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Optimization of CD34⁺ Cell Enrichment from Cryopreserved Cord Blood Units

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The majority of cord blood units (CBUs) collected do not meet the requirements to be listed for transplantation. This is primarily due to low cord blood volumes or low total nucleated cell (TNC) counts. However, these CBUs still have utility for research and development applications since they contain viable CD34⁺ cells, which are key components that drive transplant engraftment. These cells can also serve as a starting source for the derivation of a variety of cell types for use in research and the development of cell-based therapeutics. A key need is effective isolation of these cells for the research and cell therapy communities. We are carrying out studies to optimize the isolation of CD34⁺ cells from cryopreserved CBUs in comparison to fresh CBUs that are processed within 36 hours of collection. Fresh and frozen CBUs were processed by ficoll separation prior to CD34⁺ cell isolation using the Stemcell Robosep system, and analysis for TNC, CD34⁺ cell count and viability by FACs was carried out. CBUs that had been cryopreserved for up to 17 years were evaluated and had to have a viability >50% at thaw to be used for analysis. The parameters assessed were % yield, purity and viability. Isolation of CD34⁺ cells from fresh CBUs using the Robosep system gave cell viabilities and yields of 95% or greater and purities exceeding 80%. The % CD34⁺ cell yield from frozen CBUs ranged from 68 to 83%, purity ranged from 42 to 54%, and viability ranged from 77 to 84%. Both fresh and frozen CBUs gave acceptable cell yield, purity and viability, but processing of fresh CBUs was superior when compared to frozen CBU processing. Modifications to the processing of frozen CBUs for CD34⁺ cell enrichment are ongoing to improve yield, purity and viability.