FULL-LENGTH ARTICLE

Manufacturing

Study of a new device for washing and concentrating cryopreserved hematopoietic stem cells and mononuclear cells: a single center experience

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ABSTRACT

Background aims: Cryopreserved cellular products, as parts of hematopoietic progenitor cell (HPC) transplants, mononuclear cell reinjections for donor lymphocyte infusion or extracorporeal photopheresis, can be washed before being reinjected into the patient or infused directly, depending on local practices. The aim of washing is to reduce the incidence and severity of adverse reactions (ARs) due to the dimethyl sulfoxide (DMSO) used as a cryoprotective agent and other factors, such as dead cell debris. At the authors' cell therapy laboratory (CTL) in Poitiers, France, as in 76% of Etablissement Français du Sang (EFS) CTLs, all cryopreserved products undergo thawing in a water bath followed by washing with the COBE 2991. As this device will soon cease to be available, an alternative process needs to be assessed.

Methods: The authors compared two closed systems: the authors' semi-automatic system using the traditional centrifugation method (COBE 2991) and an automated device using spinning membrane filtration (Lovo). A total of 72 HPC bags available for research were used. The authors first performed a paired comparison, processing one or two HPC bags washed by each device. A second study was carried out to compare two different washing solutions generally used by EFS CTLs along with variable storage conditions. Finally, the authors studied the efficiency of the Lovo with three or four thawed bags. The main parameters studied were viable CD34+ cell recovery and viability, CD3+ cell recovery, stability up to 6 h after washing, DMSO elimination and center feasibility.

Results: The Lovo device showed better CD34+ cell recovery compared with the COBE 2991 while maintaining CD34+ viability and stability over 6 h. Moreover, Lovo efficiency seemed to be independent of the number of thawed bags processed and washing solution used in the authors' study. CD3+ cell recovery met the authors' internal specifications (cell recovery >50%), with similar results seen when processing with either the COBE 2991 or Lovo. Additionally, on average, 97% of DMSO was removed after washing with Lovo, minimizing the risk of ARs. The storage conditions post-processing indicated preferred storage conditions of $7 \pm 3^\circ\text{C}$. Finally, if processing time seemed shorter using COBE 2991 for one bag washed, the Lovo device required only one staff member regardless of the number of HPC bags processed.

Conclusions: The Lovo device seems to provide an opportunity to standardize HPC processing, ensuring patient safety, with, on average, 97% of DMSO removed, while improving recovery of cells of interest and maintaining viability over time in case of delayed transplant. The Lovo device consequently seems to be a serious alternative to the COBE 2991.

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Introduction

Autologous hematopoietic stem cell transplantation is widely used to treat malignant diseases, such as lymphomas, myelomas and solid tumors, for hematopoietic reconstitution after high-dose cytotoxic therapy. The stem cells (obtained from peripheral blood or bone marrow) have to be frozen and stored in liquid nitrogen for variable periods of time (usually less than a year) before reinjection into the patient. Other types of cell therapy products can also be cryopreserved for other disease indications. The authors' cell therapy laboratory (CTL) cryopreserves products for autologous or allogeneic HPC transplantation, donor lymphocyte infusion (DLI) and extracorporeal photopheresis (ECP) for graft-versus-host disease, dermatological indications and lung rejection after transplant (see supplementary Table 1) [1]. Currently, the Poitiers CTL and two French hospital CTLs are authorized by the Agence nationale de sécurité du médicament et des produits de santé (ANSM) to use cryopreserved mononuclear cells (MNCs) for ECP treatment.

To protect cryopreserved cells from intracellular ice formation and dehydration during cryopreservation, a cryoprotectant is required, and dimethyl sulfoxide (DMSO) is most often used. After thawing, immediate infusion of hematopoietic stem cell grafts into the patient can lead to several types of adverse reactions (ARs), which have been highlighted and widely described in the literature [2–4]. The reported ARs can be attributed to DMSO toxicity as well as other factors, such as dead cell debris and cytokines released by damaged or dead cells [5]. DMSO-related ARs range from mild (gastrointestinal, respiratory and dermatological complaints) to severe (rare cases of fatal cardiac reactions).

