cells/kg. The mean peripheral CD34 count prior to the use of P was 3.3/μl, and increased to 8.8/μl 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 C+G in terms of optimal timing and dosing of chemotherapy utilized.

150 SUCCESSFUL AUTOLOGOUS STEM CELL TRANSPLANT AFTER 21 YEARS OF CRYOPRESERVATION

Cherry, M.A.1, Kern, W.2, Wageman, B.2, Epstein, R.1, O’neal, C.2, Kratscheill, K.2, Selby, G.1, Holter, J.1, 1 Oklahoma University Health Sciences Center, Oklahoma City, OK; 2 Oklahoma University Health Sciences Center, Oklahoma City, OK

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 autologous transplantation 21 y after cryopreservation.

Case: The patient is a 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 Chlorambucil. 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 x 10^8 cells/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 developed 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 cyogenetic or histological changes. The patient was prepared with Busulfan IV at 0.8/kg q 6 hours x 4 days and Cyclophosphamide 60mg/kg IV x 2 days. The BM was infused and samples from the infused marrow showed 65-75% viability by Trypan blue assay. White cell engraftment occurred on day 17 and platelet reached 20,000/μL by day 30.

Follow-up 2 months post transplant revealed WBC of 2.6 x 10^9/μL with ANC 1.5 x 10^9/μL, Hgb 9.8 g/dL and platelets of 43,000/μL. 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 BM is being investigated on a larger scale in the future.

151 TUMOR-INFILTRATING LYMPHOCYTES ARE PRESENT IN CANCER RELAPSE AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPANTATION (alloHSCT), OF DONOR ORIGIN, DISTINCT FROM PERIPHERAL BLOOD DONOR LYMPHOCYTES AND EXHIBIT EFECTOR FUNCTION WITH CD8+ TUMOR CELLS. AN IN VIVO STUDY

Hardy, N.M., Hakim, F.T., Fellowes, V.S., Roe, J.J., Gres, R.E., Bishop, M.R. National Cancer Institute, Bethesda, MD

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 tumor-infiltrated cells 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 2 x 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 expansion 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 allo

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

St. John, L.S., He, H., Quintanilla, K., Ma, Q., Shpall, E.J., Alatrash, G., Clise-Dwyer, K., Moldrem, J.J. The University of Texas M.D. Anderson Cancer Center, Houston, TX

Relapse remains a significant problem following allogeneic stem cell transplantation (SCT) for AML. Adoptive transfer of leukemia-specific cytotoxic T lymphocytes (CTL), such as PRI-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 PRI-CTL derived from umbilical cord blood (UCB) based on our observation that UCB PRI-CTL are increased 100- to 1,000-fold compared to adult peripheral blood (PB), suggesting UCB might be a rich source of PRI-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 PRI-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 PRI-CTL from UCB could be obtained by PRI/HLA-A2 tetramer-based cell sorting and infused without further expansion to mediate GVL. To test this, CD8+ T cells from HLA-A2+ UCB were first enriched via whole blood negative immunoselection. Enriched cells were sorted (> 98% purity) into PRI-CTL and PRI-CTL-depleted CD8+ cells (PDC) and briefly activated ex vivo for 48 hours with soluble anti-CD3/anti-CD28 + IL-2. After 48 hours, PRI-CTL specifically lysed PRI-pulsed T2 cells although 95% of PRI-CTL and PDC contained a CC2R+/CD45RA+ naive phenotype. Next, 1 x 10^6 cells were infused into NOD/SCID mice engrafted for 7 days with 2 x 10^9 human AML blasts. Three separate experiments were performed. Two