Preservation and Storage of Cells for Therapy: Current Applications and Protocols

Jason P. Acker, Mykola Bondarovych, Ricarda Brunotte, Iryna A. Buriak, Barry J. Fuller, Birgit Glasmacher, Anatoliy M. Goltsev, Jiří Gregor, Oleksandr Gryshkov, Kieran Herrity, Barbora Honegrová, Charles J. Hunt, Miroslava Jandová, Brian H. Johnstone, Peter Kilbride, Miriam Lánská, Jennifer Mann, Pavel Měřička, Kelsey G. Musall, Vitalii Mutsenko, Olga Mykhailova, Yuriy Petrenko, Jakub Radocha, Aubrey M. Sherry, Glyn Nigel Stacey, Lubomír Štěrba, Doris Vokurková, Nishaka William, and Erik J. Woods

Contents

J. P. Acker
Canadian Blood Services, Centre for Innovation, Edmonton, AB, Canada

Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, AB, Canada
e-mail: jacker@ualberta.ca

M. Bondarovych · I. A. Buriak (✉) · A. M. Goltsev
Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, Kharkiv, Ukraine
e-mail: bondarov.nikolay@ukr.net; buriakiryna@gmail.com; Anatoliy.Goltsev@gmail.com

R. Brunotte · B. Glasmacher · O. Gryshkov · V. Mutsenko
Institute for Multiphase Processes, Center for Biomedical Engineering, Leibniz Universität Hannover, Hannover, Germany
e-mail: brunotte@imp.uni-hannover.de; glasmacher@imp.uni-hannover.de; glasmacher@ifv.uni-hannover.de; gryshkov@imp.uni-hannover.de; mutsenko@imp.uni-hannover.de

B. J. Fuller (✉)
Division of Surgery, UCL Medical School, Royal Free Hospital Campus, London, UK
e-mail: b.fuller@ucl.ac.uk

J. M. Gimble et al. (eds.), *Cell Engineering and Regeneration*, Reference Series in Biomedical Engineering, https://doi.org/10.1007/978-3-319-37076-7_68-2

Abstract

In this chapter we consider the particular preservation storage procedures applied to a range of cell types used to produce cell-based medicines. Specifically, it deals with the scientific considerations for preserving each cell type and the kinds of cryopreservation protocols used to successfully preserve these different cell types. The cell types addressed include both those commonly in current use for

J. Gregor · B. Honegrová · M. Jandová · P. Měřička · L. Štěrba
Tissue Bank, University Hospital Hradec Králové, Hradec Králové, Czech Republic
e-mail: jiri.gregor@fnhk.cz; barbora.honegrova@fnhk.cz; miroslava.jandova@fnhk.cz; pavel.mericka@fnhk.cz; lubomir.sterba@fnhk.cz

K. Herrity · P. Kilbride
Cytiva, Cambridge, UK
e-mail: Kieran.Herrity@nhsbt.nhs.uk; peter.kilbride@cytiva.com

C. J. Hunt
Sawston, Cambridge, UK
e-mail: charleshunt1950@outlook.com

B. H. Johnstone
Ossium Health, Indianapolis, IN, USA

Department of Biomedical Sciences, College of Osteopathic Medicine, Marian University, Indianapolis, IN, USA
e-mail: brian.johnstone@ossiumhealth.com

M. Lánská · J. Radocha
The 4th Department of Internal Medicine-Haematology, Medical Faculty, Charles University, University Hospital Hradec Králové, Hradec Králové, Czech Republic
e-mail: miriam.lanska@fnhk.cz; jakub.radocha@fnhk.cz

J. Mann
Synthace Limited, London, UK
e-mail: jennifer_man@hotmail.co.uk

K. G. Musall · A. M. Sherry
Ossium Health, Indianapolis, IN, USA
e-mail: kelsey.musall@ossiumhealth.com; aubrey.sherry@ossiumhealth.com

O. Mykhailova
Canadian Blood Services, Centre for Innovation, Edmonton, AB, Canada
e-mail: olga.o.mykhailova@gmail.com

Y. Petrenko
Institute of Experimental Medicine of the CAS, Prague, Czech Republic
e-mail: Yuriy.petrenko@iem.cas.cz

patient treatment, such as whole blood and hematopoietic stem cells and also examples of new cell-based medicines including tissue progenitor cells (MSCs), (The use of the term Mesenchymal Stem Cell (MSC) has been hotly debated in the literature as it actually applies to several different cell types. The term "tissue specific progenitor cells" has been proposed as a more accurate term (Robey 2017) and where the abbreviation "MSC" appears elsewhere in this chapter it can be assumed this is a reference to Mesenchymal Stromal cells or the more generic term for this group of cell types Tissue-Specific Progenitor Cells (TSPCs).) tissue engineered constructs, CAR-T cells and pluripotent stem cells. However, the chapter does not consider the preservation and storage of organs or cells and tissues used in reproductive medicine. A second part of the chapter addresses best practice in meeting regulatory requirements for preservation and storage of both unfrozen and cryopreserved materials, including core requirements for the design of storage facilities. Also considered is best practice for packaging and shipment of cell-based medicines and their reception at the clinic and control within the hospital environment under pharmacy rules. The authors have used examples of regulatory documents primarily from the European Union and the USA, but also include reference to key international standards and WHO guidance.

Abbreviations

ACT	Adoptive cell therapy
ATMPs	Advance therapy medicinal products
B-ALL	B-cell acute lymphoblastic leukemia
BC	Blood component

G. N. Stacey (✉)
International Stem Cell Banking Initiative, Barley, UK

National Stem Cell Resource Centre, Institute of Zoology, Chinese Academy of Sciences, Beijing, China

Innovation Academy for Stem Cell and Regeneration, Chinese Academy of Sciences, Beijing, China
e-mail: glyn.stacey@iscbi.org

D. Vokurková
Department of Clinical Immunology and Allergology, Medical Faculty, Charles University, University Hospital Hradec Králové, Hradec Králové, Czech Republic
e-mail: doris.vokurkova@fnhk.cz

N. William
Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, AB, Canada
e-mail: nishaka@ualberta.ca

E. J. Woods
Ossium Health, Indianapolis, IN, USA

Department of Biomedical Sciences, College of Osteopathic Medicine, Marian University, Indianapolis, IN, USA

Department of Medical and Molecular Genetics, Indiana University School, Indianapolis, IN, USA
e-mail: erik@ossiumhealth.com

BM	Bone marrow
CAR-T cell	Chimeric antigen receptor T-cell
CB	Cord blood
CFU-GM	Colony forming unit-granulocyte/macrophage
cGMP	Current good manufacturing practice
CIDOCD	Cryopreservation-induced delayed-onset cell death
CPA	Cryoprotective agent
DCs	Dendritic cells
DMSO	Dimethyl sulfoxide
DOCD	Delayed-onset cell death
EBMT	European Society for Blood and Marrow Transplantation
ECM	Extracellular matrix
EG	Ethylene glycol
EMA	European Medicines Agency
ESCs	Embryonic stem cells
FBS	Fetal bovine serum
FFP	Fresh frozen plasma
GCS-F	Granulocyte colony-stimulating factor
GDP	Good distribution practice
GF	Growth factors
GMP	Good manufacturing practice
GS	Granulocytes
GVHD	Graft-versus-host-disease
HCBCL	Human cord blood cell leucoconcentrate
HES	Hydroxyethyl starch
hESCs	Human embryonic stem cells
hiPSCs	Human induced pluripotent stem cells
hPSCs	Human pluripotent stem cells
HSA	Human serum albumin
HSC	Hematopoietic stem cells
LN_2	Liquid nitrogen
LS-HP	Liquid state hypothermic preservation
MCR	Mean cooling rates
MMP	Metalloproteinase
MPCs	Multipotent progenitor cells
MSCs	Mesenchymal stromal cells
NC	Nucleated cell
OBC	On board courier
PBMC	Peripheral blood mononuclear cells
PBPCs	Peripheral blood progenitor cells
PBSC	Peripheral blood stem cells
PC	Platelet concentrate
PCTS	Precision-cut tissue slices
PSCs	Pluripotent stem cells
PVP	Polyvinylpyrrolidone

RBC	Red blood cell
RCC	Red cell concentrate
ROCKi	Rho-associated kinase inhibitor Y-27632
RT	Room temperature
SOPs	Standard operating procedures
SUC	Sucrose
TE	Tissue establishment
TECs	Tissue-engineered constructs
TSPCs	Tissue specific progenitor cells
TWEs	Transient warming events
U.S. FDA	United States Food and Drug Administration
UW	University of Wisconsin
WB	Whole blood
WHO	World Health Organization

1 Introduction

This section describes a range of interventional therapies where cryopreservation plays an important role in delivery of cell therapies, and which are in wide use from a global perspective.

The explosion in the development of advanced therapies and bioengineered constructs within medicine and biotechnology increasingly involves the in vitro manipulation of living cells and complex manufacturing processes. Successful cryopreservation is crucial to enable provision of reproducible starting cell stocks and cell-based products which can be thawed after quality and safety testing is completed and at the time they are needed by patients. Storage of large batch numbers of cells in a biologically stable state that can be recovered with consistent functional properties is crucial to delivering acceptable and effective cell-based medicines (Stacey et al. 2017a). While the technologies for isolation and manufacture of cell-based products have seen dramatic advances in recent years, biopreservation of these complex cell preparations remains an area for research and development and is a key road-block in the capability to deliver widespread access to the exciting potential benefits of these new medicines. In this chapter both nonfrozen live cell storage and cryopreserved storage methods are considered for a range of different cell-based medicines already in use and others that may form the basis of future medicines. While a detailed review of regulations applicable to storage and shipment of cells in different jurisdictions is beyond the scope of this chapter, we have considered the strategies to address regulatory considerations for the development of storage and shipment of cell-based products with reference to specific regulations in the European Union (EU) and the USA.

