292. A NOVEL STRATEGY FOR SELECTIVE DEPLETION OF HOST-REACTIVE DONOR LYMPHOCYTES BY PHOTODEPLETION IS EFFICIENT AT CLINICAL SCALE CONDITIONS AND PRESERVES foxP3+ REGULATORY T CELLS


Using an anti-CD25 immunotoxin (TT) for ex vivo selective depletion (SD), we recently showed that removal of host-reactive donor T cells is clinically feasible. Nevertheless, there was a concern that the removal of CD4+ CD25+ foxP3+ regulatory T cells (Tregs) by CD25-IT might favor GVHD. We therefore investigated photodepletion (PD), an alternative approach whereby allo-reactivated cells are labeled with the photosensitizing rhodamine-based dye THP402, and exposure to light eliminates cells retaining the dye. PD may offer the possibility of conferring donor immunity without GVHD, permitting allo-SCT without the need for post-transfusion immunosuppression. Stimulator cells from human subjects were prepared from leukapheresis MNCs and cultured using anti-CD3 and 100 U/IL-2.Responder cells (leukapheresis MNCs) from HLA-matched volunteers were cocultured 1:1 with irradiated stimulators for 3 days. Cultured cells were incubated in THP402 (5.0 and 7.5 microM), followed by exposure to 514 nm light in the PD light source (Celmed) at a cell concentration of 5 X 10^6/ml in FEP plastic bags (American Fluoroseal). Results were compared with the established small scale standard method. Depletion efficacy was studied by FACS and MLR. Effects on Tregs were investigated using foxP3 RT-PCR and intracellular foxP3 staining. In two large-scale experiments using cells from two donors, both the standard small-scale conditions and the large-scale conditions resulted in a 9-18-fold and 21-42-fold reduction in outcomes were seen with the standard red cell depleted (RCD) UC, whether washing was employed after thawing or not. However, no data exist on the utility of post-thaw washing for UC that were depleted of plasma (PD) but not depleted of red blood cells.

A retrospective analysis was performed on the outcomes of 84 patients in remission without history of prior transplants, who received either washed (W; n = 43) or non-washed (NW; n = 40) PD UCB units for HSCT. Adverse events of any grade occurring more than once during infusion include hemoglobinuria (9 NW, 1 W), hypertension (6 NW, 4 W), hives (1 NW, 1 W), nausea/vomiting (2 NW, 1 W) and dyspnea (1 NW, 1 W). One patient developed severe and encephalopathy following infusion of a NW PD UCB that resolved without any sequelae, although relationship to UCB infusion was uncertain. Total nucleated cell recovery after thawing as reported by transplant centers is higher for NW (median 95% vs 73%). Unadjusted cumulative incidence (U.C.I.) of neutrophil engraftment was similar for both groups, 91% ± 5% for NW (n = 36) versus 93 ±4% for W (n = 41), but median time to neutrophil (20 vs 26 days) and platelet engraftment (platelet 20k 47 ± 55 days and platelet 50k 55 vs 63 days) occurred earlier for NW. U.C.I. for platelet 20k engraftment was higher for NW (n = 28; 92 ± 6%) than W (n = 39; 75 ± 7%). Acute grade III-IV GVHD were 10% (NW) and 19% (W), and extensive chronic GVHD were 0% (NW) and 22% (W). TRM were 18 ±6% (NW) and 20 ± 7% (W), with relapse rate for malignant cases at 11 ± 7% for NW and 25 ± 8% for W. One-year OS was 75 ±7% (n = 40) versus 72 ± 8% (n = 43), and 1-year DFS was 69 ± 10% (n = 23) versus 54 ± 9% (n = 34) for NW and W, respectively. No clear benefits of post-thaw washing for PD UCB prior to HSCT was found, except for the anticipated lower incidence of transient hemoglobinuria. HSCT with NW PD CBU was at least as efficacious as that using PD units with respect to neutrophil engraftment, TRM, relapse rate, 1-year OS, and DFS. Moreover, washing may have a negative impact on U.C.I. of platelet engraftment and the speed of neutrophil and platelet engraftment.

