Related versus unrelated allogeneic HPC graft cryopreservation: a single-center experience in the context of the global COVID-19 pandemic

B. Mfarrej1,2 · C. Lemarié1,2 · A. Granata1,3 · T. Pagliardini1,3 · C. Malenfant1 · P. Lignée1 · M. Fays1 · D. Blaise3 · C. Chabannon1,2,3 · B. Calmels1,2

Received: 30 November 2020 / Revised: 15 December 2020 / Accepted: 17 December 2020 / Published online: 12 April 2021
© The Author(s), under exclusive licence to Springer Nature Limited 2021

To the Editor:

Under normal conditions, and despite being an organizational challenge, the vast majority of allogeneic hematopoietic progenitor cell (HPC) grafts collected from related donors (RD) or unrelated donors (URD) are freshly infused within hours of collection, while cryopreservation is restricted to exceptional conditions related to donor unreliability/unavailability [1]. However, since the beginning of the global COVID-19 pandemic, recommendations from professional societies (AABB, EBMT, ASTCT, WMDA, and FACT) were issued to cryopreserve allogeneic HPC grafts. The rationale behind is to minimize the risk of harvesting a cell product from a SARS-CoV-2 positive donor (by physical examination and RT-PCR testing of a nasopharyngeal swab) as well as to exclude potential transport setbacks (due to closed national borders or other reasons) before initiation of conditioning regimen. Recent studies reported on the minimal impact cryopreservation of allogeneic grafts has on hematopoietic recoveries, risks for acute graft-versus-host disease, non-relapse mortality or overall survival of patients with hematological malignancies, despite the heterogeneity of these assessments [2, 3].

At our institution, cryopreserved HPC grafts (autologous and occasionally allogeneic) are thawed and washed before infusion, according to standardized, and mostly automated procedures, generating cell products with high CD34+ cell recovery and viability [4]. We hereby report our single-center experience of cryopreserved HPC allogeneic graft manipulation, from both RD and URD, in the peculiar context of the global COVID-19 pandemic.

Data from our cell processing facility were compiled for 42 allogeneic rHuG-CSF-mobilized peripheral blood HPC products (28 RD and 14 URD) processed between March and July 2020. RD HPC grafts were collected at our apheresis unit, while URD HPC grafts were collected in centers in Europe and subsequently temporarily stored and transported at +4–10 °C. Upon reception, systematic platelet depletion/volume reduction, suspension in 6% HES 10% DMSO at 100 ml per bag, and cryopreservation in a controlled-rate freezer were performed, before storage in vapor phase of nitrogen containers. All products tested negative for microbiological contamination (aerobic and anaerobic cultures on both fresh and thawed/washed cell products). Dry-thawing was performed on Smart-Max (Cytiva Europe GmbH), followed by automated washing using Sepax-2 (Cytiva Europe GmbH).

Viable CD34+ and CD45+ cell counts were determined by single-platform flow cytometry assay using Stem-Kit (Beckman Coulter) according to the modified International Society of Hemotherapy and Graft Engineering (ISHAGE, now ISCT) protocol which includes the use of 7-AAD and Flowcount Fluospheres (Beckman Coulter) for absolute viable CD34+ cells counting of a sample from the washed bag immediately before infusion [5]. As a validated test for potency, colony-forming unit (CFU) assay was performed on all washed products followed by automated counting on STEMVision (Stemcell technologies) at the end of the 14-day incubation period, as previously reported by our...
CD34 recovery and viability, CD45 viability and potency assay assessment of HPC grafts of both RD and URD after thawing and washing. Single-platform flow cytometry-based analysis was used for viable CD34+ and CD45+ cells enumeration and measurement of viability, while a 14-day culture and automated cell counting were performed to report CFU counts and clonogenicity. CD34+ cell recovery was calculated as the ratio of absolute count of post-wash viable CD34+ cells to pre-cryopreservation absolute counts of viable CD34+ cells. Clonogenicity was calculated as the percentage of CFU count ($10^4$) to post-wash viable CD34+ cells absolute count ($10^6$). Data are presented as median ± interquartile ranges. Recovery, viabilities and clonogenicity are reported as percentages using the left y-axis. CFU is reported as count ($10^7$/kg) using the right y-axis. Open symbols represent RD, closed symbols represent URD. p values are reported above the groups using Mann–Whitney test for null hypothesis testing.