2 Applications

2.1 Cryopreservation of Hematopoietic Progenitor Cells: From the Past to Future Perspectives

Cryopreservation of bone marrow (BM) has been connected with the HPC transplantation since its beginning (Thomas et al. 1957; Westbury et al. 1959; Berz et al. 2007). In the late 1970s peripheral blood progenitor cells (PBPCs) (Barr et al. 1975) were started to be used alternatively (Fliedner et al. 1977; Weiner et al. 1977; Körbling et al. 1980). After introduction of the granulocyte colony-stimulating factor (GCS-F) (Gianni et al. 1989) for PBPC mobilization and introduction of phenotyping and viability assessment of HPC progenitors by flow cytometry (Sasaki et al. 1987; Serke et al. 1991) they became a dominant HPC source for both autologous (To et al. 1987; Bláha et al. 1990; Garritsen et al. 2009) and allogeneic (Amouzegar et al. 2019) transplantations. At present autologous HPC transplantation is used predominantly in patients with multiple myeloma (Barlogie et al. 2010; Radocha et al. 2013; Cowan et al. 2020) and malignant lymphoma (Armitage et al. 1986; Kessinger et al. 1989; von Tresckow and Engert 2011) as in leukemias allotransplantations led to better results (Stockschläder et al. 1997; van Gelder et al. 2017; Canaani et al. 2019) and in solid malignancies HPC autotransplantations (Sumida et al. 1984; Vaňásek et al. 1998; Chakraborty et al. 1999) were replaced by more efficient therapies. Usually the HPC dose sufficient for more than one autotransplantation is collected (Areman et al. 1990; Barlogie et al. 2010; Měřička et al. 2017) and the cells are stored by cryopreservation for months or years for repeated use. Since 2020 cryopreservation of allogeneic HPCs has been introduced on basis of the European Society for Blood and Marrow Transplantation (EBMT) recommendation to prevent the COVID-19 transmission.

Cord blood (CB) (Gluckman et al. 1989; Rubinstein et al. 1995; Passweg et al. 2015) represents always the second option used in cases in which the standard HPC donor is not available (Fales et al. 2005; Bhattacharya and Stubblefield 2009; Sputtek 2008). Because of low cell content it is preferably used in pediatric hemato-oncology (Smythe et al. 2007; Paviglianiti et al. 2016) and in the treatment of inborn immunity defects (Kögler et al. 2009). Due to the high content of early stages of HPC prolongation of the engraftment is frequently encountered (Kindwall-Keller and Ballen 2020).

2.1.1 HPC Collection and Processing for Cryopreservation

The collected volume, nucleated cell (NC) concentration, hematocrit (Table 1), and contamination with fat and tissue debris are the main variables influencing the need of HPCs secondary processing.

The BM is collected by repeated punctures of iliac crest bones under general anesthesia into a set containing anticoagulant (Thomas and Storb 1970). Intraoperatively the NC number is assessed to achieve the target of 1–2 $\times$ 10^8 NC/kg of the patient's weight. After filtration the pure marrow is sent to the tissue establishment (TE), where red blood cell (RBC) depletion can be performed in case

Table 1 Comparison of parameters of collected HPCs and mature MNCs – median values

	Volume (mL)	NC concentration (10^9/L)	Hematocrit
Bone marrow for allotransplantation (5 cases)	1300	14.89	0.356
Autologous peripheral blood progenitor cells (30 cases)	200	216.99	0.0225
Allogeneic peripheral blood progenitor cells (10 cases)	341.5	189.97	0.0265
Cord blood (6 cases)	95	10.96	0.3255
Peripheral blood mononuclear cells (18 cases)	154	76.71	0.022

Countries of origin: BM: Czech Republic (3), Germany (2). Allogeneic PBPC: Czech Republic (3), Germany (5), Poland (1), Turkey (1). Allogeneic PBMC (donor lymphocytes): Czech Republic (9), Germany (6), Poland (2), Great Britain (1). All autologous PBPC and CB collections originated from the Czech Republic, only

of AB0 incompatibility or RBC depletion followed by volume reduction in case of cryopreservation. If BM for manufacturing advanced therapy medicinal product (ATMP) is collected, usually small volumes (10–20 mL) are taken under local anesthesia (Pudil et al. 2005; Marquez-Curtis et al. 2015; Šponer et al. 2018). PBPCs are collected by leukapheresis after mobilization (Körbling et al. 1980; Gianni et al. 1989; Ikematsu et al. 1992). ACD-A solution is used to prevent coagulation. Due to low hematocrit the need of RBC depletion in case the AB0 incompatibly is avoided. In case of low HPC yield, the process may be repeated several times. The target is to achieve 2×10^8 NC and/or 4×10^6 CD34+ cells/kg of patient's weight. High NC concentration and low hematocrit, however, make the collected HPCs highly susceptible to damage due to longer duration of storage at +4 °C and/or subsequent cryopreservation (Keung et al. 1996; Liseth et al. 2005; Lioznov et al. 2008). For this reason the NC concentration should not exceed 400×10^9/1 L, if the product is processed within 24 h and 200×10^9/1 L, if the product will be processed after this limit. In such cases the product is usually diluted by the donor plasma. On the other hand in NC concentrations below 100×10^9/1 L volume reduction is recommended.

Similar issues may be encountered in case of collection of non-mobilized peripheral blood mononuclear cells (PBMCs) for manufacturing of ATMPs. Cryopreservation should be performed as soon as possible after collection and volume reduction may be necessary.

In the case of CB usually a volume of 80–150 mL is collected from umbilical veins into a set containing anticoagulant. Collections below 60 mL (without anticoagulant) are regarded not suitable for cryopreservation. High hematocrit indicates that RBC depletion is necessary in case of AB0 incompatibility. The sufficient transplantation dose is 2×10^7 NC/kg of patient's weight.

2.1.2 HPC Cryopreservation

The first experimental BM cryopreservation protocol (Barnes and Loutit 1955) used 15% glycerol and slow cooling – 1 K/min to −15 °C and −10 K/min to −79 °C. Thawing was rapid and glycerol was removed by the concentrated glucose solution. In the case of human BM freezing of large volume was unavoidable and special devices able to achieve an acceptable cooling rate were constructed (Pegg and Trotman 1959). Glass ampoules were replaced by laminated foil bags (Playfair and Pegg 1962), metal containers (Rowe et al. 1967; Malinin et al. 1970; Měricka et al. 1991) and plastic bags enclosed within metal cassettes (Fig. 1) to guarantee uniform temperature profile during slow freezing and rapid thawing by immersion into the water bath. Double plastic bags are obligatory now (Sputtek et al. 2011).

Slow controlled rate freezing is achieved in programmable freezers based on cooling by liquid nitrogen (Scheiwe et al. 1981; Areman et al. 1990; Měřička et al. 1995) and later also on the Stirling cooling principle (Massie et al. 2014; Hunt 2019). Figure 2 shows a controlled rate cooling protocol being used.

At present DMSO in concentration of 5–10% is a dominant cryoprotectant for HPCs (Horacek et al. 2009; Fry et al. 2015; Carreras et al. 2019). Safety of application of thawed HPCs can be achieved by strict control of the DMSO load that should not exceed the daily dose of 1 g/kg of the patient's weight (EDQM 2019;

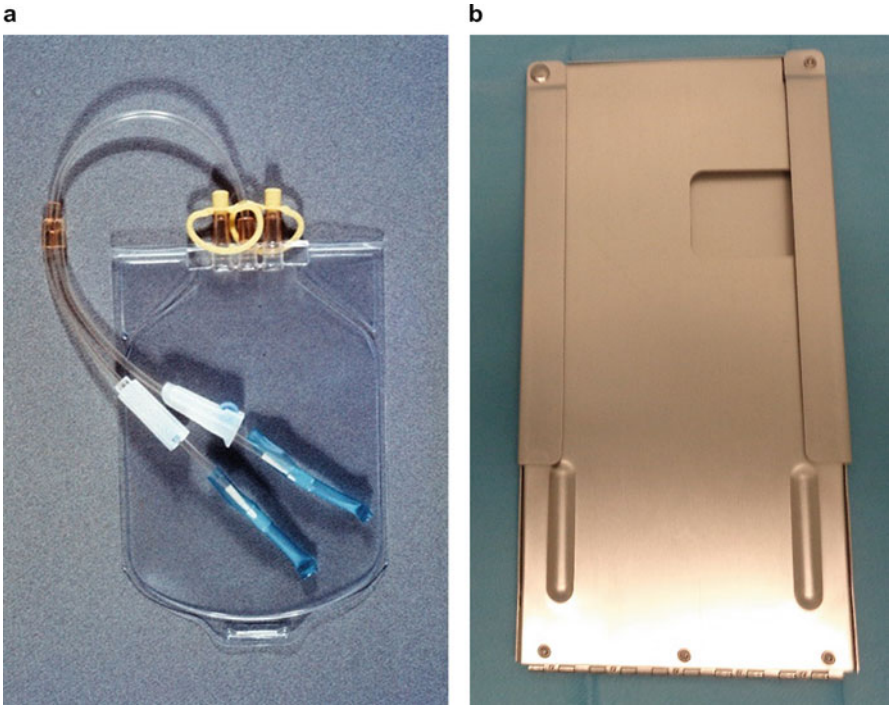

Fig. 1 Plastic Cryocyte bag used by the authors since 1994 (**a**). A more advanced version has a label pocket and bags are closed into a cassette with an observation window (**b**)

