GRAFT PROCESSING

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MOBILIZED PERIPHERAL BLOOD CONTAINS PRIMITIVE HEMATOPOIETIC CELLS THAT CAN BE ENUMERATED AND ISOLATED USING A FLUORESCENT SUBSTRATE FOR ALDEHYDE DEHYDROGENASE ACTIVITY

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High dose therapy followed by autologous or allogeneic transplantation with peripheral blood stem cells (PBSCs) has been used to treat patients with a variety of diseases. Selection of primitive stem cells and progenitors from PBSC collections is useful for reducing the transplant volume and decreasing the number of contaminating tumor cells or T-cells. We have developed a novel approach for enumerating and enriching primitive mobilized peripheral blood cells that express high levels of the enzyme aldehyde dehydrogenase (ALDH). Mobilized cells were stained with a fluorescent ALDH substrate, termed BODIPY-aminoacetaldehyde (BAAA), and then analyzed or sorted using flow cytometry. A population of cells, termed SSCloALDHbr cells, was readily discriminated and comprised a mean of 3.1 +4.8% of the collected events. A mean of 73.4 +11.7% of the SSCloALDHbr population expressed CD34 and 56 + 24.5% of all the mobilized CD34+ cells resided within the SSCloALDHbr population. The SSCloALDHbr population was largely depleted of cells with mature phenotypes and enriched for cells with immature phenotypes. The BAAA staining procedure did not diminish the viability or clonogenic activity of hematopoietic progenitors and caused no toxicity to cells or animals in a variety of pre-clinical toxicology studies. Sorted SSCloALDHbr and SSCloALDHbr CD34+ cells were enriched for progenitors with the ability to 1) generate CFUs and LTC derived CFUs, 2) expand in primary and secondary LTCs and 3) generate multiple cell lineages. In order to test whether the number of PBSC SSCloALDHbr cells would predict engraftment in actual human transplants, the total number of SSCloALDHbr cells infused per kg in 21 cancer patients who had undergone autoPBSC transplantation were compared to the times to an ANC>500 and a platelet count >20, 000 using a Cox proportional hazard analysis. The time to neutrophil and platelet engraftment were both highly correlated with the number of SSCloALDHbr cells/kg ($p<0.015$ and 0.003 respectively) and the number of SSCloALDHbr CD34+ cells/kg ($p<0.013$ and 0.0016 respectively). In summary, PBSC SSCloALDHbr cells have the phenotypic and functional properties of primitive hematopoietic cells and their number correlates with short term engraftment following autoPBSCT transplantation.

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NORMAL AND NEOPLASTIC B CELLS ARE UNDULY SUSCEPTIBLE TO PHOTODYNAMIC CELL THERAPY

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The favorable effect associated with the infusion of a graft purged of its neoplastic cells might be jeopardized by the concomitant elimination of cell populations responsible for (1) the eradication of residual lymphoma cells, (2) the prevention of viral and fungal infections, and (3) rapid hematologic reconstitution post-transplantation. In previous studies, we have shown that TH9402, a rhodamine-derived photosensitizer, selectively eliminates activated T cells, and spares resting T cells. Interestingly, P-glycoprotein (Pgp) modulates TH9402-mediated cytotoxicity by extruding this photosensitizer from the intracellular milieu. The absence of Pgp expression in most NHL cells prompted us to evaluate photodynamic cell therapy (PDCT) with TH9402 for the selective elimination of B-lineage lymphoma cells. To identify optimum treatment conditions, the uptake/retention kinetics and cytotoxicity profile of TH9402 were evaluated with B-lineage cell lines, NHL patient cells harboring or not a bcl-2/IgH rearrangement, and normal B lymphocytes. In clonogenic assays, more than 4 logs of indolent bcl-2/IgH rearranged (RL and DHL-16) and aggressive (Namalwa and Raji) NHL cell lines and patient (n=27) NHL cells were eradicated following PDCT using TH9402. Although normal B lymphocytes retained TH9402 in amounts similar to T cells, as measured by flow-cytometry, PDCT eliminated 4 logs of B cells but only 1 log of T lymphocytes, which were spared for immune reactivity. Importantly, PDCT conditions of maximum intensity that preserved more than 50% of CFU-GM, BFU-E and CFU-Mix colonies, and greater than 75% of LTC-IC progenitors for hematopoietic reconstitution, demonstrated lower levels of retention of TH9402 in CD34+ cells. The discrepancy between TH9402 retention levels and cytotoxicity indicates that the exquisite sensitivity of B lineage cells to TH9402 phototherapy must rely on a retention-independent mechanism of target cell elimination. The specificity of TH9402 for B lymphocytes indicates that this strategy should be evaluated for ex vivo purging of autologous stem cell grafts from patients with NHL and also for the treatment of autoimmune B cell disorders.