To reduce the occurrence of ARs, most Etablissement Français du Sang (EFS) CTLs, according to Société Francophone de Greffe de Moelle et de Thérapie Cellulaire recommendations, add a washing step before graft infusion into the patient [6]. Currently, the equipment used by the authors' CTL as well as 76% [7] of EFS CTLs to wash and prepare the cells is the COBE 2991 (Terumo BCT, Lakewood, CO, USA), a semi-automatic device, using a traditional centrifugation method. The other device, used by 24% of CTLs, involves a manual method of centrifugation. The announced cessation of the marketing of the COBE 2991 led the authors to consider its replacement. A closed and automated device, allowing maximum elimination of DMSO and optimal cellular recovery, was targeted.

To this end, the aim of this study was to evaluate the efficiency of a new device, the Lovo, developed by Fresenius Kabi (Bad Homburg, Germany). Pre-clinical assessment of the Lovo served as a reference for this study for general knowledge about the operation of the device, especially the use of packed cell volume (PCV), DMSO quantification and critical process parameters validated in this study [8].

As part of a national examination, other alternatives are being studied by other EFS CTLs. The authors chose to perform a paired comparison between the Lovo and the authors' current device, the COBE 2991, focusing on several parameters: viable CD34⁺ cell recovery and viability as the main indicator for graft intake in autologous/allogeneic HPC transplantation, CD3⁺ cell recovery for DLI [9,10] and ECP [11–14], DMSO elimination for reduction in ARs, stability over time in case of delayed injection and feasibility for operation.

Methods

Collection and cryopreservation

A total of 72 human granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood HPC product bags collected and cryopreserved between 2011 and 2019 and available for research (deceased patients/no more graft indication; informed consent) were used. This study was approved by the national EFS director of cell therapy processes.

The authors chose to perform the study with HPC products mainly from patients suffering from multiple myeloma (thirteen patients, 34

bags) and non-Hodgkin lymphoma (eight patients, 24 bags), which are the most frequent indications for autologous HPC transplant. G-CSF-mobilized peripheral blood collected by apheresis from voluntary donors was also used (four donors for acute myeloid leukemia [12 bags] and one donor for plasma cell leukemia [2 bags]) as a means of representing allogeneic transplant. Multiple myeloma and non-Hodgkin lymphoma for autografts and acute myeloid leukemia for allografts represent more than 80% of the indications for HPC engraftment (data from 2019 CTL activity). Therefore, these are the most represented indications among grafts available for research.

Within 24 h after collection, HPC products underwent volume reduction using a manual method of centrifugation, plasma press and reconstitution or dilution with albumin [6]. Anticoagulant citrate dextrose solution A (ACD-A) (5% of total cell volume to cryopreserve) was then injected before slowly adding volume-to-volume cryoprotectant solution (20% DMSO/80% albumin stored at $7 \pm 3^\circ\text{C}$ for 1 h minimum). Final volumes were between 100 mL and 120 mL per cryopreserved bag (DF700; MedHemScience B.V., Emmen, the Netherlands). Cryopreservation was performed in a controlled-rate freezer (Nicol Freezal; Air Liquide, Paris, France or Mini-Digitcool; Cryo Bio System, Saint-Ouen-Sur-Iton, France). Temporary storage in a -150°C semi-liquid nitrogen tank (Air Liquide, Paris, France) and final storage in a liquid nitrogen tank were carried out.

Experimental plan

An adaptive test was performed between May 2019 and November 2020, which means that process conditions (washing solution, storage temperature, etc.) were gradually adapted according to the results obtained. After thawing in a water bath (set to 40°C to obtain at the end of thawing 37°C in the water bath), HPC products were washed using either Lovo 3.0 or COBE 2991.

An important piece of input data used by the Lovo to adjust its washing parameters to optimize cellular recovery is the PCV percentage. Prior to washing with Lovo, the PCV percentage calculation was performed by the device based on the pre-freezing counts: $([\text{Total volume of cellular material: red blood cells, white blood cells and platelets}] / [\text{total source product volume}]) \times 100$.

