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cells/kg. The mean peripheral CD34 count prior to the use of P was 3.3/ul, and increased to 8.8/ul after its use.

Conclusion: Our limited single-center outcomes data suggests that the addition of P as a salvage agent may improve mobilization outcomes in poor mobilizers. Further evaluation is needed to combine P with CY+G in terms of optimal timing and dosing of chemotherapy utilized.

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SUCCESSFUL AUTOLOGOUS STEM CELL TRANSPLANT AFTER 21 YEARS OF CRYOPRESERVATION

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Introduction: Successful transplantation of cryopreserved hematopoietic stem cells can be regularly achieved provided sufficient numbers of cells are administered. The duration of hematopoietic stem cell viability is unclear. Evidence of autologous repopulation has been seen at 14 years after bone marrow transplant and 12 years after peripheral stem cell transplant. We report a successful autologus transplantation 21 y after cryopreservation.

Case: The patient is 43 year old man found to have follicular lymphoma with bone marrow involvement in 1989 at age 22. He achieved complete remission after treatment with two cycles of Chorambucil. Bone marrow (BM) procurement and cryopreservation was performed at that time for possible subsequent infusion. The procured BM consisted of a total cell count of 1.21×10^8 cells/per kg body weight with a total volume of 354 ml. Equal parts of 20% DMSO were combined with marrow to a final concentration of 10% DMSO. The BM was stored in the liquid phase of nitrogen until date of infusion 21 years later. Our patient relapsed in 1996, and underwent treatment in 2006 with six cycles of Fludarabine and Rituximab, achieving a complete remission. He continued Rituximab maintenance and then developped pancytopenia. Work-up confirmed MDS with 5q- and t(6q21;17p13) in 20/20 cells by karyotype analysis. Assessment of previously cryopreserved marrow was undertaken showing no evidence of cytogenetic or histological changes. The patient was prepared with Busulfan IV at 0.8/kg q 6 hours $\times$ 4 days and Cyclophosphamide 60mg/kg IV $\times$ 2 days. The BM was infused and samples from the infused marrow showed 65-75% viability by Tryptan blue assay. White cell engraftment occurred on day 17 and platelet reached 20,000/uL by day 30.

Follow-up 2 months post transplant revealed WBC of 2.6×10^3/uL with ANC 1.5×10^3/uL, Hgb 9.8 gr/dl and platelets of 43,000/uL. FISH analysis for 5q performed showed 85/200 cells positive for 5q-. BM biopsy confirmed dysplastic features consistent with his pre-transplant BM.

Our case illustrates that even in the setting of marginal numbers of infused marrow components and after prolonged cryopreservation, repopulation can readily occur. To our knowledge, this is the oldest successful cryopreserved autologous bone transplant at 21 years post preservation. As novel uses of stem cells advance, optimal storage of various cellular components is necessary. This should be further investigated on a larger scale in the future.

CLINICAL CELLULAR THERAPY

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TUMOR-INFILTRATING LYMPHOCYTES ARE PRESENT IN CANCER RELAPSE AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (ALLOHSCT), OF DONOR ORIGIN, DISTINCT FROM PERIPHERAL BLOOD DONOR LYMPHOCYTES AND EXHIBIT EFFECTOR FUNCTION WITH CD3/CD28 COSTIMULATION *EX-VIVO*