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MOBILIZATION OF TYPE 1 AND TYPE 2 DENDRITIC CELLS BY GM-CSF ALONE OR IN COMBINATION WITH G-CSF

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Dendritic cells (DC) are the only antigen-presenting cells capable of priming naive T cells with antigens. Generally, DC does not express lineage-specific markers (lineage negative), but express HLA-DR. There are two subsets of DC: one of lymphoid lineage that induces Th1-like responses and expresses CD11c (DC1) and another of myeloid lineage that induces Th2-like responses and expresses CD123 (DC2). Previous studies reported that G-CSF preferentially mobilized DC2, and not DC1, in normal subjects. To determine the effect of GM-CSF alone or in combination with G-CSF on the mobilization of DC1 and DC2, we studied DC markers in 7 subjects received 10 mcg/kg/day of GM-CSF for 4 days and another 5 subjects received 10 mcg/kg/day of GM-CSF and 10 mcg/kg/day of G-CSF for 4 days. Peripheral blood was obtained from all subjects before treatment and 24 hours after the final dose of the mobilization regimen. The numbers of DC1 and DC2 were determined by staining blood with lineage cocktail antibodies, anti-HLA-DR, anti-CD11c, and anti-CD123, and analyzed by 4-color flow cytometry. Compared with the number of total DC prior to treatment with G-CSF plus GM-CSF, the post-treatment number of DC was significantly higher (32 +/- 6 per mcl versus 143 +/- 35 per mcl; $p = 0.020$). The increase in total DC post-mobilization with G-CSF and GM-CSF was due to a significant increase in DC2 (8 +/- 3 per mcl versus 37 +/- 19 per mcl; $p = 0.046$), and not in the number of DC1. By comparison, mobilization of DC, DC1, and DC2, with GM-CSF alone was only marginal. The preferential expansion of DC2 by the combination of G-CSF and GM-CSF as with G-CSF alone may have important consequences on sensitization of the host and on the engraftment.

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EFFECTIVENESS OF METRONIDAZOLE ON CYCLOSPORINE-INDUCED GINGIVIAL HYPERPLASIA IN BONE MARROW AND KIDNEY TRANSPLANT PATIENTS:A PROSPECTIVE, DOUBLE-BLINDED CONTROLLED CLINICAL TRIAL

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Background: Cyclosporine-induced gingival hyperplasia (CIGH) regression has been reported with some antibiotic therapies, especially Metronidazole and Azithromicine. **Object**: To evaluate the efficacy of metronidazole on CIGH regression, **Method**: we performed a prospective double-blinded study on 22 patients with CIGH, following kidney or bone marrow transplantation. None of the patients received calcium-channel blockers or anticonvulsants. Other causes of gingival enlargement were ruled