294. ACTIVATION AND ENHANCEMENT OF EX VIVO (E-E) EXPANDED CRYOPRESERVED CORD BLOOD (CB) NATURAL KILLER (NK) CELLS, CYTOLYTIC POTENTIAL, AND NK RECEPTOR (NKR) EXPRESSION: POTENTIAL ROLE FOR CB NK IN ADOPTIVE CELLULAR IMMUNOTHERAPY (ACI)


NK subsets exhibit differential NKR profiles including KIR, C-lectin (NKG2), and natural cytotoxicity receptors (NCR) involved with tumor recognition (Farag et al, Blood, 2002). NKRs such as NCR (NKP44, NKP46) and NKG2 surface receptors NKG2D induce NK cell activation and mediated cytolysis (Morretta et al, Curr Opin Immunol, 2004). CB is limited by the absence of available donor effector cells for infusion after UCBT (Cairo et al, Transfusion, 2005). We demonstrated the ability to EvE in short term culture with IL-2, IL-7, IL-12, and anti-CD3 (ABCY) cryopreserved, thawed, re cryopreserved, rethawed, and EvE (CTCTE) CB with increases in CD56^dim^/CD3^+^ NK cells displaying enhanced NKR expression KIR1D1L1, KIR2DL1/S1, KIR2DL2, and CD94/NKG2A/A (Ayello/ Cairo et al, BBMT, 27A, 2004). We now compared short-term with prolonged cultures (4-10 days) on expanding the expression of NKR, NK, and cytolytic mechanisms in CTCTE CB. Rethawed CB cells (Kurtzberg/Cairo, Transfusion, 2005) were cultured (2-10 days) in media alone or with anti-CD3 (30 ng/ml), IL-2 (5 ng/ml), IL-7 (10 ng/ml), and IL-12 (10 ng/ml). NKR expression (CD94, NKG2C, NKG2D, NKP44, NKP46, KIR2DS4) and intracellular perforin and granzyme B activity were determined by flow cytometry. Europium release assay measured NK and LAK cytotoxicity. NK activating KIR2DS4 was measured day 10 versus 2 in ABCY in both CD3^+^/CD56^+^ NK cells (16.9 ±0.4 vs 21.1 ± 0.2%) and 22.3 ±0.3 vs 0.9 ± 0.2% (P < .001, respectively). CD94/NKG2D expression was increased day 7 versus 2 in ABCY EvE (41.4 ± 0.45 vs 23.7 ± 2.0%) (P < .001). NK CD56^dim^/CD3^+^ KIR3DL1 subset was increased day 10 versus 2 (38.3 ± 2.8 vs 18.9 ± 6.37%, P < .05). CD56^dim^/CD3^+^
NKp44 subset NCR expression was decreased day 10 versus 2 in ABCY day 10 versus 2 (55.7 vs 71.5), NK: 71.5 vs 84.3, P < .001). Granby M was increased from day 2 to 10 (25.8 ± 1.8 vs 45.1 ± 1.7%, P < .001) yet perforin was decreased in ABCY day 10 versus 2 (55.7 ± 18 vs 84.3 ± 13%, P < .001). ABCY CB NK and LAK cytotoxicity was increased day 10 versus 2 (NK: 71.5 ± 1.6 vs 53.8 ± 10.3%, P < .001; LAK: 63.2 ± 0.24 vs 11 ± 1.8%, P < .001). In summary, CB MNC may be thawed at UCBT, CT at a later date, EvE and activated for 7–10 days to yield viable NK subsets. ABCY 10 day EvE CB yielded increased NK KAR (CD56dim/bright) and granby M expression but decreased NK C-lectin CD94/NKG2D, NCR NKp44 and NKp46, and perforin expression.