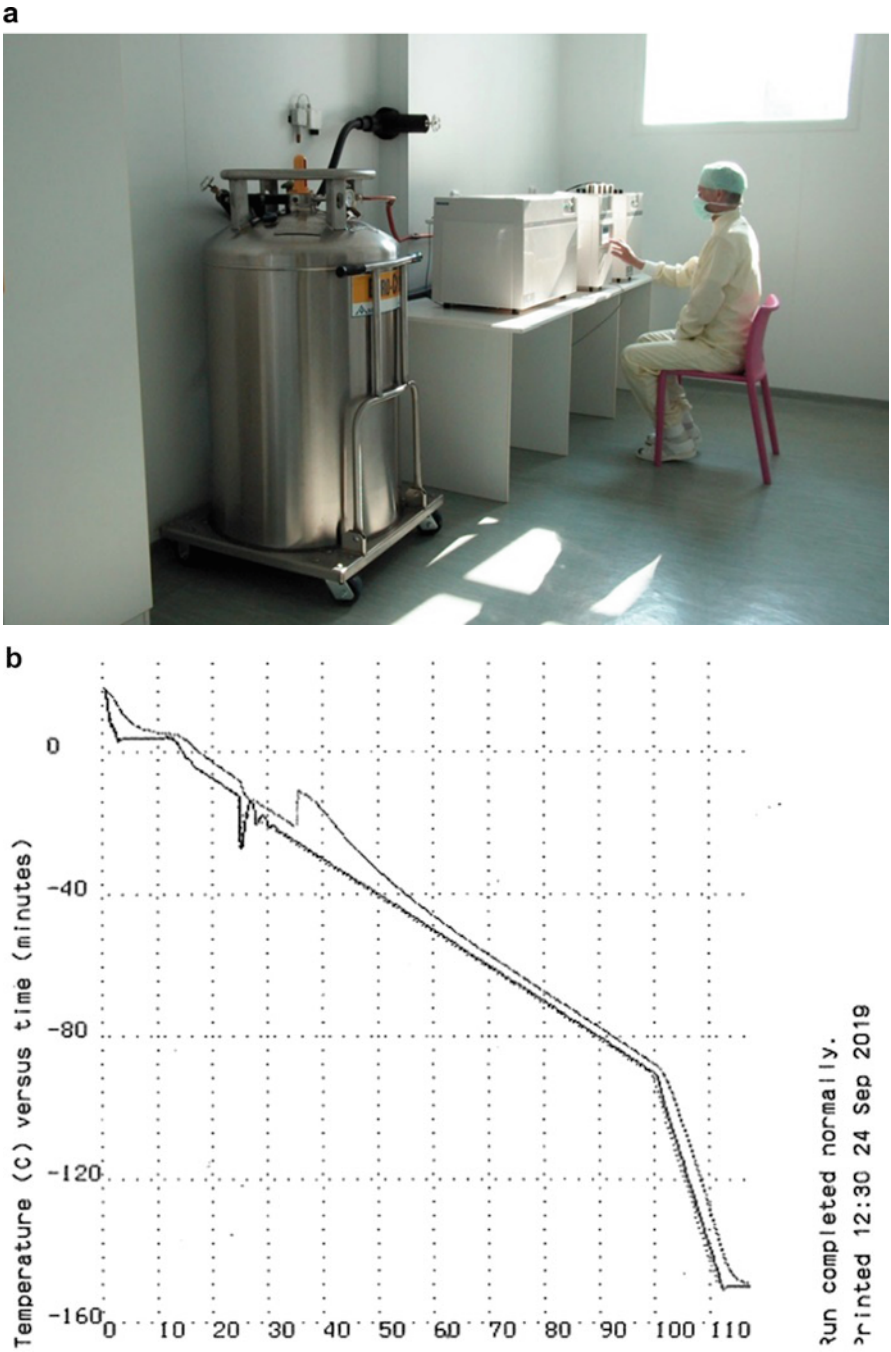

Fig. 2 The programmable freezer with the backup vessel in the clean room of the tissue establishment (**a**) and the freezing protocol used (**b**). The temperature is monitored in a control bag filled with a 10% DMSO solution

Měřička et al. 2019a). Good cryoprotection of non-mononuclear cells is also important as their damage may cause adverse reactions, too (Milone et al. 2007). Post-thaw DMSO should be removed in special cases, such as in patients suffering from severe cardiac diseases or from chronic renal failure, for example, due to secondary amyloidosis of kidneys, a frequent complication of multiple myeloma (Akkök et al. 2009).

Removal of DMSO with automated cell washers (Perotti et al. 2004) and reducing of the DMSO concentration by addition of other cryoprotectants, such as hydroxyethyl starch or trehalose are other contributions to the safety of application of cryopreserved HPCs. In future the use of DMSO-free media is expected (Awan et al. 2020).

2.1.3 Storage, Warming, and Clinical Delivery of Cryopreserved HPCs

Since 2006 the storage of HPCs at −80 °C (Stiff et al. 1987; Makino et al. 1991; Měřička et al. 2000) has not been recommended due to reports on impaired viability and membrane integrity (Rowe et al. 2006; Sputtek and Rowe 2006; Měřička et al. 2010). Submerged storage in liquid nitrogen was replaced by storage in the vapor phase, after the description of the risk of cross contamination (Tedder et al. 1995; Fountain et al. 1997; Blaha et al. 2003) and the new containers using a copper heat shunt for stimulation of evaporation able to guarantee more uniform temperature field above the level of liquid nitrogen became available (Hunt and Pegg 1996). The risk of recrystallization because of temperature fluctuations during storage has been reduced by introduction of automatic filling. Another technical solution avoiding the possibility of the direct contact of the liquid nitrogen with the stored material is based on placing a stainless steel internal vessel with frames for cassettes with cryobags into a container. Liquid nitrogen is only in the narrow space between the wall of the vessel and the internal wall of the container. Adequate number of control samples with temperature history as close as possible to the history of bags for re-check of HPC viability and colony-forming capacity must be stored to assure safe application after years or decades. Figure 3 shows the typical durations of PBMC storage in our practice.

Rapid thawing in the water bath of 37 °C has been recommended since the beginning (Barnes and Loutit 1955; Pegg and Trotman 1959; Hunt 2019). It is usually completed within 3–5 min. Now, this method is being replaced by use of programmed thawing device (Hunt 2019; Yakoub-Agha et al. 2020). In the past, when metal containers were used, the thawed concentrate was transferred into a National Blood Service jar or transfer bags, now it is infused directly from the cryobag, or, in case of DMSO removal, from a transfusion transfer bag. The transport to the clinical department (Sputtek et al. 2002) is performed in the vapor phase of liquid nitrogen in simple isolation boxes or in dry shippers (Hunt 2019; Yakoub-Agha et al. 2020) at temperatures below −140 °C. In the wards the bags should be thawed successively, in case of any complication it should be possible to postpone thawing of the next bag and to provide the patient adequate treatment.

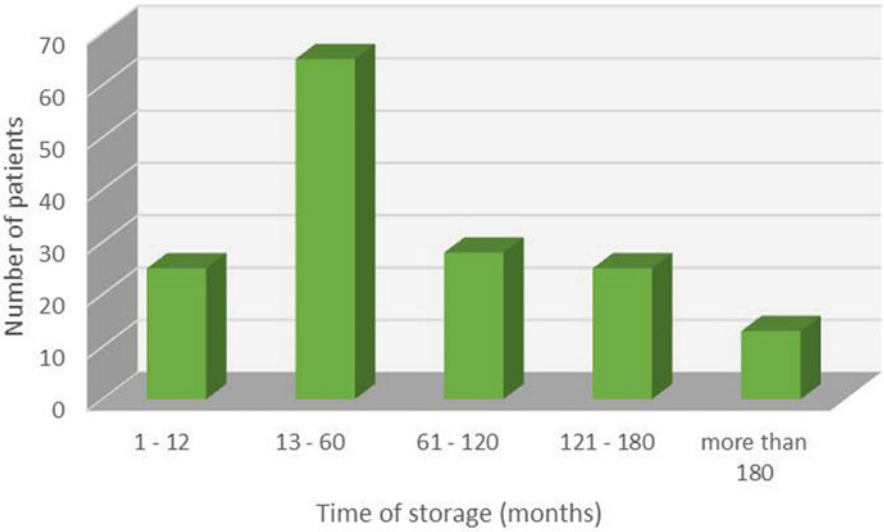

Fig. 3 Distribution of storage times for autologous PBPC concentrates. The most patients (66) are in the group of 13–60 months, storage times up to 180 months are not an exception. The figure documents long survival of patients after autologous transplantation. The concentrates stored for more than 5 years can be used clinically only after the re-check of viability and colony-forming capacity of HPC progenitors (Měřička et al. 2017)

2.1.4 Quality Control of the HPC Collection, Cryopreservation, and Storage Processes

The precise control of all processes is crucial for the final result of the HPC transplantation. The data of WBC count including detailed differential results of phenotyping and membrane integrity assessment by flow cytometry, as well as of HPC colony-forming capacity (Yang et al. 2005; Reich-Slotky et al. 2008) and sterility, are essential for the release of the cryopreserved HPC concentrates for clinical application. The accreditation manuals (FACT–JACIE 2018) and the European Directorate for Quality of Medicines (EDQM) recommendations (EDQM 2019) require that the tissue establishments have validated processes with declared acceptability criteria for individual parameters. Shelf life of stored HPCs should be determined by a cryostability study and repeated test for HPC membrane integrity and colony-forming capacity should be performed before clinical application after long-term storage. The product released for clinical application must be properly labeled using TE identification code (Měřička et al. 2019b), donation identification sequence, and product code as given in the ISBT 128 product list. Besides estimation of recoveries (Table 2) and of cell doses from thawed control samples (Yang et al. 2005) it is recommended to perform additional control of thawed HPC concentrates at infusion (Wagner et al. 2006; Měřička et al. 2011, 2019a). In this way it is possible to identify possible deviations from the estimated

Table 2 Recovery of PBPC/PBMC after cryopreservation – estimation from thawed control samples

	Viable NC (%)	Viable MNC (%)	Viable CD34+ cells (%)	CFU-GM (%)
Allogeneic unrelated PBPCs – 10 cases				
Mean	79.44	92.77	91.08	64.79
SD	13.05	14.78	21.50	14.26
Median	78.38	95.53	87.93	63.69
Autologous PBPCs – 30 cases				
Mean	70.01	96.41	93.74	75.19
SD	16.36	18.15	24.97	21.01
Median	72.77	93.08	90.55	71.90
Allogeneic non-mobilized PBMCs – 18 cases				
	Viable NC (%)	**Viable MNC (%)**	**Viable CD3+ cells (%)**	
Mean	92.67	92.30	98.03	
SD	11.66	11.16	23.04	
Median	92.96	92.74	93.30	

Note: Cell phenotyping, assessment of the number of nucleated and mononuclear cells and of nucleated and mononuclear cell viability before and after freezing was performed according to the European Pharmacopoeia articles (Czech Pharmacopoeia 2009 Supplement 2015a, b, c). *CFU-GM* granulocyte-macrophage colony-forming units

HPC doses that may occur for different reasons (Douay et al. 1986; Lioznov et al. 2008; Wats et al. 2020) and to perform efficient corrective actions.

Recoveries of nucleated, mononuclear CD34+ and CD3+ cells were calculated according to the Eq. 2.1:

$$\text{Recovery\%} = \frac{\text{Total number of cells} \times \text{viability post-freeze}}{\text{Total number of cells} \times \text{viability pre-freeze}} \times 100 \qquad (1)$$

CFU-GM recovery (%) was calculated as the (number of colony-forming units post-freeze/number of colony forming units pre-freeze) × 100

There has been a substantial progress in the HPC collection, processing, and cryopreservation techniques in the last decades. While at the beginning the major HPC source was BM and the cryoprotectant glycerol, the most autologous and allogeneic transplantations are performed, now with PBPCs and the dominant cryoprotectant is DMSO. A new technical background is represented by the use of clean rooms for HPC processing, reliable double plastic bags, new generation of programmable freezers, and improved storage containers avoiding the contact of the stored material with liquid nitrogen. Cryopreservation is performed in licensed or accredited tissue banks/establishments. Combination of these factors together with application of current methods of assessment of HPC content, viability, and colony-forming capacity leads to achieving of high HPC recoveries (Měřička et al. 2021a, b). For the future a significant challenge will be the development of protocols for cryopreservation of the starting material for ATMP manufacturing and storage of the manufactured products distributed in the cryopreserved form (Hunt 2019; Yakoub-Agha et al. 2020; Jandová et al. 2020).