out among our patients. Before being allocated into the study groups, the patients had undergone a course of dental treatment programs for three weeks in order to reduce the amount of superimposed dental inflammation as much as possible. Thereafter, the patients were randomized into intervention and control groups. Metronidazole tablets 250 mg/TDS were given to the intervention group, while the control group received the same amount of placebo. Patients were examined by a blinded periodontologist, at days 0, 7, 14, and 21, to determine the gingival overgrowth scores. **Results**: The two groups were identical in age, gender, cause of receiving cyclosporine (kidney or bone marrow transplantation), duration of receiving cyclosporine, plasma level of cyclosporine and gingival overgrowth scores. Gingival enlargement improved in 7 patients, of whom 6 were in our intervention group (54.5%). The response to treatment was different in two groups significantly (P= 0.03). No association was found between gender, cause of receiving cyclosporine, duration of receiving cyclosporine, plasma level of cyclosporine and gingival overgrowth scores variables and response to treatment. **Conclusion**: In conclusion, our findings suggest that we can benefit the advantages of metronidazole administration in CIGH. Although we think that higher doses of metronidazole in association with oral hygiene programs and dental treatment planning could show better results in such patients.

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RECOVERY OF LEUKOCYTES FROM CORD BLOOD UNITS AFTER CONTROLLED RATE FREEZE IN DMSO AND CRYOPRESERVATION IN THE VAPOR PHASE OF LIQUID NITROGEN

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American Red Cross, Western Area Community Cord Blood Bank has established a program to assure the quality of cryopreserved volunteer donor cord blood units (CBU). Recovery (Rec) of leukocytes (WBC) and WBC viability after CBU thawing are important surrogate measures CBU quality. We determined the Rec of WBC in cryopreserved CBU immediately post-thaw and after dilution and washing in Dextran 40/5% Albumin (D/A), which is widely employed. Methods: CBU were collected from consented volunteer donors. CBU that were eligible for transplant, but had less WBC than required by storage criteria (6 E8) were studied. CBU were processed within 48 hours by Rubinstein's method. The CBU volume after addition of 10% DMSO was 25 ml. CBU were cryopreserved by controlled rate freezing and stored in vapor phase liquid nitrogen for up to 1 year. Twelve CBU selected for study were removed from storage and immediately placed in a 37 C waterbath. After thawing, the CBU was maintained at 4 C and a sample was immediately removed to assess post-thaw WBC (% NC-Rec) and cell viability by Trypan blue dye exclusion (% TB-viable). The CBU was diluted over 2 min in 25 ml D/A, samples removed, then the CBU was diluted to 100 ml in D/A. The CBU was centrifuged at 400 xG at 4 C and resuspended in 25 ml of D/A. WBC Rec and NC viability were assessed post-thaw, after the 1:1 dilution in D/A, and post wash and resuspension. In separate experiments, the Rec of CD34-bearing cells was measured. Results: There was a median 22% loss of NC after thawing, and additional NC loss after dilution and washing. Post thaw trypan blue dye viability was slightly reduced. In 6 separate experiments the Rec of CD34+ cells was 107 ± 31% after thawing and 106 ± 33 after washing. Conclusions: Thawing of cryopreserved CBU was associated with loss of approximately 22% of the total NC and reduction in overall cell viability. Further cell loss accompanied washing. Recovery of CD34 bearing cells was superior to total NC. Hematopoietic progenitor growth in culture after CBU thawing was consistent with the above.

Time	Post Thaw	Post Thaw	Post Dilution	Post Dilution	Post Wash	Post Wash
Data	% NC Rec	% TB viable	% NC Rec	% TB viable	% NC Rec	% TB viable
Mean ± SD	78±12	90±6	76±12	90±8	65±18	90±7
p	0.02	0.002	0.019	0.014	0.001	0.005

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EX VIVO ENGINEERING OF PREVIOUSLY THAWED AND CRYOPRESERVED UMBILICAL CORD BLOOD (UCB) WITH INTERLEUKIN (IL)-2, IL-7, IL-12 AND ANTI-CD3 FOR EXPANSION OF CYTOTOXIC T LYMPHOCYTES (CTL): PROMISING STRATEGY FOR ADOPTIVE CELLULAR IMMUNOTHERAPY (ACI) POST UCB TRANSPLANTATION(T)