Comparability of automation devices with one- or two-bag processing

A paired comparison was performed using one bag ($n = 5$) and two bags ($n = 5$) that were thawed and then washed with sodium chloride (NaCl). The staff added ACD-A to the thawed product at 10% of the thawed bag volume prior to washing with both Lovo and COBE 2991. For consistency, the final volume was held constant at 200 mL. The Lovo and COBE 2991 final products were stored at room temperature ($18\text{--}24^\circ\text{C}$) under agitation (using a platelet agitator) for 6 h (similar conditions as described by Mfarrej *et al.* [8]).

For DMSO quantification, 1 mL of thawed product was collected after washing with either COBE 2991 or Lovo. Residual DMSO was evaluated with the Sciex P/ACE capillary electrophoresis system (Beckman Coulter, Brea, CA, USA) as described by Mfarrej *et al.* [8]. The percentage of DMSO elimination was calculated relative to the mean DMSO amount from five cryopreserved samples (cryopreserved at the same concentration as five HPC products used for this study) as follows: $(1 - [\text{value after washing} / \text{value before washing}]) \times 100$.

Determining washing solutions and storage conditions (with the Lovo device only)

A paired comparison was performed with one bag thawed and washed with either NaCl (ACD-A 10% of thawed bag volume added to thawed bag before washing) or NaCl 60%/albumin 30%/ACD-A 10% and then stored at room temperature under agitation ($n = 3$) or at $7 \pm 3^\circ\text{C}$ under no agitation ($n = 2$). The final volume from each procedure was 200 mL. For DMSO quantification ($n = 9$), the same method described by Mfarrej *et al.* [8] was used with the Sciex P/ACE capillary

electrophoresis system. For better accuracy regarding pre-washing DMSO quantity, 1 mL of thawed product was collected before and after washing with the Lovo device. The percentage of DMSO elimination was calculated as follows: $(1 - [\text{value after washing} / \text{value before washing}]) \times 100$.

Comparability after wash solution selection and storage condition identification

A paired comparison of Lovo versus COBE 2991 was performed with one bag ($n = 7$) thawed and washed with NaCl 60%/albumin 30%/ACD-A 10% and then stored at $7 \pm 3^\circ\text{C}$ under no agitation. The final volume from each procedure was 200 mL. This procedure was repeated with three bags ($n = 1$), and the final volume targeted was 300 mL. Moreover, as no additional bags could be used, four bags were processed under identical processing conditions using Lovo only ($n = 3$). The final volume was set at 200–400 mL and adjusted according to cellularity.

Flow cytometry testing

A sample was taken from the thawed product immediately after resuspension (hour 0) and at hour 2, hour 4 and hour 6. Registration and staining were performed within 1 h of thawing. Total nuclear cell count was performed on an XN-9000 (Sysmex Corporation, Kobe, Japan). When required, white blood cell counts were adjusted by dilution in cold phosphate-buffered saline/0.5% bovine serum albumin to target a concentration lower than 40×10^3 cells/ μL . Cell viability by 7-aminoactinomycin D and CD34+, CD45+ and CD3+ cell counts were determined using a single-platform flow cytometry assay. Samples were prepared using a BD stem cell enumeration kit (BD Biosciences, Franklin Lakes, NJ, USA) according to the manufacturer's instructions and the modified International Society of Hematotherapy and Graft Engineering protocol. Sample acquisition was performed on a Navios flow cytometer (Beckman Coulter) with a protocol based on the International Society of Hematotherapy and Graft Engineering modified gating strategy. CD34+ and CD3+ cell recovery (%) was calculated as follows: $([\text{post-wash viable absolute CD34} + \text{or CD3+ cell count}]) / ([\text{pre-cryopreservation viable absolute CD34} + \text{or CD3+ cell count}]) \times 100$

Processing time

Processing time for one to four thawed bags was evaluated when processing with Lovo compared with COBE 2991. Time was measured from the installation of the kit to washing procedure completion and resuspension for final volume adjustment. The whole staff was certified to proceed with COBE 2991. Runs using the Lovo were carried out by staff trained by Fresenius Kabi.

When a paired comparison was performed, bags were simultaneously processed with COBE 2991 and Lovo by two different staff members. The same staff member performed the manipulations on the Lovo while different staff members performed the washing process with the COBE 2991 (depending on staff availability).