2.2 Tissue-Specific Progenitor Cells

Multipotent progenitor cells (MPCs) often referred to as mesenchymal stromal cells (MSCs) provide wide opportunities for regenerative medicine and tissue engineering due to their ability to stimulate the regeneration processes in damaged tissues. Besides three-lineage differentiation capacity, the therapeutic action of MSCs is associated with their ability to produce a cocktail of growth factors, cytokines, micro-vesicles, and exosomes in response to microenvironment of the target tissue (Lunyak et al. 2017; Parekkadan and Milwid 2010; Petrenko et al. 2020). Such interaction may provide essential support for the resident cells, improve angiogenesis, prevent cell death, and modulate the inflammatory processes or immune response (Petrenko et al. 2017b; Marquez-Curtis et al. 2015). MSC-like cells with similar properties have been isolated from the variety of adult or perinatal human and mammalian tissues and usually the correspondence of fibroblastic cells to MSCs in separate studies is based on the minimal criteria proposed for characterization of MSCs in research studies by the International Society for Cellular Therapy (Dominici et al. 2006).

Before clinical application, MSCs should be thoroughly analyzed to confirm their identity and safety, paying special attention to the absence of viral and microbial contamination. The microbiological sterility control is usually validated in each laboratory according to European Medicinal Agency (EMA) or FDA guidelines (Gálvez et al. 2014). It is favorable that the final determination of sterility is prepared in the finished cell-based graft, meaning after cell harvesting and packaging. Otherwise, the additional cell manipulations (re-seeding, passaging, washing) in opened environment may lead to the need of repeated quality control analysis. Despite this, the FDA has recently reported the possibility of using several rapid tests for fast detection of microorganisms in liquid systems (e.g., BacT/ALERT®, BACTEC™, Rapid Milliflex®) and there is a strong need to preserve cellular properties during at least 48–72 h, necessary to perform the tests. The storage of harvested MSC suspensions in normothermic conditions is challenging, since without the substrate the aggregation or apoptotic processes in MSCs usually occur. Therefore, the cryobiological strategies can be considered as the only possibility to preserve functional and structural properties of MSCs for clinical applications.

Two distinct approaches can be used to preserve the viability of MSCs during short or long periods. The hypothermic storage in nonfrozen conditions represents probably the most reproducible system for short-term cell preservation. However, in nonspecific conditions such a stressful hypothermic environment would lead to perturbation of ion balances, osmotic cell swelling, oxidative stress and pH switch, driving cell apoptosis, and death (Fuller et al. 2013; Guibert et al. 2011). Therefore, the development of specially formulated media for hypothermic preservation of MSCs has become a demand in MSC-based research and therapy. Currently there are several commercially available solutions with confirmed protective effects during hypothermic storage of MSCs. The University of Wisconsin Solution (UW solution), available on the market under the trademarks VIASPAN®, SPS-1® (Organ Recovery Systems, USA), and Belzer UW® Cold Storage Solution (Preservation Solutions Inc., USA) is a gold standard medium for organ and tissue preservation prior to transplantation. Several reports have shown the use of UW solution for hypothermic preservation of MSCs (Tarusin et al. 2015; Corwin et al. 2014). HypoThermosol® FRS (BioLife Solutions, USA) represents another effective option for the storage of cells and tissues. The preservation has been confirmed for several cell types, including MSCs monolayers (Ginis et al. 2012), suspensions (Petrenko et al. 2019), and for the hypothermic storage of MSCs (Petrenko et al. 2019). The mentioned storage media usually are based on specially formulated ionic composition, extracellular supporting substitutes (sugars and/or high molecular weight compounds), buffering agents, and/or additional compounds (antioxidants and others), aimed to preserve the ionic balance in stored cells and provide the essential protection. However, despite the special cold storage solution showing efficiency in MSC preservation, their clinical application has several challenges associated with the need to confirm their safety when administered to a patient.

Cryopreservation is the only established method to ensure long-term preservation and rapid delivery of MSC grafts for clinical use. The most applied research and clinical-grade cryopreservation method is based on the application of 10% of DMSO in saline solutions of various compositions (Marquez-Curtis et al. 2015; Thirumala

et al. 2013; Hunt 2011). However, it is already well documented that high concentrations of DMSO are toxic toward cells at close to normothermic temperatures and may cause different adverse reactions when administered to a patient (Notman et al. 2006; Cox et al. 2012; Awan et al. 2020). Although it is now common practice to remove DMSO prior to application, in research practice, the washing of cells for clinical use from CPA is associated with several challenges, discussed previously. Many efforts have been made to reduce or completely remove the DMSO from the preservation media aiming to neutralize the possible toxicity of cellular grafts. In a recent comprehensive review, authors thoroughly analyzed the use of DMSO in cryobiology and discussed different ways for the reduction or exclusion of this highly effective CPA during cell cryopreservation, including MSCs (Awan et al. 2020). Summarizing the available information, the commonly applied strategy for reducing toxic concentration of DMSO is based on the supplementation of cryoprotective media with different non-permeable substances, including sugars (dextrose, trehalose, sucrose, raffinose), high molecular weight compounds (PVP, PEG), macromolecules (HES, albumin, dextran, ZENALB 4.5), and others (Naaldijk et al. 2012; Yuan et al. 2016b; Liu et al. 2011; Marquez-Curtis et al. 2015; Awan et al. 2020). For clinical application, components of CPA solutions should be suitable as raw materials for the manufacture of GMP products and the validation of cryopreservation procedures (including safety and toxicity studies) should be performed. For example, the addition of HES, commonly used in clinic as plasma expander, is allowed to be used to reduce DMSO concentration during cryopreservation of MSCs but this change will require validation (Naaldijk et al. 2012). Besides reducing the concentration of DMSO during MSC cryopreservation, more and more studies are currently focused on complete removal of this CPA from the medium, aiming to develop xeno- and DMSO-free solutions that may ensure off-the-shelf availability of the cellular graft (Pollock et al. 2017; Matsumura et al. 2013; Freimark et al. 2011; Petrenko et al. 2014). Using carboxylated poly-l-lysine authors showed successful preservation of MSCs, compatible to 10% DMSO (Matsumura et al. 2013). Successful results have also been shown using the mixture of proline and ectoin in the presence of methylcellulose (Freimark et al. 2011) or the mixture of ethylene glycol, glucose, and sucrose (Shivakumar et al. 2016). Several alternative methods have been recently proposed, which are based on the intracellular delivery of sugars by either electroporation (Dovgan et al. 2017; Mutsenko et al. 2019) or 24 h of in vitro pretreatment. These authors showed the preservation of functional properties of MSCs after cryopreservation in DMSO- and xeno-free conditions (Petrenko et al. 2014; Rogulska et al. 2017, 2019).

Therefore, the development of nontoxic hypothermic storage and cryopreservation methods, providing ready-to-use availability of MSCs as well as efficient and safe protective solutions, capable to be administered to a patient as a vehicle for cell delivery would have great advantages in faster translation of MSCs-based therapeutics to clinical settings.

2.3 Human Pluripotent Stem Cells

Human Pluripotent Stem Cells (hPSCs), including human embryonic and induced pluripotent stem cells (hESCs and hiPSCs), are still at a very early stage of clinical application. They are not intended for clinical use in their undifferentiated state as they have the potential to generate teratomas, but as differentiated cell products they are now being used in numerous clinical trials (Kobold et al. 2020). Undifferentiated hPSCs have been cryopreserved by both nonequilibrium vitrification and classical slow-cooling techniques (Hunt 2011, 2017; Li and Ma 2012). The colonial nature of both types of PSCs in culture and their propensity, in early comparative studies, for increased differentiation and cell death when dissociated into single cell suspensions for classical freeze/thaw (Richards et al. 2004; Zhou et al. 2004), led initially to the adoption of vitrification protocols, based on those for bovine embryos (Reubinoff et al. 2001). These protocols adopted the manual cutting methods used for passaging, vitrifying the PSCs as colony fragments (i.e., clusters of cells) in straws (Hunt and Timmons 2007).

A vitrification solution (VS) of EG, DMSO, and sucrose (SUC) was used in a short two-stage addition protocol ($\leq$60 s) to avoid cryoprotectant toxicity. This necessitated ultra-rapid cooling in small (~20 μL) volumes using open-straws plunged directly into liquid nitrogen (LN_2); producing a metastable glass prone to devitrification unless maintained at temperatures below the glass transition. This technically challenging, operator-dependent, lengthy process was really only suited to producing small "insurance" stocks of newly developed hPSC lines. Despite measures to reduce risks from contamination (Kuleshova et al. 2009; Nishigaki et al. 2011), such protocols posed significant problems in the application of Good Manufacturing Practice, both microbiological and logistical, while resisting scale-out/scale-up.

2.3.1 Recent Advances in Nonequilibrium Vitrification Technologies

Attempts have been made to address two major criticisms: contamination and scale out. "Bulk" vitrification (Li et al. 2010a) and surface-based vitrification of adherent hPSC colonies in specially designed dishes (TWIST), which separate the cryogen from the cells (Neubauer et al. 2017; Kaindl et al. 2019), offer two possible methods for upscaling. The latter is applicable to high-throughput, multi-well format, technologies and addresses contamination issues. Alternative high-throughput, non-contact techniques, utilizing inkjet printing onto surfaces cooled by LN_2, produce ρL-size droplets which apparently avoid recrystallization using standard warming methods (Dou et al. 2015; Shi et al. 2015; Akiyama et al. 2019). However, droplets remain prone to devitrification by accidental rewarming during transport to/from storage; a major drawback of all nonequilibrium techniques. Nano-warming coupled with alginate encapsulation has been advocated as a way to increases warming rate thereby minimizing devitrification and recrystallization and improving immediate post-thaw survival (Liu et al. 2018), while an EG/SUC VS, employing the ice recrystallization inhibitor (IRI) poly-L-lysine instead of DMSO, has been shown to be effective for hiPSCs (Matsumura et al. 2011).

2.3.2 Classical Slow-Cooling

Despite early studies reporting poor survival, others reported relatively high recovery (Lee et al. 2010; Li et al. 2010b), possibly reflecting the empirical nature of these studies and the choice/timing of viability assessment post-thaw. Intrinsic factors may also affect recovery. Genetic variation has been proposed to partially explain line-to-line variation in post-thaw recovery of mouse ESCs (Kashuba et al. 2014), while Xu et al. (2014) have highlighted differences in membrane biophysical parameters between hPSCs.

Extrinsically, the influence of undercooling (Li et al. 2020a) and controlled nucleation (Morris and Acton 2013) have both been highlighted (Ware et al. 2005; Yang et al. 2006; Huang et al. 2017), particularly in the cryopreservation of aggregates (Li et al. 2018) and adherent cells (Daily et al. 2020). Results indicated an optimal seeding range between −7 °C and −10 °C, though Li et al. (2020a) suggest an optimum of −4 °C for cooling rates between 1 °C/min and 3 °C/min.