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Limitations associated with using UCB as a source for ACI post UCBT includes the lack of donor immunoeffector cells from the original cryopreserved UCB unit and/or immaturity of CB cellular immunity. We have demonstrated that CTL can be selectively engineered and activated from fresh and cryopreserved and thawed (CT) aliquots of UCB (Robinson/Cairo et al, Exp Hem 30:245, 2002). In this study we evaluated and compared the activation and NK and LAK cytotoxicity of UCB CTL. Thawed UCB aliquots were monocyte depleted (5 x10^6 cells/ml) in serum-free (SF) AIM-V in 5% CO_2 @ 37°C. Nonadherent cells (1x10^6 cells/ml) were either cultured in SF AIM-V + anti-CD3 (50 ng/ml), IL-2 (5 ng/ml), IL-7 (10 ng/ml) and IL-12 (10 ng/ml) (AB/CY) or in SF AIM-V alone for 48 hours @ 37°C in 5% CO_2 or expanded, recryopreserved and rethawed (TECT), or not expanded but recryopreserved, rethawed and subsequently expanded (TCTE). NK subsets were analyzed by flow cytometry and NK and LAK cytotoxicity by WST-1 methodology utilizing a 10:1 E:T ratio against K562 (NK) and Daudi (LAK), respectively. A significant enhancement in NK cytotoxicity was seen when UCB cells were cultured in the AB/CY cocktail ($p<0.001$) when compared to media alone, but no difference between the modalities (TE: 0.73±0.03 vs 0.16±0.01; TECT:0.72±0.03 vs 0.16±0.01 and TCTE: 0.75±0.04 vs 0.16±0.01). Similarly, there was significant enhancement of LAK cytotoxcity with all modalities of AB/CY stimulation vs media alone ($p<0.001$) but no difference between modality (TE: 0.39±0.01 vs 0.26±0.01; TECT: 0.37±0.008 vs 0.23±0.002; TCTE: 0.41±0.01 vs 0.20±0.01). Furthermore, there was a significant increase in the CD3-/16+/56+ subset of TE, TECT and TCTE when compared to media alone (TE: 60.2±1.24% vs 47.33±0.55%, n=3, $p<0.001$; TECT: 60.67±2.4% vs 46.68±1.3%, n=3, $p<0.001$; TCTE: 60.7±3.27% vs 45.45±1.4%, n=3, $p<0.001$). These data suggest that previously cryopreserved and thawed UCB aliquots may be engineered at time of UCB transplant, ex vivo expanded and activated for cytotoxic (NK & LAK) potential and recryopreserved for later use for DLI post UCBT. Xenotransplant animal studies are underway to examine the in vivo effects of this UCB CTL population.

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ONCE AND TWICE FILGRASTIM FOR ALLOGENEIC PBSC MOBILIZATION

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Objective: To compare efficacy and safety in allogeneic peripheral blood stem cell (PBSC) donors who received 400μg/m^2/day of filgrastim administered subcutaneously once daily (Once-daily regimen) or in two-divided dose every 12 hours (Twice-daily regimen). Design: An open-label, randomized, multicenter phase III trial. Patients: Between May 2001 and May 2002, 72 PBSC donors were enrolled in this study. Eligibility criteria was as follows: Allogeneic PBSC donors to related patients aged between 16 and 65 years, eligible for the criteria described in the Japan Society for Hematopoietic Cell Transplantation (JSHCT) guideline of PBSC harvest. Intervention: Donors were randomly assigned to receive 400μg/m^2 of G-CSF (filgrastim) subcutaneously once daily or in two-divided doses every 12 hours on 3 consecutive days. PBSC was collected by leukapheresis using COBE Spectra on day 4. Main Outcome Measures: The primary endpoint of this study was CD34 positive cell dose per kg of donor's body weight collected by leukapheresis on day 4. Additional endpoints were total nucleated cell (TNC) counts in the leukapheresis products, total pain burden experienced by donor measured with area under curve (AUC) of visual analogue scale (VAS), and total dose of analgesic drugs. Safety endpoints: Incidence and severity of