Statistical analysis

Data were expressed as median and interquartile range (IQR). Two-tailed Wilcoxon–Mann–Whitney test or Student's *t*-test was used to compare two paired groups with Prism 9.1.0 (GraphPad Software, San Diego, CA, USA). $P \leq 0.05$ was considered significant. A synopsis of the experimental objectives, values analyzed and experimental variables can be found in Table 1.

Results

Characteristics (pre-cryopreservation counts, indications, patient age, etc.) of HPC products are detailed in supplementary Table 2. HPC bag PCV ranged from 2.2% to 9.9%.

Comparability of automation devices with one- or two-bag processing

A comparative study of Lovo versus COBE 2991 was initiated with one and two thawed HPC bags. The main washing media used by EFS CTLs in France are NaCl/albumin/ACD-A 76% and NaCl 12% [7]. The authors chose to start with the NaCl solution. This solution was recently validated for the washing/processing of cryopreserved products at the authors' institution, as the prior solution (NaCl 0.9% + glucose 0.2%; Bioluz, Saint-Jean-de-Luz, France) lost its European Union authorization. Nevertheless, this is also the preferred solution for supply chain security and cost of goods. The outcomes

Table 1

Experimental plan and variables for consideration used in this adaptive study to test the Lovo and COBE 2991 systems in accordance with the use conditions and environment of the EFS.

Purpose	Automation technology	Cell Type	Assessment	Wash solution	Storage conditions	Runs, n
Study 1: comparability of automation devices with one- or two-bag processing	Lovo versus COBE 2991 (paired)	CD34+	Viability Recovery Stability DMSO removal	NaCl	Room temperature/ agitation	
Study 2: determining wash solution and storage conditions	Lovo	CD34+	Viability	NaCl	Room temperature/ agitation $7 \pm 3^\circ\text{C}$	3
			Recovery			2
			Stability		Room temperature/ agitation $7 \pm 3^\circ\text{C}$	3
			DMSO removal			2
Study 3: comparability after solution selection and storage condition identification	Lovo versus COBE 2991 (paired)	CD34+	Processing time	NaCl 60%/albumin 30%/ACD-A 10%	Room temperature/ agitation $7 \pm 3^\circ\text{C}$	
			Viability			
			Recovery			
			Stability			
	Lovo	CD3+	DMSO removal	NaCl 60%/albumin 30%/ACD-A 10%	$7 \pm 3^\circ\text{C}$	
			Processing time			
			Viability			
			Recovery			
	Lovo	–	Stability	Same conditions		
			Processing time			
			Same parameters assessed			
						3 (4 bags)

Figure 1. Viable CD34+ cell recovery from one ($n = 5$) and two ($n = 5$) thawed HPC products washed with NaCl at hour 0 using Lovo versus COBE 2991. Data are expressed as a percentage of pre-cryopreservation counts. $P \leq 0.05$ was considered significant.

showed significant superiority of the Lovo device at hour 0 in terms of viable CD34+ cell recovery percentage (Figure 1). However, poor stability over time regardless of equipment used led the authors to carry out a second study (Figure 2).

Determining wash solution and storage conditions

Two different washing solutions (NaCl 60%/albumin 30%/ACD-A 10% versus NaCl) and storage conditions after thawing were compared to improve stability (viable CD34+ cell recovery) over time. The addition of a macromolecule (albumin) to the washing solution did not improve stability compared with storage at $7 \pm 3^\circ\text{C}$ (room temperature $n = 3$, $7 \pm 3^\circ\text{C}$ $n = 2$) (Figure 2). Moreover, the outcomes showed satisfactory stability over time regardless of washing solution used when the HPC products were stored at $7 \pm 3^\circ\text{C}$ after the thawing/washing process.

Based on these outcomes and to verify this trend, further tests ($n = 7$) were carried out at $7 \pm 3^\circ\text{C}$ with NaCl/albumin/ACD-A, which is the most widely used washing solution at EFS CTLs and seems to give the best cell recovery yields based on the national EFS study ($n = 1921$ final products for transplantation) [7]. Stability was improved, with significant superiority of the Lovo device up to 4 h (Figure 3), while maintaining the viability of CD34+ cells at hour 0

(Figure 4). As indicated in Figure 3, the product was stable up to 6 h. In addition, these outcomes met the authors' internal specifications (cell recovery $>50\%$). Moreover, in the event of a delayed injection, the product can be stored at $7 \pm 3^\circ\text{C}$ according to the authors' validated process and EFS national recommendations.

