

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-γ and TNF-α) 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

describe a serious vapor ST failure, as well as the improvements implemented in the storage procedure to increase the safety and quality of cryopreserved CT products. **Method:** This is a case report of a vapor ST failure (CBS, isothermal V5000-AB series). The records were reviewed to obtain information regarding the frozen units. **Results:** On 07-dec-2021, the technical staff observed the accumulation of liquid nitrogen (LN2) at the bottom of the ST. The engineering team was contacted and adjusted the ST's maximum LN2 level. The problem recurred on 23-dec-2021. The engineering and maintenance company carried out a series of tests on the ST. On 06-Jan-2022, it was requested emptying and defrosting of the ST for 48 hours for corrective maintenance of the LN2 level sensor. Planning to transfer the inventory to another ST was started, including bags with serology/NAT reagent overwrapping. However, on 23-Jan-2022 (Sunday), the LN2 level sensor stuck at level zero leading the automated LN2 filling of the ST to start and not stop. There was an overflow of LN2 inside the vapor ST, and 3 of 4 rack positions were submerged in LN2. The engineering team performed the filling interruption manually, after being called by the concierge. There were 553 frozen bags from 307 patients/donors stored in the ST at the time of failure. Out of the 553 bags, 87 (15.7%) had positive serology or NAT tests. Of these, 11 (1.9%) were anti-HIV positive (NAT HIV negative) and two (0.3%) were HBsAg and NAT HBV reagents. Out of the 553 bags, 10 (1.8%) had a positive microbiological test, including four (0.7%) for multi-sensitive *Salmonella* sp. Excess of LN2 was manually removed from the ST and the actions to transfer the inventory to the backup ST were intensified, including changing the bags with positive tests (PT) to positions A or B of the racks, as well as finishing these bags overwrapping. The inventory was transferred and stored in the backup ST for approximately 72 hours. Surveillance actions were performed, including virtual meeting and discussion with the Brazilian National Health Surveillance Agency, visual examination to disclose leaking or breakage of seals, thawing of the unit with positive HBV NAT (authorized disposal) to confirm the absence of leaking or breakage of seals, monitoring external signs of vacuum loss, and discussion of the possibility of cross-contamination with the bag supplier. **Discussion:** The data provided by this study are unique and should be considered when evaluating the cryopreserved cell therapy products storage procedure, including ST monitoring, detection of a failing ST, and contingency plan. After this ST failure, improvement actions in the storage procedure were implemented in our institution, including the modification of the storage location of the bags in quarantine and those with PT (positions A and B of the rack) and implementation of the routine use of overwrap in bags with PT. All activities conducted to resolve the issue and the improve actions were documented in non-compliance. **Conclusion:** Vapor ST may fail, leading to an unexpected accumulation of LN2. Improvements in the storage procedure can avoid the serious consequences of this failure, including cross-contamination.

**TRANSPLANTE DE CÉLULAS-TRONCO
HEMATOPOIÉTICAS NO HAPLOIDÊNTICO:
PERCEPÇÃO E DESAFIOS DAS ENFERMEIRAS
FRENTE AO CUIDADO DE ADOLESCENTES
ACOMETIDOS POR ANEMIA APLÁSICA SEVERA
- RELATO DE EXPERIÊNCIA DE UM HOSPITAL
FEDERAL DO ESTADO DO RIO DE JANEIRO**

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Objetivo: Relatar a experiência de trabalho de enfermeiros(as) de uma unidade de Transplante de Células-Tronco Hematopoiéticas com adolescentes acometidos pela Anemia Aplásica Severa. **Material e Métodos:** Trata-se de um relato de experiência de abordagem qualitativa e descritiva de enfermeiras assistenciais de uma unidade de internação de um Centro de Transplante de Células-Tronco Hematopoiéticas do Estado do Rio de Janeiro. **Resultados:** Pode-se perceber que o trabalho do(a) enfermeiro(a) dentro de um centro de transplante tão especializado é notadamente diferenciado pois o mesmo absorve a internação da criança até o idoso. Nesta perspectiva percebe-se a forma especial que o adolescente precisa ser abordado e requer níveis diferentes de atenção e cuidados. O tempo de internação, a gravidade do tratamento, as diversas perspectivas como o medo e a insegurança no sucesso do TCTH são situações que aumentam a carga emocional e de trabalho do enfermeiro (a) frente ao paciente gerando desgastes e desafios diários na evolução do transplante. Dessa forma torna-se necessária, a implantação de estratégias de alívio do estresse de ambos os lados para que a relação paciente e enfermeiro(a) seja a melhor possível, ocasionando o alívio da dor emocional, do sofrimento, do acolhimento, enfim gerando um relacionamento de cumplicidade com o objetivo final do sucesso do tratamento físico e mental. **Discussão:** É relevante entender que a carga emocional que recai sobre os enfermeiros (as) encontra-se em patamar semelhante da sua articulação intelectual para efetuar os cuidados com essa clientela adolescente. Considerando a saúde mental do trabalhador como uma demanda de extrema importância na atualidade vale ressaltar que propostas devem ser criadas e implementadas como suporte para essa realidade. As fases a serem ultrapassadas neste tipo de transplante por um adolescente é bastante difícil, pois recai na sua sobrevivência, sonhos, autoimagem, estudos enfim tudo o que se espera de uma vida plena e saudável. Em contrapartida o(a) enfermeiro(a) está presente para identificar cada fase que está surgindo a fim de que não haja progressão das reações advindas do TCTH, movimento esse que causa grande tensão diária ao profissional que necessita de cooperação do adolescente assim como de sua destreza para não aumentar a ansiedade natural do curso do tratamento. **Conclusão:** As vivências do enfermeiro(a) sendo aquele(a) que está sempre a frente do cuidado demonstram a importância da saúde do trabalhador juntamente com a instituição em identificar esses profissionais e suas demandas. Principalmente num setor onde trata-se as