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We theorized that in patients with persistent or relapsed cancer following alloHSCT, tumor would be a source of tumor-reactive donor T lymphocytes that could be expanded *ex vivo* to provide a potent T cell therapy. We recently reported our initial clinical results demonstrating feasibility, safety and biologic activity of this approach in patients with refractory B cell tumor relapse after alloHSCT (ASH 2010). We report here the characterization of the initial tumor infiltrate as compared to circulating T cell populations, and as compared to expanded products from tumor and blood. We determined that tumor-infiltrating donor T cells (confirmed by chimerism assays) differed from peripheral donor T cell populations in individual patients in terms of T cell frequency, naive/memory phenotype, ratio of CD4:CD8 cells and frequencies of effector and regulatory T cell populations. Following α-CD3/CD28 antibody-coated bead expansion with low dose IL-2, PCR-based chimerism assessment of products demonstrated that the costimulated lymphocytes were of donor origin; by flow, products were greater than 95% CD3+ T cells. CD4+ T cells usually predominated, but there was significant patient variation in expansions from both tumor infiltrates and blood. Both expanded tumor and blood products demonstrated increased expression of markers of activation and effector function (CD4: CD40L; CD8: CD137, perforin, NKG2D). The proportion of FoxP3+ Treg cells declined and IFN-producing TBet+ Th1/Tc1 effectors became the dominant T cell population. While the percentage of cells expressing CD27 and CD28 declined proportionate to the duration of culture, their expression was retained in a significant proportion of cells after our typical 12-day culture. Our findings support the hypothesis that, after allotransplant, donor lymphocytes can be identified in residual/progressing tumor, and appear to be distinct from circulating T cell populations. Furthermore, these tumor-infiltrating T cells can be effectively expanded, even from minor populations, to become T-Bet+ T effectors, plausibly circumventing a mechanism of GVL resistance. Costimulated tumor-derived donor lymphocytes may be a therapeutic option for patients without a source of, or who have not responded to, donor lymphocyte infusion for disease progression after alloHSCT.

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NON-EXPANDED PR1-SPECIFIC CTL SORT-PURIFIED DIRECTLY FROM CORD BLOOD LYMPHOCYTES DEPLETE HUMAN AML *IN VIVO*

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Relapse remains a significant problem following allogeneic stem cell transplantation (SCT) for AML. Adoptive transfer of leukemia-specific cytotoxic T lymphocytes (CTL), such as PR1-specific CTL, might be used to treat persistent leukemia after SCT by enhancing graft versus leukemia (GVL) while minimizing graft versus host disease (GVHD). A limitation of this approach is the limited persistence of adoptively transferred T cells in the recipient, due in part to the lengthy *ex vivo* expansion of low frequency cells necessary to obtain a sufficient cell number. We chose to study PR1-CTL derived from umbilical cord blood (UCB) based on our observation that UCB PR1-CTL are increased 100- to 1,000-fold compared to adult peripheral blood (PB), suggesting UCB might be a rich source of PR1-CTL. Because UCB is associated with a decreased risk of GVHD, in part because of the predominance of naive T cells, it may also be a preferred platform to transfer GVL with minimal risk of GVHD. We found the frequency of PR1-CTL in UCB to be 0.007 to 0.345% (mean 0.117%; n = 57) of CD8+ cells compared with a frequency of $< 0.001\%$ in healthy adult PB. Therefore, we hypothesized that a sufficient number of PR1-CTL from UCB could be obtained by PR1/HLA-A2 tetramer-based cell sorting and infused without further expansion to mediate GVL. To test this, CD8+ T cells from HLA-A2+ UCB units were first enriched via whole blood negative immunodensity separation. Enriched cells were sorted ($>$ 98% purity) into PR1-CTL and PR1-CTL-depleted CD8+ cells (PDC) and briefly activated *ex vivo* for 48 hours with soluble anti-CD3/anti-CD28 + IL-2. After 48 hours, PR1-CTL specifically lysed PR1-pulsed T2 cells although 95% of PR1-CTL and PDC retained a CCR7+CD45RA+ naive phenotype. Next, 1×10^4 cells were infused into NOD/SCID mice engrafted for 7 days with $2\text{-}4 \times 10^6$ human AML blasts. Three separate experiments were performed. Two

weeks after adoptive transfer, AML in bone marrow (BM) of PR1-CTL treated mice decreased 27% (range 21%-32%; $p < 0.02$) compared to untreated mice, and by 23% (range 4%-47%; p = NS) compared to mice receiving PDC. Moreover, AML in the PB of PR1-CTL treated mice decreased 47% (range 30%-61%, $p < 0.04$) compared to untreated mice, and by 58% (range 48%-68%; $p < 0.01$) compared to mice receiving PDC. These data justify clinical studies to determine whether PR1-CTL isolated directly from UCB can be used to enhance GVL without increased GVHD.