In addition to the CD34+ recovery and viability assessments, the authors noticed significant ($P = 1.1 \times 10^{-5}$) platelet depletion using Lovo (median, 76.4%, IQR, 70.1–79.4) compared with COBE 2991 (median, 37%, IQR, 35.8–39). Although no correlation could be found with improved viability and/or cellular recovery, this parameter might help to improve cell recovery. An Italian CTL observed these outcomes, with 72.9% of the initial platelet contamination found in the waste bag [15].

Thawing more than two bags

At the authors' CTL, $>70\%$ of all transfusions derive from three to four HPC bags. Therefore, the efficiency of the Lovo needed to be assessed for three and four HPC bags thawed for the same transfusion. The availability (at the author's CTL) of six or eight cryopreserved bags intended for destruction is more challenging. As such, the number of runs was smaller, and a COBE 2991 versus Lovo comparison was only possible for three thawed bags.

The outcomes for four bags processed using only Lovo ($n = 3$) met the authors' internal specifications ($>50\%$) in terms of CD34+ cell recovery (median, 66.8%, range, 52.9–78.8) and viability (median, 93%, range, 91–95) (see supplementary Table 2). One run comparing COBE 2991 and Lovo was performed with three thawed bags. The outcomes were not conclusive in terms of viable CD34+ cell recovery for either COBE 2991 (22.8%) or Lovo (23.5%).

Viable CD3+ cell recovery

Viable CD3+ cell recovery through 6 h was quite stable, with less than 10% loss from hour 0, when processing with Lovo ($n = 7$). Similar results were observed with COBE 2991, which was slightly superior (Figure 5). These outcomes were in compliance with the authors' internal specifications (viable CD3+ cell recovery $>50\%$) for DLJ and ECP on cryopreserved cell activity.

DMSO removal

In terms of DMSO elimination, there was no significant difference between Lovo (97.06 ± 1.66) and COBE 2991 (98.60 ± 0.83) for one processed bag ($P = 0.27$) compared with two processed bags ($P = 0.003$) (Table 2). According to this second study, more than 97%

Figure 2. Viable CD34+ cell recovery from one thawed HPC product washed with NaCl or NaCl/Alb/ACD-A using the Lovo device. Stability was expressed up to 6 h under varying storage temperatures ($7 \pm 3^\circ\text{C}$ [$n = 2$] and room temperature/agitation [$n = 3$]). Data are expressed as a percentage of pre-cryopreservation counts. Mean $\pm$ SEM is indicated. Alb, albumin; SEM, standard error of the mean.

Figure 3. Viable CD34+ cell recovery from one thawed HPC product washed with NaCl/Alb/ACD-A using Lovo versus COBE 2991 and stored at $7 \pm 3^\circ\text{C}$ up to 6 h ($n = 7$). Data are expressed as a percentage of pre-cryopreservation counts. $P \leq 0.05$ was considered significant. Alb, albumin.

Figure 4. Viable CD34+ cell recovery and viability from one thawed HPC product washed with NaCl/Alb/ACD-A at hour 0 using Lovo versus COBE 2991 ($n = 7$). Data are expressed as a percentage of pre-cryopreservation counts. $P \leq 0.05$ was considered significant. Alb, albumin.

Table 2

DMSO removal in study 1 after washing one or two bags with NaCl using Lovo versus COBE 2991.

	Before ^a washing	After washing COBE 2991	Lovo	Result, % ^b COBE 2991	Lovo	After washing COBE 2991	Lovo	Result, % ^b COBE 2991	Lovo
HPC bags thawed, n				1				2	
DMSO, g/L, mean $\pm$ SD	96.56 $\pm$ 4.94	1.36 $\pm$ 0.80	2.84 $\pm$ 1.60	98.60 $\pm$ 0.83	97.06 $\pm$ 1.66	2.28 $\pm$ 0.57	4.56 $\pm$ 0.50	97.64 $\pm$ 0.59	95.28 $\pm$ 0.52

Study 1 $n = 5$.

SD, standard deviation.

^a Mean of DMSO amount from five samples cryopreserved at same concentration of five HPC products used.

^b Percentage of DMSO elimination.

