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Cord Blood Collection, Manufacturing and Cell Engineering

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Abstract 16

Insights into Highly Engraftable Hematopoietic Cells from 27-Year Cryopreserved Umbilical Cord Blood

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Introduction: Cord blood banking has consistently outpaced the utilization of cord blood units (CBUs). Thus, the average duration of cryopreservation among banked CBUs will likely continue to increase. It remains unclear how long cryopreserved CBUs remain functional, and it is critical to determine whether duration of cryopreservation should be used as an exclusionary criterion during selection for clinical use or if alternative post-thaw metrics can identify potent cryopreserved CBUs regardless of age.

Objectives: Our goal was to determine whether long-term (27-year) cryopreserved CBUs retain viable and functional hematopoietic stem (HSCs) and progenitor cells (HPCs). We further sought to leverage differences in HSC/HPC function (measured by in vivo engraftment) to demonstrate the utility of using omics approaches to identify candidate genes for use as molecular potency markers.

Methods: We performed comprehensive *ex vivo*, in vivo, and molecular analyses on the numbers, viability, and function of three 27-year cryopreserved CBUs using 3-year cryopreserved and fresh CBUs for comparison. Assays included viability staining, immunophenotyping by flow cytometry, primary and secondary colony forming unit (CFU) assays, *ex vivo* expansion of immunophenotypic HSCs/HPCs/CFUs, limiting dilution transplantations into immune-deficient mice, secondary transplantations, and RNA-sequencing of sorted HSCs and multipotent progenitor cells.

Results: Compared to fresh and recently cryopreserved CBU controls, long-term cryopreserved CBUs yield statistically similar numbers of viable immunophenotypic HSCs, multipotent HPCs, and committed myeloid and lymphoid HPCs. They retain highly functional cells, demonstrating similar primary and secondary CFU numbers and expansion capacity compared to controls, as well as robust engraftment, SCID repopulating cell frequency, and secondary engraftment capacity in mouse models of transplantation. Transcriptomic modelling revealed 18 genes, including *MALT1* and *MAP2K1,* and several gene programs, including lineage determination programs and oxidative stress responses, that are strongly enriched in high engrafting HSCs/HPCs.

Discussion: CBUs cryopreserved for up to 27 years retain highly functional HSCs/HPCs. Thus, duration of cryopreservation alone is not an ideal exclusionary criteria for selection of CBUs. Preserving older CBUs may help to maintain a large and diverse pool of donors for clinical selection. Further, transcriptomics can identify candidate genes associated with engraftment for elucidation of possible CBU potency markers regardless of the duration of cryopreservation.