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ANTIGEN PRESENTING CELL-MEDIATED EX VIVO EXPANSION OF HUMAN UMBILICAL CORD BLOOD CELLS YIELDS SIGNIFICANT EXPANSION OF NATURAL KILLER CELLS WITH ANTI-MYELOMA ACTIVITY

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Background: Allogeneic natural killer (NK) cells are active in various hematologic malignancies and may have an important role in multiple myeloma (MM). Umbilical cord blood (UCB) is a potential source for allogeneic NK cells and ex vivo expanded UCB-derived NK (UCB-NK) cells demonstrate activity comparable to that of peripheral blood-derived NK cells. Here we demonstrate the ability to expand fresh and frozen UCB-NK cells to clinical grade by novel techniques using artificial antigen presenting feeder cells modified to express IL-21 ("K562-cl9-mIL21") or IL-15 ("K562-mb-15-41BBL"). These UCB-NK cells demonstrate appropriate phenotype and are active against a variety of MM cell lines.

Methods: UCB-NK cells were expanded from 1) fresh or 2) frozen cord blood units. 1) Fresh cord blood mononuclear cells (CBMCs) were cultured in 10% human serum albumin media with IL-2 (500 IU/ml) and irradiated K562-cl9-mIL21 feeder cells (2:1 feeder: CBMC ratio) for 21 days. Thereafter, cells were subjected to CD3-immunomagnetic depletion. 2) Frozen CBMC's were grown in a gas permeable culture flask with IL-2 (100 IU/ml) and irradiated K562-mb-15-41BBL feeder cells (2:1 feeder: CBMC ratio). Cells were CD3-depleted on day 7, replated with the same conditions and grown until day 14. CD3-negative cells were then used as effector cells in functional assays. Flow cytometry was used to confirm NK cell purity (C56+/CD3- cells) and a standard chromium-51 assay was performed to determine NK cell cytotoxicity. Targets included K562 cells and MM cell lines RPMI 8226, ARP-1 and U266.

Results: Expansion of fresh and frozen CBMCs yielded a > 2000 and > 200 fold expansion of NK cells, respectively, compared with only 47 fold expansion of fresh CD56-selected cells cultured with IL-2 alone. After CD3 depletion, fresh and frozen-derived UCB-NK cultures were comprised of 92% and 94% CD56+/CD3- cells respectively. APC-expanded UCB-NK cells from fresh and frozen cords demonstrated cytotoxicity against the classic NK cell target K562 as well as MM cell lines RPMI 8226, ARP-1 and U266.

Conclusions: UCB-NK cells can be expanded ex vivo to clinically relevant doses for allogeneic NK cell therapy via co-culture with K562-cl9-mIL21 and K562-mb-15-41BBL feeder cells. Expanded UCB-NK cells are cytotoxic to K562 cells and various myeloma cell lines. Further study of fresh and frozen-derived UCB-NK cells as an adjunct therapy in stem cell transplantation for myeloma is warranted.

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TRANSFER OF SPECIFIC CELLULAR AND HUMORAL ANTI-TUMOR IMMUNITY AFTER STEM CELL TRANSPLANT (SCT) BY VACCINATING STAGE IV BREAST CANCER PATIENTS WITH Her2/*Neu* TARGETED T CELLS AND TRANSFERRING IMMUNE T CELLS INTO THE PATIENTS AFTER SCT