Cooling rate studies have indicated an optimum in the range 0.5–5 °C/min (Ware and Baran 2007; T'Joen et al. 2012). Two-step cooling without seeding (Orellana et al. 2015) and multistep cooling with/without seeding (Katkov et al. 2011; Li et al. 2020a) have also been reported to achieve satisfactory outcomes with most steps within the above range. To date, the use of IRIs and antifreeze proteins with slow-cooling has not been reported for hPSC. However, the antioxidant catalase, incorporated into the CPA, has been shown to improve recovery (Fernandes et al. 2019) indicating a potential avenue of future research.

Cryopreservation outcomes cannot be divorced from culture conditions including hPSCs passaging and state prior to and following cryopreservation (Ko et al. 2018; Liu et al. 2019). Reversible adaptation to feeder-free monolayer culture allows successful cryopreservation of hPSCs as single cell suspensions (Kunova et al. 2013) as has mild and enzyme-free dissociation (Ware et al. 2005; Beers et al. 2012).

Originally used during subculture to minimize dissociation-induced apoptosis and improve re-attachment (Kurosawa 2012; Liu et al. 2019), the Rho-associated kinase inhibitor (ROCKi) Y-27632 significantly improves cryopreservation outcomes assessed by standard viability methods (Li et al. 2008; Baek et al. 2019). However, though its routine use in culture does not significantly change the mutation rate of hPSCs (Thompson et al. 2020), negative effects have been reported during passaging (Gao et al. 2019) and for non-hPSCs, post-cryopreservation (Heng 2009; Bueno et al. 2010); arguing for its removal shortly after both. A considerably cheaper, clinically approved alternative to Y-27632, Fastudil, has also been shown to improve outcomes for hPSCs (So et al. 2020). Nicotinamide, used in the treatment of a wide range of diseases, possibly acting in its capacity as a kinase inhibitor, has also been shown to promote cell survival in hPSCs (Meng et al. 2018).

These improvements in classical cryopreservation outcomes, together with the development of xeno-free, serum-free defined medium (e.g., E8) has allowed not only derivation and banking of hPSCs (Crook et al. 2007; Baghbaderani et al. 2016; De Sousa et al. 2016) but also their scale-out/scale-up in 2D culture and 3D bioreactor systems (Pandy et al. 2020; Rivera et al. 2020) under current Good Manufacturing Practice (cGMP). Such cryopreserved hPSCs have demonstrated

both stability and maintenance of differentiation capacity in long-term culture (Shafa et al. 2020).

2.3.3 Issues with DMSO

CP-induced toxicity (at 37 °C) and osmotic damage have both been demonstrated in hPSCs (Katkov et al. 2011; Valbuena et al. 2008). These can be ameliorated by reducing exposure temperature, multistep addition/elution protocols and/or lowering the CP concentration (Lee et al. 2010; T'Joen and Cornelissen 2012; Imaizumi et al. 2014).

Transient, reduced levels of the pluripotency marker Oct-4 have been detected after cryopreservation in DMSO (Katkov et al. 2006), though this is reversible by using ROCKi (Martin-Ibañez et al. 2008). Changes to gene expression have been reported following cryopreservation in DMSO (Wagh et al. 2011), as have effects on differentiation potential and the epigenetic status of ESCs (Iwatani et al. 2006; Pal et al. 2012; Chatterjee et al. 2017). While these changes are generated by exposure times/temperatures not usually associated with cryopreservation, a recent study has suggested that changes may occur after as little as 2 h exposure to DMSO (Verheijen et al. 2019). This, together with other safety considerations (Awan et al. 2020), have led to calls for care in the continued use of DMSO in novel cellular therapies (Stacey et al. 2017a) and a search for DMSO-free alternatives (Weng and Beauchesne 2020). Lists of suitable cGMP-compliant alternatives to DMSO have been published (Hunt 2019; Awan et al. 2020).

2.3.4 Cryopreservation-Induced Delayed-Onset Cell Death (CIDOCD)

Figure 4 shows the effect of classical slow-cooling on a hiPSC line in the absence of ROCKi. Immediately post-thaw, viability (as measured by a membrane permeability assay) is high. However, 24 h after seeding, the surviving cells form two subpopulations: those that have adhered (~2%) and those that remain viable but fail to re-attach (~7%). The viability of the unattached cells declines with time in culture while the reattached cells will, after a period in culture and passaging, expand and re-constitute the cell line.

The results are typical for slow-cooling hPSCs in the absence of a ROCK inhibitor and emphasize the difficulties associated with both the type and timing of assays used to determine viability. Neither assays undertaken immediately post-thaw nor those carried out following an extended period in culture will accurately reflect the level of cryopreservation-induced delayed-onset cell death or the severe loss of cells that can occur in the hours post-thaw. The results also indicated that, even in the absence of ROCK, there is a subpopulation of cells that, while unable to attach in the early post-thaw period, are nonetheless still viable, at least by the standard of live/dead staining, and potentially rescuable.

This pattern of post-thaw cell loss is well documented in hPSCs (Xu et al. 2009), occurring through the activation of apoptotic pathways post-thaw through various stresses imposed by the cryopreservation process. CIDOCD has been the subject of a number of reviews (Baust et al. 2016, 2017b). Inhibitors have been added to the CP solutions to target the various pathways involve in activating the apoptotic cascade.

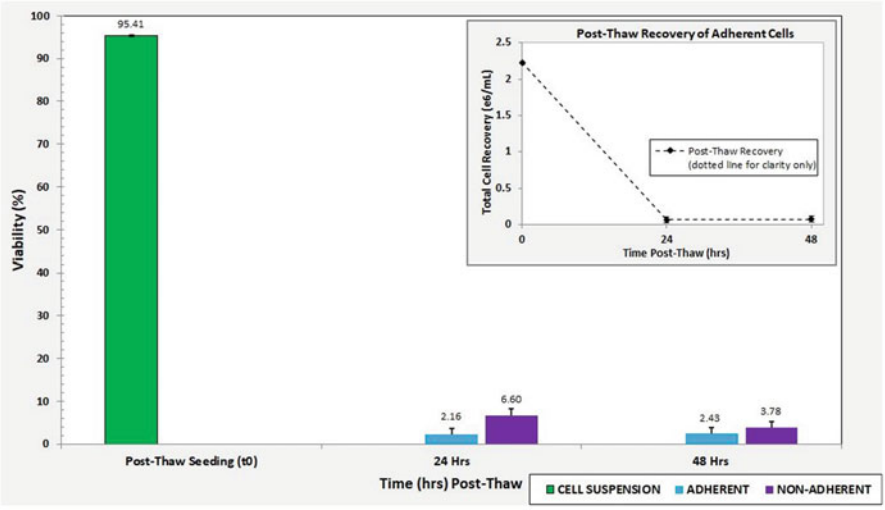

Fig. 4 Post-thaw viability of slowly-cooled hiPSCs in the absence of a ROCK inhibitor. The hiPSC line NIBSC5, generated by mRNA/miRNA reprogramming of a human fetal fibroblast cell line MRC-9, was cultured on Matrigel-coated plates in mTeSR™1. Undifferentiated cells were sub-cultured every 5 days using 1× TrypLE™ Express. Colonies at p29 were dissociated into a mixed population of single, duplet and triplet cells and resuspended in pre-cooled 90% (FBS)/10% DMSO at +4 °C. After 10 min equilibration, aliquots of 3×10^6 cells/mL were cooled at 1 °C/min to −80 °C in a Mr. Frosty and stored in the gas phase of LN_2. Samples were thawed at ~160 °C/min and assayed using an AO/DAPI live/dead stain (membrane integrity test) in a Nucleocounter® NC3000™ imaging cytometer capable of counting not only single cells but cells in aggregates. Experiments were performed in triplicate, with three biological replicates from each of three separate cryovials per experiment, at three time points: immediately after thawing/prior to seeding (t0) and after 24 and 48 h in culture. Inset shows total cells recovered post thaw (the dotted line is only shown for purposes of clarity). Similar results were obtained for the hESC cell line H9 and for NIBSC5 cooled in a controlled-rate freezer (data not shown). Immediately after thawing and prior to seeding, over 95% of the cells were viable (i.e., excluded DAPI). Twenty-four hours after seeding total cell recovery was low (see inset), for cells both adherent to the Matrigel coated plates (~3%) or free-floating within the culture medium (~12%). Viability assessment of these two subpopulations indicated that over 75% of the adherent cell population was viable, while the nonadherent population also showed a significant proportion of viable cells (>50%). After 48 h the proportion of viable, attached cells showed no significant increase while the "viable" cell population of non-adherent cells had significantly declined. Surviving attached cells exhibit typical hPSC pluripotency stem cell markers when assayed some five passages post-thaw (data not shown). This work was undertaken as part of the TSB project "Development of a GMP cryogenic cold chain for clinical delivery of regenerative medicine therapeutics" (project no. 101103) (Data courtesy of J. Mann).

These inhibitors and the mechanism by which they operate are detailed in a review by Bissoyi et al. (2014).

Figure 4 also exemplifies the dilemma over viability assessment: what to assess and when to assess it. Timing is crucial if reliable evaluation is to be achieved and the type of assay should reflect the functional outcomes expected of the cell or therapy. Baust et al. (2017a) have argued for a four-tier approach involving assays for

membrane integrity, molecular (apoptotic/necrotic) mechanisms, functionality, and genomic/epigenetic assessment. This fits well with regulatory requirements to demonstrate identity, potency, and stability of cell therapy products.

2.3.5 Storage of hPSCs

Storage at −80 °C or above typically leads to progressive deterioration and loss of viability, with storage limited to weeks or, at best, a few months. Though the use of Ficoll 70 in the CP solution has been shown to extend storage times for hPSCs to at least 1 year (Yuan et al. 2016a), long-term storage below the glass transition is required in gas-phase LN_2 or ultra-low(−140 °C) mechanical refrigerators to maintain cell viability.

A number of recent studies suggest alternatives to cryopreservation where short-term storage is logistically preferable. Deep-supercooling has been shown to preserve human stem cells for periods up to 7 days (Huang et al. 2020). This process relies on undercooling the cell suspension in supplemented UW solution without CPAs. Freezing is avoided by preventing surface-catalyzed nucleation through the layering of an immiscible oil phase onto the surface of the sample. However, this nonequilibrium approach to undercooling leaves open the possibility of uncontrolled nucleation should the immiscible oil phase be breached.

