively sorted for differentiation determined cells. The obtained enriched population of bone marrow precursor cells (BMPC) was stably transfected with ecotropic retrovirus carrying TK-dmDHFR and GFP-Luc chimeric genes. Transfected cells were positively selected on FACSVantage using GFP-expression signal. 10^4 BMPC were reinfused as a rescue of lethally irradiated C57BL/6-Ly.2 recipient mice. D-luciferin was administered either in vivo, immediately post-transplant, or ex vivo, directly to the BMPC, in two animal groups: Bioluminescent imaging (BLI) was started in 5 minutes after the BMPC. For the follow up studies, d-luciferin was administered I.V. to all animal groups. Control group included irradiated animals without BMPC support. BLI revealed a weak, but distinctive signal accumulation in the projection of lungs and mediastinum with animals in supine position. Dilution of BMPC local concentration resulted in disappearance of any detectible signal between hours 36 and 60. Further observation demonstrated BMPC localization to the final hematopoietic sites. Higher signal was registered in the animals inoculated with the ex vivo d-luciferin preincubated BMPC. No specific signal was identified in the control group. Weak sources of photons associated with the projection of open skin/mucosa areas corresponded to the subinfrared thermal photon background. Early migration of a small number of stem cells can be efficiently monitored using highly sensitive bioluminescence approach. Systemic BMPC dose lower limits for sensitivity of BLI registration were identified. Triple