Table 3

DMSO removal in study 2 after washing one bag with NaCl or NaCl/albumin/ACD-A using Lovo.

	After thawing	After washing	Result, % ^a	After thawing	After washing	Result, % ^a
Washing solution		NaCl		NaCl/albumin/ACD-A		
Runs, n		4		5		
DMSO, g/L, mean $\pm$ SD	103.72 $\pm$ 14.18	2.56 $\pm$ 0.46	97.50 $\pm$ 0.50	107.19 $\pm$ 4.28	2.32 $\pm$ 0.03	97.84 $\pm$ 0.09

NaCl $n = 4$. NaCl/albumin/ACD-A $n = 5$.

SD, standard deviation.

^a Percentage of DMSO elimination.

Figure 5. Viable CD3+ cell recovery from one thawed HPC product washed with NaCl/Alb/ACD-A using Lovo or COBE 2991 and stored at $7 \pm 3^\circ\text{C}$ ($n = 7$). Data are expressed as a percentage of pre-cryopreservation counts. Mean $\pm$ SEM is indicated. Alb, albumin; SEM, standard error of the mean.

Table 4

Total processing time depending on number of bags thawed and device used.

HPC bags thawed, n	Lovo, minutes (Runs, n)	COBE 2991, minutes (Runs, n)	P value
1	45.2 $\pm$ 4.0 (5)	29.0 $\pm$ 2.3 (4)	0.01
2	42 $\pm$ 10.61 (2)	36 $\pm$ 1.41 (2)	–
3	35 (1)	55 (1)	–
4	73 $\pm$ 3.54 (2); one staff member required	90 (1); two staff members required	–

Mean $\pm$ SD or mean is indicated.
SD, standard deviation.

of DMSO was removed from HPC products ($n = 9$, median, 97.8%, IQR, 97.6–97.9) after processing with Lovo regardless of the washing solution used ($P = 0.27$) (Table 3). Similar outcomes were observed by Mfarreje et al. [8] and were in compliance with recommendations for patient safety [16]. The outcomes from these two studies showed an average of 97% elimination of DMSO after washing with Lovo.

Processing time

Processing time was estimated for one to four thawed bags. The technical time between the two devices was significantly different when processing a single bag (Table 4).

Discussion

The announced withdrawal from the market of the COBE 2991 device and the lack of a replacement have led to a nationwide EFS effort to find an alternative to this device. The essential requirement for this procedure is to eliminate as much DMSO as possible while ensuring satisfactory recovery and viability of cells of interest. In the current study, the outcomes showed highly effective removal of DMSO using Lovo, with an average of 3% residual in the final graft, minimizing the risk of patient ARs while meeting the authors' specifications for cell product quality: CD34+ recovery and viability and CD3+ recovery.

Regarding HPC autologous and allogeneic transplantation, the authors initially observed improvement in viable CD34+ cell recovery while maintaining viability after washing with Lovo compared with COBE 2991. The authors' CTL administers products within 30 min post-processing in 99% of cases; however, it is desirable to show stability for longer periods in case of delayed administration. Very good stability was observed for up to 6 h at $7 \pm 3^\circ\text{C}$ regardless of the

washing solution used. The study was continued with NaCl/albumin/ACD-A washing solution for harmonization of practices, as this is the most widely used washing solution at EFS CTLs and seems to give the best cell recovery yields based on the national EFS study [7]. Additionally, the automated consolidation of a final product into a single container allows for reduced operator steps. Significantly higher platelet depletion using Lovo compared with COBE 2991 might contribute to cell recovery enhancement and stability over time.

The Lovo seems to be reliable insofar as the effectiveness of the cell recovery rate appears to be independent of the number of thawed bags or total cells processed. Indeed, it may happen in the authors' facility (and other CTLs) that more than two bags of products need to be thawed for the same injection to reach the prescribed dose, especially in cases involving patients who are poor mobilizers. For three thawed bags, the results were similar whether COBE 2991 or Lovo was used, suggesting a product/patient-dependent outcome that is independent of the device used. Further trials would be needed to confirm this hypothesis. Based on the outcome of another study [17] concerning performance qualification of Lovo for washing and concentrating three and four cryopreserved HPC bags, CD34+ cell recovery and CD34+ viability were more than satisfactory.