Seven-day storage at ambient temperature (20–25 °C) of hESCs has also been achieved using a technique termed spheropreservation (Jiang et al. 2017). Here, hESC colonies were cut into small aggregates and cultured on low-attachment plates to produce cell spheres which were stored in hPSC medium (E8)-filled cryovials for up to 7 days. Alginate encapsulation has also been used to store PSCs at ambient temperature for up to 5 days in culture medium (Chen et al. 2013) and 7 days at 4 °C using Hypothermasol as the storage solution (Correia et al. 2016).

2.3.6 Cryopreservation of Organoids

hPSC-derived organoids have been generated for a wide variety of organ systems. Their ability to increasingly mimic the three-dimensional structure and complexity of human organs has great potential in many areas of pharmaceutical medicine, beyond that of traditional human and animal model systems, for evaluation of drug efficacy/metabolism and toxicity testing (Miranda et al. 2018; Kim et al. 2020). Unlike spheroids (3D clusters of cells) or embryoid bodies (hPSC aggregates which spontaneously generate the three germ lineages), organoids are self-organizing structures generated by guided differentiation of embryoid bodies or hPSCs during 3D culture in extracellular matrices such as Matrigel™ (Lancaster and Knoblich 2014).

Successful cryopreservation, using conventional slow cooling, has been reported in a number of studies: Reichman et al. (2017) for hiPSC-derived retinal organoids; Han et al. (2017) for small intestinal organoids treated with ROCKi and Lu et al. (2017) for encapsulated gastrointestinal organoids – the last of these showing a significant improvement in post-thaw recovery compared to organoids cryopreserved using a standard non-scalable process in Matrigel™.

Cryobiologically, the size, 3D architecture, and multicellular nature of organoids present a unique set of problems beyond those of single cell suspensions. The effect of extracellular (i.e., interstitial) ice, as well as differences in behavior with respect to intracellular freezing between similar cells, in tissue and in suspension, are well documented (Pegg 2020). Moreover, the osmotic response and the influence this exerts on intracellular ice formation have been demonstrated in hepatic spheroids (Korniski et al. 1999) and cell aggregates (Li et al. 2018), while the size (diameter) of neural stem cell spheres has been shown to influence post-thaw recovery (Ma et al. 2010).

Given the variety of organoids generated from both hPSC and adult stem cells, if optimal recovery is envisaged, it is unlikely that all will be amenable to a one-size-fits-all approach to cryopreservation. The difficulties likely to be encountered and the approaches available to improving cryopreservation outcomes are similar to those encountered in the cryopreservation of pancreatic islets (Taylor and Baicu 2009; Kojayan et al. 2018) which are, in terms of size and complexity, comparable to many organoids and for which a wealth of information on cryopreservation, using both slow cooling and vitrification approaches to preservation, exist.

2.4 Preservation and Storage of Whole Blood and Blood Components

2.4.1 Blood Products in Transfusion Medicine

Blood component (BC) therapy has become the standard of care in medicine for the treatment of the anemic, bleeding, or cytopenic patient. The widespread adoption and retention of BC therapy have been driven by innovations in refrigeration, blood bag design, anticoagulant and preservative solution composition, infectious disease testing, and other means of donor screening (Mollison and Engelfriet 1999; Freedman 2014). Whole blood (WB) of healthy donors is often separated into BCs including a red cell concentrate (RCC), platelet concentrate (PC) or plasma with post-processing modifications including leukocyte, pathogen and volume reduction, gamma irradiation, washing, pooling, or cryopreservation (Acker and Razatos 2020). However, WB can be stored without further processing for up to 35 days and has recently seen increased use in prehospital and military trauma resuscitation (Spinella et al. 2016; Cap et al. 2018; Chipman et al. 2020).

2.4.2 Blood Component Preparation and Storage

WB collection and separation systems are designed in different configurations depending on the needs of the blood manufacturer. All current methods for preparation of the major BCs – RCCs, PCs, and plasma – rely on one or more semi- or automated centrifugation and extraction steps. Apheresis devices are continuous systems that remove WB from a donor, isolate the component of interest, and return the remaining components back to the donor, allowing the concurrent collection of BCs from a single donor depending on the regulatory approval of each individual device. Automated production devices can improve the standardization of BCs by

fully controlling all the centrifugation and extraction steps, providing more consistent preparation of BCs.

Hypothermic storage of BCs: While the availability of donated BCs has facilitated widespread transfusion, the ex vivo storage of BCs in an artificial environment impairs natural processes required for cell viability and function. Room temperature (RT) or refrigerated storage of BCs, aimed to minimize cell injury, can suppress biochemical reactions with residual metabolic activity eventually resulting in nutrient depletion and accumulation of cell waste (Tinmouth and Chin-Yee 2001; Scott et al. 2005). This is termed the "storage lesion" and has been well documented for BCs (Germain et al. 2017; Alshalani et al. 2018; Yoshida et al. 2019). Acceptable standards for the hypothermic storage of BCs to ensure product safety and quality are outlined in government regulations and by standard-setting organizations. In general, RCCs can be stored the longest – for up to 56 days at 1–6 °C – which ensures there is no more than 1% hemolysis at expiry (0.8% in Canada and Europe) and 75% in vivo cell recovery (Hess and BEST 2012). WB units are also stored at 1–6 °C for up to 35 days. PCs have a shelf life about 5 days (or up to 7 days if bacterial screening is performed) at 20–24 °C with constant agitation. Granulocyte-rich white cell concentrates can be stored only up to 24 h at room temperature.

Cryopreservation of BCs: Cryopreservation remains the only technology that provides preservation and long-term storage of cell quality and biological function. However, inappropriate logistics or handling of cryopreserved samples during routine banking operations can cause transient warming events (TWEs) (Gulliksson and Nordahl-Källman 2014; Briard et al. 2016; Chabot et al. 2017) and affect resulting product quality. Rising evidence of TWE-induced product impairment caused by ice recrystallization emphasizes the need for more robust storage technologies that can support expanding regulations on the length of stable storage of BCs.

Cryopreservation of RCCs is used for the long-term storage of rare blood products or products of a strategic nature (i.e., military or strategic stockpile of universal RCCs) (Hess and Thomas 2003). Currently, there are two methods used for the cryopreservation of RBC products: low glycerol/rapid cooling (Rowe et al. 1968) and high glycerol/slow cooling (Meryman and Hornblower 1972; Valeri and Zaroulis 1972).

The more common cryopreservation method found in the USA and most international blood centers is the use of a high concentration of glycerol (40%) in conjunction with slow (~1 °C/min) and following storage at ≤ -65 °C. In each method, controlled addition and removal of glycerol using semi- or automated methods are used to prevent osmotic lysis of the RBC and to minimize patient exposure to the chemical cryoprotectant. Cryopreserved RBCs must be stored at less than −65 °C and expire after 10 years unless a policy is in place for release of these units beyond their expiry. European regulations permit the cryogenic storage of cryopreserved RBCs for up to 30 years (EDQM 2017). Rare RBC products that were thawed and deglycerolized can be refrozen and rethawed when needed without adversely affecting the recovery of the products (Valeri 1973).

With the lifespan of platelet concentrates for several days, cryopreservation can be used to extend the storage of these labile BCs. Since firstly reported in 1974

(Valeri et al. 1974), cryopreservation of platelets with 5–6% DMSO and storage for up to 4 years at −80 °C remains the most widely used method in clinical practice (Valeri et al. 1974, 2005; Cid et al. 2016). Removal of DMSO prior to freezing allows PCs to be thawed and reconstituted immediately with plasma making these units suitable for military and civilian trauma use (Valeri et al. 2005; Noorman et al. 2016). Although PC cryopreservation is time-consuming and more expensive than standard RT storage, clinical trials are underway to support platelet cryopreservation (Dumont et al. 2013; Reade et al. 2013; Slichter et al. 2018).

Rapid freezing of plasma without the addition of any cryoprotectants can be accomplished using a blast freezer, dry ice, or a mixture of dry ice with either ethanol or antifreeze. In Europe, Fresh Frozen Plasma (FFP) has an expiry time of 36 months when stored at <-25 °C, or 3 months at −18 °C to −25 °C (EDQM 2017). In the USA, FFP can be stored for up to 12 months when stored at ≤ -18 °C and up to 7 years at −65 °C.

Despite controversial findings for granulocytes (GC) transfusion efficacy, this product is widely used to treat neutropenic patients, antibiotic-resistant infections, patients under chemotherapy, and others (Yoshihara et al. 2016; Gea-Banacloche 2017). The shelf life of 24 h places limits on the availability and inventory management of GCs. Unfortunately, traditional approaches to cryopreservation do not provide a high recovery of GCs after freeze-thawing (Boonlayangoor et al. 1980; Moss and Higgins 2016).

2.4.3 Emerging Blood Products

As transfusion practices continue to evolve, there is a constant need for new and innovative approaches to the collection, manufacturing, storage and distribution of BCs. Two products that are being actively pursued are cold-stored PCs and cultured RBCs. The short shelf life of PCs and risks of bacterial contamination, despite universal bacterial testing and pathogen reduction (Jones et al. 2019), requires alternative strategies to meet emerging transfusion needs and inventory requirements (Acker et al. 2016). Storage of PCs at 1–6 °C has a number of advantages over RT storage include prolonged shelf life due to reduced metabolism, enhanced hemostatic activity, improved bacteriologic safety, and ease of storage and transport (Waters et al. 2018). Despite the shorter circulation lifespan of cold-stored PCs, they can improve inventory flexibility by supporting trauma or other bleeding patients (Stubbs et al. 2017).

Cultured RBCs can be produced by ex vivo erythroid cell generation from different primary cells, such as human peripheral blood mononuclear cells (Heshusius et al. 2019) or from cord blood CD34+ cells by co-culturing with macrophages (Fujimi et al. 2008). The ability to produce universally compatible RCCs by large-scale production of cultured RBCs would dramatically affect the current donor-centric approach to BC manufacturing (Giarratana et al. 2011; Zeuner et al. 2012) and new developments in the differentiation of RBCs and platelets from pluripotent stem cells opens up exciting possibilities for the future (Focosi and Amabile 2018).

Transfusion of blood components is likely to remain the single largest cell therapy used globally. With more than 118.5 million blood donations made per year (according to WHO 2020), proper preservation and storage of these important products remains an important priority for the transfusion medicine community.

2.5 Cryopreservation of T Cells for Adoptive Therapies

Cryopreservation of blood-derived T lymphocytes has been of interest for several decades. Early studies showed that success could be achieved with slow cooling methods (Thomson and O'Connor 1971; Knight et al. 1972; Tsutsaeva et al. 1978). Today, the expanding clinical adoption of T-cell therapies has once again relied on robust cryopreservation techniques.

