modality in the treatment of cutaneous HPV infections and may become an important treatment option in immunocompromised patients or recalcitrant cases, although there is need for further studies to investigate therapeutic efficacy, safety, and cost-benefit of CDV.

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Successful Autologous Stem-Cell Transplantation After 21 Years of Cryopreservation

Successful transplantation of cryopreserved hematopoietic stem cells (HSCs) can be achieved provided sufficient numbers of cells are administered. However, the optimal conditions for preparation, freezing, and thawing remain to be defined (*1*, *2*). This becomes increasingly important as variations in the cryopreserved stem-cell sources and subsets of cell types with specific functions are infused (*3*). The duration of cell viability under current procedures is unclear. For bone marrow transplantation, the measure of viability remains in vivo hematologic recovery after HSC infusion. Evidence of successful repopulation has been shown in animal studies for autologous peripheral blood stem cell after 12 years, marrow 14 years, and human cord blood stem cells in reconstitution studies in severe combined immunodeficiency mice up to 15 years (*4*). Clinical reconstitution has been achieved with bone marrow stored for 8 years and peripheral blood stem cell for 12 years (*5*). We report a patient in whom autologous cryopreserved marrow with total nucleated cell dose of 1.21×10E8/kg was infused 21 years after collection. Collection and storage were carried out by our standard procedure using dimethyl sulfoxide (DMSO) as the cryoprotectant and controlled rate freezing followed by liquid N_2 preservation.

The patient was a 43-year-old man diagnosed at the age of 22 years with follicular lymphoma and bone marrow involvement. He achieved complete remission after treatment with two cycles of chlorambucil. Marrow procurement and cryopreservation were performed in 1989. The total nucleated cell count obtained was 1.21×10E8 cells/kg body weight. Colony forming unit—granulocytic monocytic was determined to be 0.77×10E5/kg. Equal parts of 20% DMSO and marrow were combined to a final concentration of 10% DMSO in a total volume of 354 mL. Aliquots were placed in 100 mL cryo bags and were frozen in aluminum cassettes. A PLANAR PLC KRYO freezer, programmed to freeze at a rate of 1°C/min to the heat of fusion followed by a boost of liquid N_2 to minimize the time of phase change, was used. Aliquots were cooled at 1°C/min to −40°C and 3°C/min to −100°C and were stored in the liquid N_2. The patient relapsed 7 years later but did not undergo treatment until 2006 with six cycles of fludarabine and rituximab, achieving a complete remission. Rituximab therapy was given as maintenance every 6 months for 2 years. During rituximab therapy, pancytopenia was noted. Workup confirmed myelodysplastic syndrome with 5q- and translocation of long arm of chromosome 6q21 and short arm 17p13 in 20/20 cells by karyotype analysis. He was offered an allogeneic sibling transplant or an autologous transplant using the cryopreserved marrow. He elected to proceed with the cryopreserved marrow transplant. Because of the concern for myelodysplasia in the cryopreserved cells, cytogenetics and histology were performed on the stored marrow. No dysplastic features were noted. The patient received 0.8 mg/kg busulfan intravenously daily for 4 days and 60 mg/kg cyclophosphamide intravenously for 2 days as a preparative regimen. The marrow was thawed in a 37°C water bath and each aliquot infused in less than 20 min. Samples from the infused marrow showed 65% to 75% viability by trypan blue exclusion. Cytogenetic abnormalities were not noted in preserved marrow by karyotype and fluorescence in situ hybridization analysis. White blood cell (WBC) engraftment occurred on day 17, and platelets reached 20,000 by day 30. WBC count remained stable between 2000 and 4000 and platelets remained more than 25,000 for the next 30 days.

Two months after transplant, persistent mild pancytopenia with WBC count of 2600×10E9/L with ANC 1500×10E9/L, hemoglobin 9.8 g/dL, and platelets of 43,000×10E9/L were noted. Fluorescence in situ hybridization analysis showed 85 of 200 cells positive for 5q-. Bone marrow biopsy confirmed dysplastic features consistent with myelodysplastic syndrome.

Early experiences outlining the causes of cellular loss during cryopreservation were critical to expansion of marrow transplantation. Factors that could affect cell survival of stem cells and lymphocyte components have been identified. These include of cryopreservatives such as DMSO, sucrose and protein containing additives, final storage temperature, and methodology of thawing and infusion. These factors have been reviewed extensively (*6–8*). As seen in oocyte, sperm, and embryonic stem-cell preservation, each component reacts differently to the various factors identified above (*9*, *10*). Optimal methods to determine in vivo viability of stem cells from different sources also remain unclear. This report illustrates that in a setting of marginal numbers of infused marrow components, followed by prolonged cryopreservation, repopulation can occur. Because of the early recurrence of the myelodysplastic clone, no firm conclusions can be drawn regarding the long-term engraftment. However, the presence of rapid reconstitution at the expected time point after ablative preparation, transfusion independence, and the in vitro viability results speak to the initial function of the infused HSC. This success of marrow reconstitution after infusion of human marrow cryopreserved for 21 years is the longest duration of preservation of such cells reported to date.

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