A colony-forming unit–granulocyte-macrophage assay was not performed in this study, as it is not part of the authors' routine since results are known after hematopoietic reconstitution. However, this could be evaluated in a further study to estimate functionality of the cells after the washing process.

By contrast, focusing on the other activities of the authors' unit, the satisfactory cell recovery of viable CD3+ cells when processing with the Lovo device suggests the possibility of its use for “modified” DLI [9]. This process involves the cryopreservation of increasing doses of CD3+ cells from an allogeneic G-CSF-mobilized peripheral blood HPC graft surplus. Indeed, with regard to the authors' practice, the reinjected dose of an allograft generally ranges between 2×10^6 CD34 cells/kg and 6×10^6 CD34 cells/kg. With the exception of myelofibrosis, beyond 6×10^6 CD34 cells/kg, the surplus of the graft is cryopreserved. This allows for administering the dose of CD34+ cells that is strictly necessary, thereby limiting the dose of reinjected CD3+ cells and the risk of graft-versus-host disease. After this, doses of DLI are quickly available in the event of signs of malignant hemopathy relapse or partial chimerism without having to resolicit the donor for a second cytopheresis.

Similarly, although the authors have not conducted tests with non-mobilized MNCs, it can be assumed that Lovo could be used for processing “classical” DLI prepared from MNCs and ECP on cryopreserved cells. However, further testing will need to be carried out to verify this hypothesis.

Finally, because the Lovo device is automatic compared with COBE 2991, which is semi-automatic, once the kit is installed, very few staff interventions are necessary, which standardizes the process and saves time. Moreover, although the authors are limited by the number of bags that can be connected to the COBE 2991 kit (maximum of three), the Lovo can process up to 21 L, which allows more bags to be processed. In the authors' CTL, one staff member processes one to three bags with the COBE 2991, whereas two staff members are required in case of a transfusion of four bags. In this scenario, the bags are processed two by two using two COBE 2991 devices at the same time and then pooled. By contrast, processing with the Lovo device requires only one staff member regardless of the number of HPC bags processed. In addition, the practicality of Lovo allows for faster training and empowerment of technical staff. Moreover, the ability to record production data on the Lovo device allows traceability tracking for process security.

In spite of its efficiency, the cost of the device and its kits is a significant aspect that must be taken into account before considering nationwide implementation. Currently, compared with COBE 2991, the Lovo is more expensive. Other currently identified alternatives are being evaluated (Rotea, Thermo Fischer Scientific, Waltham, Massachusetts, USA; UniFuge, Pneumatic Scale Angelus, Stow, Ohio, USA; Sefia, Marlborough, Massachusetts, USA). All of these devices can be used for advanced therapy medicinal products.

According to current knowledge, manual centrifugation remains the cheapest and most reliable solution. However, this is to be contrasted with efficiency of DMSO elimination, cell recovery and reproducibility. In addition, regardless of the efficiency of the equipment, the CE marking could be a decisive factor in the replacement of COBE 2991.

Conclusions

Lovo appears to be a serious alternative to the COBE 2991 device for washing cryopreserved cell therapy products, as it removes an average of 97% of DMSO and improves the recovery of cells of interest, with viability maintained over time in case of delayed transplant. Moreover, efficiency appeared to be independent of the number of thawed bags and washing solutions used in the authors' study. Further studies need to be carried out to confirm its effectiveness for washing non-mobilized MNCs and to exploit all other possibilities of this equipment in terms of cell therapy product processing.

Declaration of Competing Interest

MM and SB are employees of Fresenius Kabi. Fresenius Kabi provided the disposables to support the studies described in this article.

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Author Contributions

Conception and design of the study: Charron Mickaël, Chesnel Santurette Catherine, Giraud Christine. Acquisition of data: Sabrina Bouyer, Mickaël Charron, Pascal Houzé, Alexis Lavergne. Analysis and interpretation of data: Catherine Chesnel-Santurette. Drafting or revising the manuscript: Steven Binnering, Catherine Chesnel

Santurette, Christine Giraud, Mathieu Mercier. All authors have approved the final article.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:[10.1016/j.jcyt.2021.07.008](https://doi.org/10.1016/j.jcyt.2021.07.008).

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