2.5.1 Cryopreservation in CAR-T Cell Therapies

Chimeric antigen receptor T cell (CAR-T cell) therapy involves the genetic modification of T cells to express recombinant antigen receptors which target cancer cells. In August 2017, Trisagenlucleucel (Kymriah), a CAR-T cell therapy for patients up to 25 years of age with B-cell acute lymphoblastic leukemia (B-ALL), became the first autologous cell-based cancer therapy to receive approval by the US Food and Drug Administration (FDA) (Maude et al. 2018; Mullard 2018; Schuster et al. 2019). This was followed by the approval of Axicabtagene ciloleucel (Yescarta) in October 2017, as a second-line treatment for adult patients with diffuse large B-cell lymphoma (Mullard 2018; Roberts et al. 2018). These two autologous CAR-T cell therapies have demonstrated improved remission rates relative to standard chemotherapy and their stable establishment in the drug market has been contingent on the development of a reliable and robust supply chain (Levine et al. 2017; Grigor et al. 2019).

Nonmobilized peripheral blood mononuclear cells (PBMCs) are collected via leukapheresis, cryopreserved within 24 h of collection, shipped to a central manufacturing site, and enriched for T cells (Vormittag et al. 2018). Enriched T cells are then transduced with the CAR transgene, expanded in a bioreactor culture system for 7–9 days, cryopreserved in an infusible medium, and finally transported back to the treatment center (Vormittag et al. 2018). Cryopreservation is therefore crucial to both ends of the supply chain and not only promotes flexibility to the manufacturing process, but more importantly allows patients to undergo leukapheresis and receive the therapy at a time that is appropriate based on their condition. There are consequently two pertinent questions that arise in regard to the impact of cryopreservation in CAR-T cell therapies: (1) whether it affects the manufacturing and efficacy of the final CAR-T cell product, and (2) whether it affects the persistence of CAR-T cells upon infusion. In clinical protocols patient conditioning with interleukin treatment is used and thus reproducible culture processes and cryopreservation protocols that enable reliable recovery from thawing are vital to enable assure efficient matching of patient and CART preparation.

Cryopreserved PBMCs are used in functional and phenotypic in vitro immunological assays and additionally prove effective in numerous clinical applications, ranging from immunotherapy precursors to direct transplantation (Smith et al. 2007; Heo et al. 2009; Filbert et al. 2013; Kodama et al. 2013; Li et al. 2017). Therefore, an abundance of research since the 1980s has gone into not only optimizing PBMC cryopreservation protocols, but also the impact of these optimized protocols on PBMC survival and function (Milson and Keller 1982; Prince and Lee 1986; Weinberg et al. 2000, 2007; Kreher et al. 2003; Disis et al. 2006; Bull et al. 2007). High levels of delayed onset cell death (DOCD) are endemic to any cryopreservation protocols that incur a high degree of sublethal stress and are commonplace in cryopreserved PBMCs, suggesting there is room for further optimization (Baust 2002; Baust et al. 2005; Cosentino et al. 2007). Panch et al. (2019) performed a retrospective analysis on 158 autologous CAR-T products from six single-center clinical trials, revealing reduced levels of enriched T cells over the first 2 days in culture following isolation from cryopreserved PBMCs (Panch et al. 2019). This did not prove true for fresh PBMCs, suggesting DOCD took place. However, CAR-T cultures initiated with fresh PBMCs fare similarly to those initiated with cryopreserved PBMCs based on transduction efficiency, CAR-T fold expansion, and CD4:CD8 ratios upon harvest (Panch et al. 2019; Tyagarajan et al. 2019). Therefore, the use cryopreserved PBMCs likely does not pose a significant impact on either the efficacy of the final CAR-T product or the time required to generate a sufficient number of CAR-T cells. The latter point is particularly important, as extended periods of in vitro expansion (9–14 days) have proven to diminish post-thaw CAR-T cell recovery (Fisher et al. 2014; Berens et al. 2016). In the production of Kymriah, Novartis uses a multicomponent cryopreservation cocktail consisting of 7.5% DMSO, 20% Human Serum Albumin (HSA), and 10% Dextran 40 (FDA 2019). On the other hand, Kite Pharmaceuticals uses a formulation consisting of 5% DMSO and 2.5% HSA in the production of Yescarta (FDA 2020b). While the concentrations vary, the use of DMSO, Dextran, and HSA has been consistent amongst several CAR-T cell clinical trials (Singh et al. 2013; Garfall et al. 2015; Kebriaei et al. 2016; Li et al. 2019). As a part of Kymriah's quality assurance, Novartis requires that cells be frozen in an insulated freezing container at a rate of 1 °C/min to −40 °C and 10 °C/min from −40 °C to −80 °C (Chamberlain et al. 2019). Products are subsequently stored in the liquid nitrogen vapor phase and rapidly thawed in a dry thawing device compliant with cGMP regulations (FDA 2020a). Details pertaining to the cooling and warming regimens used by Kite Pharmaceuticals, as well as those used in most CAR-T cell clinical trials have not been clearly stated; however, given the relative consistency in these regimens between different cell therapy applications, they are unlikely to differ substantially.

Cryopreservation protocols for CAR-T have been derived by application of traditional controlled slow cooling methods with DMSO supplemented by pentastarch and/or human serum albumin (Panch et al. 2019; Lu et al. 2016). Such protocols have limited impact on the tumoricidal effects of CAR T cells in vitro with respect to their specificity and proliferation upon stimulation (Xu et al. 2018). However, global gene expression analysis has revealed that 1139 genes are

overexpressed in cryopreserved CAR-T cells, many of which were associated with apoptotic pathways (Panch et al. 2019). CAR-T cells are infused immediately upon thawing, making it challenging to form definitive conclusions regarding the in vivo impact of cryopreservation and resulting DOCD on the effectiveness of the therapy. There is currently no data to indicate that in vivo levels or persistence of cryopreserved CARTs are diminished; however, it is certainly possible that DOCD would occur in vivo and potentially be followed by compensatory expansion.

The clinical use of cryopreserved CAR-T cells is a logistic necessity and if ongoing trials show long-term efficacy and safety will become more widespread in the coming decade, with several ongoing clinical trials exploring novel CAR-T cell therapies for hematologic malignancies, combined with the potential application of CAR-T cells to solid tumor treatment (Chavez et al. 2019; Ma et al. 2019; Zhao et al. 2019). To further delineate the impact of cryopreservation on CAR-T cells, it is necessary that we understand how storage times as well as the frequency and duration of transient warming events alter phenotypic or functional characteristics. It will be important to pay special attention to long-term safety of CAR-T medicine as there are issues which need to be addressed (Eyles et al. 2019). Finally, as is the case with most cell therapies, there is a need to explore DMSO-alternatives to minimize infusion-related reactions.

2.5.2 Cryopreservation of Regulatory T Cells

Adoptive cell therapy (ACT) is also used for the treatment autoimmune pathologies. Experimental studies have demonstrated the effectiveness of adoptive Treg cell therapy (Eggenhuizen et al. 2020). The possibility of using these cells for allergic diseases and asthma treatment has also been demonstrated (Esmaeilzadeh et al. 2020). The suppressive effect of Tregs on immunocytes can be implemented directly during intercellular contact or indirectly, as a result of secretion of biologically active molecules (Christoffersson and von Herrath 2019). An alternative to the administration of Tregs in autoimmune diseases can be the involvement of dendritic cells (DCs) capable of inducing the expansion of the recipient's Tregs (Goltsev et al. 2019).

As mentioned above, the effect of deep cooling on a heterogeneous population of lymphocytes is ambiguous. Indeed, in the report (Chen et al. 2016), the peripheral blood mononuclear cells by phenotypic characteristics before and after cryopreservation were assessed and a higher cryostability of CD19+ B cells was established as compared to CD3+ T cells. In other studies, a decrease after cryopreservation in the percentage of naive lymphocytes (CD45RA+ CD62L+), memory T cells (CD45RO+ CD62L+), as well as HLA-DR+ CD38+, CD39− CD73+ cells among subpopulations of CD4+ and CD8+ peripheral blood lymphocytes was noted (Costantini et al. 2003; Reimann et al. 2000; Turner et al. 2020; Weinberg et al. 2009). A possible reason for the decrease in CD62L+ cells may be the activation of matrix metalloproteinase (MMP) due to DMSO effect, which causes the cleavage of L-selectin (CD62L) from the cell surface (Hattori et al. 2001) and a change in its functional activity. Sparrow et al. (2006) revealed a strong correlation between the reduced expression of the CD62L receptor and the appearance of apoptotic/SYTO16 (dim)

lymphocytes, when investigating the post-thaw cord blood lymphocytes. Essentially, the shading of L-selectin from activated T cells prevents their re-entry into peripheral lymph nodes (Galkina et al. 2003). The importance of preserving the receptor repertoire of cryopreserved lymphoid cells is emphasized by a decreased ability of murine Tregs after low-temperature exposure to bind to a partner in the CD62L-MADCAM1 system in vitro, as well as a disordered homing to secondary lymphoid organs in vivo (Florek et al. 2015). The same authors showed that during adoptive transfer, the cryopreserved Tregs protected animals from experimental lethal graft-versus-host-disease (GVHD) to a far lesser extent than native ones. This can be likely explained by a high cryolability of Tregs, shading of their surface markers and ability of triggering an abnormal secretion of cytokines after cryotherapy (Brunstein et al. 2011; Golab et al. 2013; Weinberg et al. 2009). It is notable that immediately after thawing most Tregs are assessed as viable; only a part of them exhibit apoptotic signs leading to cell death upon 12 h of cultivation (Gołąb et al. 2018).

Nevertheless, the results of successful cryopreservation of Treg without disturbing the phenotype and function are presented (Guo et al. 2015; Weiner et al. 2015), as well as the development of a method for lyophilization of human cord blood cell leucoconcentrate (HCBCL), which provides an increase in the Tregs concentration in general cell spectrum (Koval et al. 2019). At the same time Brunstein et al. (2011) did not reveal an increase in the Tregs content in peripheral blood of recipients with GVHD after infusion of cryopreserved Tregs as it was observed after infusion of "freshly isolated" Tregs. In general, the controversial results regarding the successful cryopreservation of Tregs are explained by a limited number of clinical trials with application of cryopreserved Tregs (Fraser et al. 2018; Meyer et al. 2019). Recently strategies to move adoptive T-cell therapies to an almost industrial scale have been proposed, requiring cryopreservation as an essential step in the production pathway (Iyer et al. 2018).