Fig. 1

In conclusion, cryopreservation of allogeneic HPC grafts is a reasonable option that might be implemented after benefit-risk assessment to ensure both safety of the collected HPC graft during exceptional conditions such as the COVID-19 pandemic or anticipated challenges in relation to cell procurement or transportation. While CD34+ cell loss remains unavoidable, inter-individual variability can be mitigated by robust, standardized, and automated post-collection processing. Since CD34+ cell recovery tends to be lower for URD grafts (63 versus 74% for RD grafts, Fig. 1), we therefore suggest to systematically reduce transit times when feasible and to request a slightly higher dose of CD34+ cells to be collected by donor centers for URD.
Acknowledgements  We would like to thank the transplant coordinators responsible for RD and URD identification and recruitment as well as the donors for their time and commitment.

Compliance with ethical standards

Conflict of interest  The authors declare no competing interests.

Publisher's note  Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. Frey NV, Lazarus HM, Goldstein SC. Has allogeneic stem cell cryopreservation been given the ‘cold shoulder’? An analysis of the pros and cons of using frozen versus fresh stem cell products in allogeneic stem cell transplantation. Bone Marrow Transplant. 2006;38:399–405. https://doi.org/10.1038/sj.bmt.1705462
2. Alotaibi AS, Prem S, Chen S, Lipton JH, Kim DD, Viswabandya A et al. Fresh versus frozen allogeneic peripheral blood stem cell grafts: a successful timely option. Am J Hematol. 2020. https://doi.org/10.1002/ajh.26033
3. Hamadani M, Zhang MJ, Tang XY, Fei M, Brunstein C, Chhabra S, et al. Graft cryopreservation does not impact overall survival after allogeneic hematopoietic cell transplantation using post-transplantation cyclophosphamide for graft-versus-host disease prophylaxis. Biol Blood Marrow Transplant. 2020;26:1312–7. https://doi.org/10.1016/j.bbmt.2020.04.001
4. Calmels B, Drezet A, Huynh C, Autret A, Stoppa AM, Bouabdallah R, et al. Automated washing of autologous hematopoietic stem cell grafts after thawing does not impair engraftment. Bone Marrow Transplant. 2014;49:1127–8. https://doi.org/10.1038/bmt.2014.111
5. Brocklebank AM, Sparrow RL. Enumeration of CD34+ cells in cord blood: a variation on a single-platform flow cytometric method based on the ISHAGE gating strategy. Cytometry. 2001;46:254–61.
6. Velier M, Chateau AL, Malenfant C, Oufai S, Calmels B, Chabannon C, et al. Validation of a semi automatic device to standardize quantification of Colony-Forming Unit (CFU) on hematopoietic stem cell products. Cytotherapy. 2019;21:820–3. https://doi.org/10.1016/j.jcyt.2019.06.005
7. Berens C, Heine A, Müller J, Held SA, Mayer K, Brossart P, et al. Variable resistance to freezing and thawing of CD34-positive stem cells and lymphocyte subpopulations in leukapheresis products. Cytotherapy. 2016;18:1325–31. https://doi.org/10.1016/j.jcyt.2016.06.014
8. Fisher V, Khoo H, David-Ocampo V, Byrne K, Pavletic S, Bishop M, et al. Analysis of the recovery of cryopreserved and thawed CD34+ and CD3+ cells collected for hematopoietic transplantation. Transfusion. 2014;54:1088–92. https://doi.org/10.1111/trf.12428
9. Purtill D, Antonenas V, Chiappini P, Tong D, O’Flaherty E, Bajel A, et al. Variable CD34+ recovery of cryopreserved allogeneic HPC products: transplant implications during the COVID-19 pandemic. Blood Adv. 2020;4:4147–50. https://doi.org/10.1182/bloodadvances.2020002431
10. Kim DH, Jamal N, Saragosa R, Loach D, Wright J, Gupta V, et al. Similar outcomes of cryopreserved allogeneic peripheral stem cell transplants (PBSCT) compared to fresh allografts. Biol Blood Marrow Transplant. 2007;13:1233–43. https://doi.org/10.1016/j.bbmt.2007.07.003
11. Lioznov M, Dellbrügger C, Sputtek A, Fehse B, Kröger N, Zander AR. Transportation and cryopreservation may impair hematopoietic stem cell function and engraftment of allogeneic PBSCs, but not BM. Bone Marrow Transplant. 2008;42:121–8. https://doi.org/10.1038/bmt.2008.93
12. Schmidt AH, Buk D, Platta A, van den Brink MRM. Cryopreservation for all is no option in unrelated stem cell transplantation: comment on Dholaria B, et al. securing the graft during pandemic: are we ready for cryopreservation for All? Biol Blood Marrow Transplant. 2020;26:e145–e146 Biol Blood Marrow Transplant. 2020;26:e298–e299. https://doi.org/10.1016/j.bbmt.2020.08.011