como a doença do enxerto contra hospedeiro (DECH) ou aplasia medular. Do mesmo doador do TCTH é coletado por aférese o produto DLI. No material é contado, por citometria de fluxo, a quantidade de células CD3 e uma primeira infusão é feita a fresco, se houver um remanescente pode ser criopreservado, em alíquotas, geralmente em uma dose escalonada. Historicamente, a padronização da técnica de criopreservação do DLI se baseou na experiência com criopreservação das células troncos, mas ainda não há um consenso da melhor fórmula. Em nossa análise, apesar do número pequeno de amostras, todas apresentaram viabilidade pós-descongelamento acima de 75%, consideramos adequada essa taxa. Não houve intercorrência associada ao procedimento de criopreservação. **Conclusão:** A nova metodologia mostrou ser eficiente e efetiva para o procedimento de criopreservação de linfócitos, mantendo-se dentro de critérios aceitáveis de viabilidade e foi aprovado para utilização em nossos produtos de DLI no Hospital Santa Marcelina.

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## COMPARISON OF DIFFERENT METHODOLOGIES FOR EXPANSION OF NATURAL KILLER CELLS FROM PERIPHERAL BLOOD AND UMBILICAL CORD BLOOD FOR IMMUNOTHERAPY APPLICATIONS

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In Immunotherapy, several clinical studies with Natural Killer (NK) cells have shown promising results in the treatment of onco-hematological diseases due to their high cytotoxicity against tumor cells. There are several protocols for the expansion of these cells, but there is no consensus of a gold standard protocol that provides large numbers of functional NK cells required for conducting clinical studies. Thus, we propose the development of a protocol for the expansion of these cells with the subsequent intention of applying them as cell therapy product in the Hematology Service of the Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HC-FMUSP). Our aim was to compare different methodologies and establish a standardized protocol for the isolation and expansion of NK cells from peripheral blood (PB) and umbilical cord blood (UCB). We also characterized the expanded cells according to their functionality and cytotoxicity. The experiment was carried out at the department of Hematology and Cell Therapy of the HC-FMUSP, in the Laboratory of Medical Investigation (LIM-31), fresh PB samples were obtained from Fundação Pró-Sangue leukapheresis healthy donors and the cryopreserved UCB units were obtained from Hospital Sírio-Libanês Umbilical Cord Blood bank. Mononuclear cells were isolated by Ficoll density gradient centrifugation. The isolation and expansion of UCB-derived and PB-derived NK cells was performed with the selection of CD56+ CD3- cells by immunomagnetic separation. Cells were expanded ex vivo for 14 days in RPMI-1640 medium supplemented with different combinations of cytokines, such as interleukin IL-2 alone, IL-2 and IL-21, IL-15 and IL-18 and IL-12, co-cultured or not with CSTX002 feeder cells in a 1:2 ratio (NK:aAPC). The number of NK cells increased significantly when they were co-cultured with aAPC in comparison to conditions without feeder cells. Pre-activation conditions with IL-12, IL-15 and IL-18 for "cytokine-induced memory-like" (ML NK) cells in both PB-NK and UCB-NK expansion yielded a significantly greater fold expansion (12-fold and 8-fold) when compared to expansion with IL-2 alone or IL-2 and IL-21. Moreover, the addition of IL-21 did not have a significant impact in the expansion of CD56+ CD3- cells when compared to conditions that used IL-2 alone. Furthermore, aAPC-mediated expansion yielded a predominantly CD56bright CD16bright mature phenotype in both PB-NK and UCB-NK. To evaluate the functionality NK cells, in vitro assays against tumor target cells was performed and UCB-NK conditions with IL-2 alone or IL-2 and IL-21 showed enhanced degranulation capacity and lytic granules (Granzyme B and Perforin) and cytokine production (IFN-y and TNF-a) than PB-NK cells. ML NK cells from PB produced higher amounts of cytokines and lytic granules when compared to ML NK cells from UCB. This may be related to a lower expression of NKp46, NKG2D and CD69 activation markers in ML NK UCB, and possibly because the UCB units were cryopreserved. The results suggest that ML NK cells show good ex vivo expansion, but do not show proper functionality against tumor cells. It is possible that with the addition of IL-2, ML NK cells could improve the production of cytokines and lytic granules, as seen in the IL-2 alone and IL-2 and IL-21 conditions.

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## CRITICAL FAILURE OF A CELL THERAPY PRODUCTS STORAGE TANK: DESCRIPTION, INVESTIGATION AND IMPLEMENTED IMPROVEMENTS

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**Aim:** Cryopreserved cell therapy products are usually stored in vapor or liquid storage tanks to maintain long-term viability. Storage tank (ST) failure can lead to the loss of cryopreserved cell therapy (CT) products, which means the only possibility of treating serious illnesses. We aimed to