LANTCET: NOVEL LASER NANOTECHNOLOGY FOR GRAFT PURGING

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We introduce a novel ex vivo method, Laser-Activated Nano-Thermolysis Cell Elimination Technology (LANTCET), for selective killing of individual tumor cells in bone marrow and peripheral blood samples. LANTCET assumes cell-specific delivery of gold nanoparticles (GNP) into tumor cells and irradiation of cell sample with laser pulse. Nanoparticles absorb laser pulse, instantly heat, and produce micro-bubbles of vapor that instantly heat, and produce micro-bubbles of vapor that are targeted to individual and targeted to individual cells, thereby being safe for surrounding normal cells. The mechanism of cell-specific delivery of GNP to targeted tumor cells uses primary and secondary monoclonal antibodies for binding the GNP to cell membrane and further cationization of GNP into compact groups. In experiments, we used human ALL and AML and normal bone marrow samples to evaluate efficacy and safety of LANTCET. Accumulation of GNP in cells was studied with flow cytometry, fluorescent microscopy, and electron microscopy. Using specially developed incubation protocols for 30-nm GNP, we provided controlled forming of GNP clusters in tumor cells only, while only single GNP were discovered in normal stem cells due to non-specific binding. After incubation with GNP, the cell samples were irradiated with single laser pulse (532 nm, 10 ns, 0.5–1.0 J/cm²). Cell damage levels were measured for tumor and normal cells with flow cytometry (necrosis and apoptosis). At specific laser fluence total damage of ALL cells and 84% of AML cells was achieved while among normal cells only 16% were damaged after single laser pulse. This method is now at the stage of the development of an experimental system for purging transplants (grafts) of bone marrow and blood.

AUTOMATED SEPARATION OF CORD BLOOD MNC FRACTION IN A CLOSED SYSTEM: THERMOMEDICINE AXP™ SYSTEM


Background: Compliance with current good tissue practices (cGTP) is essential to achieving product uniformity in cord blood banks. A critical step in the processing of cord blood is volume reduction, to allow standardization of the freezing rate and help maintain stem cell viability and to optimize liquid nitrogen storage space utilization. To enable instrumented cord blood volume reduction, ThermoGenesis developed the AutoXpress™ (AXP™) System consisting of a microprocessor-controlled device and disposable closed blood bag set. The AXP™ fits standard refrigerated blood bank centrifuge buckets. Cord blood is transferred to the bag set and centrifuged. With the AXP™ system, up to 6 units of cord blood can be processed at one time with a standard blood bank centrifuge. Study Design: A study was performed to determine the efficiency with which cord blood hematopoietic progenitor cells can be recovered, using the CD34 cell marker and colony-forming unit (CFU) counts as indices. Twenty-three consecutive cord blood units were processed with the AXP™. The blood volume, hematocrit, total nucleated cells (TNC), mononuclear cells (MNC), CD34+ cells, and colony-forming units (CFU) counts as indices. Twenty-three consecutive cord blood units were processed with the AXP™. The blood volume, hematocrit, total nucleated cells (TNC), mononuclear cells (MNC), CD34+ cells, and colony-forming units were determined in samples from cord blood units before and after AXP™ separation. Results: Results are presented as the mean ± S.D. (N = 23) for all values. The AXP™ process achieved mononuclear (MNC) fraction volumes of 19.7 ± 0.3 ml with a final average hematocrit of 29.8 ± 2.6%. The recovery was 98.2 ± 8.0% (CD34+ cells), 94.6 ± 7.0% (CFU), 97.9 ± 4.9% (MNC), and 84.8 ± 9.2% (TNC). Less than 1% of TNC were found in the excess plasma bag. Fifteen percent of TNC were lost and recovered in the red cell bag, and were mostly granulocytes. Less than 0.5% of the CD34+ cells were found in the RBC bag. Conclusions: The AXP™ cord blood volume reduction process automatically, efficiently, and reproducibly separated cord blood mononuclear cells, CD34+ cells, and CFU activity. It achieved high recoveries (>94%) in a closed system without requiring hetastarch. The volume target was de-livered into the freezing bag with high precision. Thus, AXP™ can meet the volume reduction quality standard for automated cord blood processing.