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Women with stage IV metastatic breast cancer (BrCa) have limited treatment options. Innovative treatment strategies are needed to improve anti-tumor responses. We have recently shown that specific T cell immunity can be induced in patients by "vaccinating" the patients (pts) with infusions of anti-CD3 × anti-Her2/*neu* bispecific antibody (Her2Bi) armed activated T cells (aATC). This study investigated whether memory cytotoxic T lymphocytes (CTL) and antibody (Ab) directed at BrCa antigens can be transferred into patients after SCT by evaluating the cytotoxic and Ab responses after aATC infusions prior to SCT and after SCT. After infusions of aATC, peripheral blood lymphocytes (PBL) exhibited high levels of cytotoxicity directed at SK-BR-3 breast cancer cells and high serum levels of Th1 cytokines and IL-12. Three weeks after aATC infusions, ATC were expanded from the second leukopheresis and cryopreserved for re-infusion after SCT. The expanded ATC from 6 patients at an E:T ratio of 25:1 exhibited cytotoxicity ranging from 3.7-25.8 (mean13.6%) directed at the SK-BR-3. After SCT, pts received multiple infusions with a mean total of $54x10^9$ ATC (range 16-110 $\times 10^9$). Cytotoxicity ranged from 4.7 to 70 % from 2 weeks to 12 months post SCT. We tested PBL for the transfer of humoral immunity after SCT by co-culturing PBL with or without SK-BR-3 tumor cells before IT, mid-IT, 1 month post-IT, pre and post SCT for *in vitro* specific anti-SK-BR-3 Ab synthesis. Anti-SK-BR-3 Abs detected in the culture supernatants by ELISA ranged from 15 to 40 ng/ml of Abs prior to IT, 150-220 ng/ml mid-IT and remained high between 70-95 ng/ml at 1 month post-IT. PBL after SCT showed gradual increases in *in vitro* Ab synthesis ranging from 0-10 ng at 1-month post SCT to 70-90 ng by six-month post SCT upon stimulation with tumor cells. Serum antitumor Ab levels directed at SK-BR-3 increased from 2-4 μg to 10-12 μg post IT and between 6-9 μg after SCT. No dose-limiting toxicities, delays in engraftment, and life-threatening infections were observed. These data show that transfer of pre-immunized ATC and T cells in the stem cell product enhance tumor specific cytotoxicity after HDC and PBSCT for breast cancer. Data suggest that both cellular and Ab responses can be transferred and provide an anti-tumor immune response early after SCT. This "vaccinate" and "boost" proof-of-principal strategy may be used to design protocols to enhance anti-tumor activity.

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IMPROVED POST-THAW STABILITY VALIDATION OF PERIPHERAL BLOOD CELL PRODUCTS UTILIZING THE INTRACELLULAR-LIKE CryoStor CRYOPRESERVATION SOLUTION, AND PRELIMINARY RESULTS OF CLINICAL APPLICATION

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Collection and cryopreservation of autologous stem cells is a routine procedure in a variety of malignant diseases. Further, improved stability of cell products is critical to the development of cell and tissue based therapies as part of the growth in regenerative medicine. A growing body of evidence indicates that one key method for improved cryopreservation efficacy is the utilization of a hyperosmotic/intracellular-like cryopreservation media, as opposed to the traditional use of an isotonic/extracellular-like media such as saline as the vehicle for the cryoprotectant. In this evaluation of cryopreservation methods for clinical application, cell samples from apheresis products were cryopreserved in a conventional isotonic-based freeze media (CFM; 20% DMSO, 10% human plasma derivate in Ringer solution) or intracellular-like CryoStor CS10 (CS10; 10% DMSO, serum-free and protein-free). Immediately after thawing, the recovery of WBC was 50.7 ± 14.4% for CFM versus 70 ± 11.6% for CS10 ($p < 0.001$), and that of CD34+ cells 81.8 ± 36.1% for CFM and 101 ± 16.4% for CS10 ($p < 0.05$). In CFM, 20 to 60 min after thawing there was a dramatic loss in cell viability (-40% to -90%), up to complete clotting in 3/10 samples. By contrast, cells remained viable up to 60 min after thawing in CS10, and no clotting occurred. Because of these positive validation results, cryopreservation in the intracellular-like CryoStor CS10 was translated to clinical application for treatment of hematological malignancies. To date, seven patients have received autologous stem cells cryopreserved in CS10.