2.6 Bone Marrow in the Organ Donor Pathway

It was recognized over 60 years ago that bone marrow (BM) recovered from deceased organ donors is a potential source of viable hematopoietic stem/progenitor cells (HSPC) for transplantation that exceeds the volumes obtainable from living donors (Thomas et al. 1957; Ferrebee et al. 1959). Each year in the USA, approximately 10,000 victims of non-survivable traumas donate their organs and a further 30,000 donate their tissues, through which 30,000 organs and over a million tissues are recovered. The combination of large volumes recovered from each donor with the substantial number of donors each year gives the potential for building an extensive BM bank of diverse HLA representation.

Ossium Health is developing such a bank of organ and tissue donor-derived whole BM as well as selected BM-derived hematopoietic stem/progenitor cells (HSPCs), mesenchymal stromal cells (MSCs), and mature cells for conventional medical purposes, to promote tolerance of solid organs and vascular composite allografts and as radiation-nuclear medical countermeasures. Ossium's initial

product, hematopoietic progenitor cell marrow (HPC, Marrow), contains the full complement of HSPCs and mature immune cells required to treat hematological cancers and BM failure.

Recovery of functional BM from deceased donors is conceptually similar to the procurement of organs and tissues. Published studies have confirmed that stem and progenitor cells within deceased organ donor BM are highly viable and comparable to living donor cells (Fig. 5) (Blazar et al. 1986; Kenyon et al. 1995; Rybka et al. 1995; Söderdahl et al. 1998; Ahrens et al. 2004; Baumert et al. 2012).

Ossium has developed optimized recovery systems including specialized kits and shippers for uniform recovery by our multiple OPO partners and has characterized warm and cold ischemia times for optimum cellular yield (Woods et al. 2020). Our current processing methodology yields highly functionally viable cells from deceased donor BM, with colony-forming unit granulocyte/macrophage (CFU-GM) averaging 170 ± 40 and total CFU averaging 417 ± 50 per 105 total nucleated cells plated as compared to live donor controls that yielded 140 ± 18 and

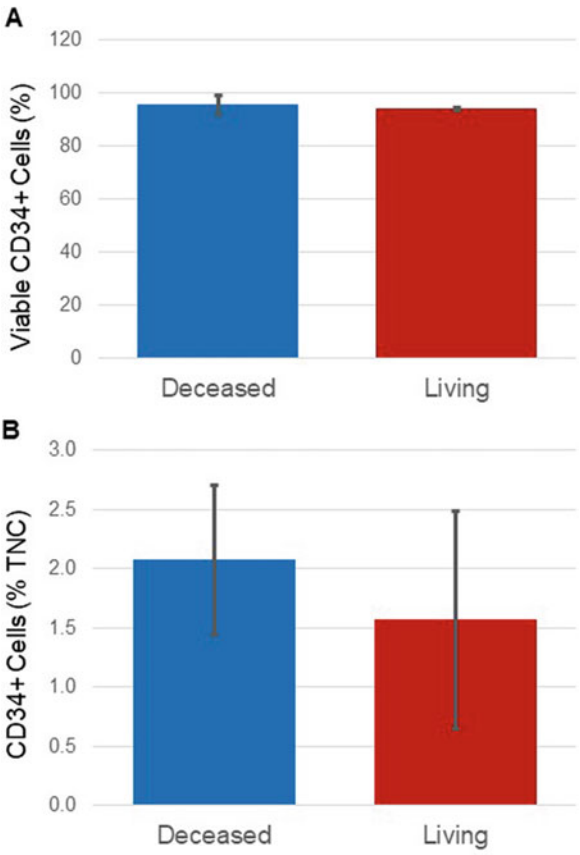

Fig. 5 Similar (**a**) viability and (**b**) number of CD34+ HSC isolated from deceased and living donor BM. Bars represent means (± standard deviations) of data from the six studies listed in the text. *TNC* total nucleated counts

205 ± 17, respectively (mean ± SEM; $n = 4$ experimental and 2 control; live donor BM purchased from Lonza) as well as stable engraftment in a humanized immunocompromised mouse model (unpublished data).

For end user ease, Ossium packages HPC, Marrow at 70 mL volumes in 250 mL cryostorage bags (Origen BioMedical, Austin, TX, USA). Multiple such units are prepared from each donor, ranging from 3 to 12 based on the donor size and recovery outcomes. For the bank to service the greatest number of patients, cryopreservation is essential. Bone marrow or mobilized stem cells have been cryopreserved and transplanted for decades, with varying protocols mostly consisting of slow cooling (1–2 °C/min) in a cryopreservative of 10% DMSO and storage in vapor phase or liquid nitrogen until prepared for use by rapidly warming, typically in a 37 °C water bath (Berz et al. 2007; Hunt 2011). Controlled rate cooling and passive cryopreservation approaches have both been used with success (Morgenstern et al. 2016). To develop the protocol for use at Ossium, initially two controlled rate cooling methods and a passive approach were considered. The VIA Freeze Quad (Cytiva, Marlborough, MA, USA) and the CryoMed (ThermoFisher Scientific, Waltham, MA, USA) were evaluated as controlled rate cooling options, and a passive approach was evaluated utilizing a "box in box" method (Shu et al. 2010). The VIA Freeze was adapted to cryopreserve six bags at a time to meet Ossium's requirements. This initial study indicated no difference among methods, with each yielding high post thaw survival with mean post thaw CD34 viability and CFU-GM equivalent among methods (Pennington et al. 2019).

Given the scale of production with multiple donors per day each yielding between 3 and 12 cryostorage bags, and the relative simplicity of the process, a version of the passive cooling approach was further investigated and subsequently validated for routine use. For this, an ultracold mechanical freezer (CryoCube F740, Eppendorf, Enfield, NC, USA) was temperature mapped using a Part-11 compliant data capture system (Ellab Denver, CO, USA). Preliminary experiments indicated a chamber setting of −86 °C to be optimum for uniform temperatures across each of the three freezer shelves. Surrogate bags containing cryoprotectant only (10% DMSO in saline with 2.5% human serum albumin) were prepared, placed into aluminum cassettes, and used in further preliminary experiments which indicated that cooling rates would be faster if cassettes were placed directly against the walls of the freezer but were consistent across the bulk of the shelves (data not shown). An experiment was designed in which surrogate bags were prepared including T-type thermocouples placed in the cryoprotectant solution with an Omega OM-USB-TC data capture system (Omega, Norwalk, CT, USA) utilized to record freeze curves. The bags were placed in cassettes which were then placed on each shelf of the freezer maintaining a minimum of 11 cm distance from the walls and held for a minimum of 6 h. The resulting freezing curves were very consistent. Mean cooling rates (MCR) for each bag across a range from −10 °C to 40 °C were determined to be −1.39 ± 0.13 °C (top shelf), −1.38 ± 0.16 °C, (middle shelf) and −1.30 ± 0.14 °C (bottom shelf) (mean ± SD), respectively.

Next, experiments were performed with three organ donor bone marrow products. For these experiments, the same protocol and temperature monitoring system was

Table 3 Post-thaw viability assessed via flow cytometry (USP<127>) and colony-forming assay

CD34 viability (%)	Bag 1	Bag 2	Bag 3	Mean (±SD)
Donor 1	96.71	97.51	97.69	97.3 ± 0.52
Donor 2	70.87	80.24	79.34	76.8 ± 5.16
Donor 3	90.04	88.05	82.06	86.7 ± 4.15
CFU-GM (per 10^5 cells)	**Bag 1**	**Bag 2**	**Bag 3**	**Mean (±SD)**
Donor 1	40	30	35	35.0 ± 5.0
Donor 2	25	35	20	26.7 ± 7.6
Donor 3	45	95	60	66.7 ± 25.7

used; however, the thermocouples were taped to the outside of the bags to avoid contaminating the products, and after 6–24 h at −86 °C the cassettes were transferred to vapor LN_2 storage. Taping thermocouples to the bags allowed for an approximation of the actual freeze curve but was less accurate than the immersion method and prone to failure due to detaching. Again, freezing curves were very consistent, with MCRs of −1.50 ± 0.17 °C ($n = 3$ bags), −1.52 ± 0.14 °C ($n = 3$ bags), and −1.56 °C (only one bag measured due to thermocouple failures) (mean ± SD), respectively.

After storage for >1 week, bags were thawed and evaluated for CD34+ cell viability and CFU-GM potential. All donors exhibited high viability and robust colony growth (Table 3).

This experiment was repeated without the temperature monitoring with three additional donors and results were confirmed with a post thaw CD34+ viability of 86.81 ± 8.35% and mean CFU-GM of 51 ± 29 colonies/10^5 cells plated (mean ± SD).

With smaller volume products (e.g., umbilical cord blood cryopreserved in 25 mL volumes), directly placing cassettes in a static −86 °C chamber could result in a cooling rate faster than desirable. Ossium's larger volume units carry sufficient thermal resistance so that the chamber temperature can be easily adjusted to result in ideal cooling rates. This robust method is relatively insensitive to user error and equipment failure as well. For clinical production at Ossium, all units banked are tested for post-thaw viability for 100% verification to allow use of this method for ongoing clinical production.

2.7 Tissues and Cell/Scaffold Constructs

2.7.1 Preservation of Unprocessed Native Tissues

Cryopreservation of unprocessed native tissues may be considered for the cases where their transplantation as a whole would be a more preferred treatment choice (e.g., oocytes vs. ovarian tissues in cancer or prepubertal patients). From a practical standpoint, compared with the storage of isolated cells, cryopreservation of a whole tissue implies minimal processing times and has a major advantage of maintaining cells within their natural microenvironment. Ultimately, upon demand such frozen

specimens may be subsequently used as a source of cells to be isolated and expanded for experimental or clinical purposes. This strategy has been the focus of many studies. For the detailed description of the techniques used for the cryopreservation of ovarian tissue and whole ovaries the reader may refer to Isachenko et al. (2009) and the recent review by Arav and Patrizio (2019). Vermeulen et al. summarize the challenges and perspectives associated with cryopreservation of immature testicular tissues (Vermeulen et al. 2017). By the refinement of a vitrification protocol, great success has also been achieved in preservation of intact human articular cartilage with high chondrocyte recovery and preserved cartilage structure as Jomha et al. report (Jomha et al. 2012). Slow freezing of intact mucosal tissues using 10% DMSO has been shown a superior performance versus vitrification; with cryopreservation of tissue pieces higher cell yields and viability were gained compared to isolated cells (Hughes et al. 2018). Two-step freezing of cervical explants and their thawing in pre-warmed (37 °C) culture medium resulted in comparable with fresh tissues viability over time (Fox et al. 2017). Cryopreservation of cornea has met with varying degrees of success as Armitage pointed out; the feasibility of using low cooling rates (0.2 K/min) and impermeable CPAs in slow freezing and some progress in vitrifying corneas has been reported (Armitage 2009). In a review on the cryopreservation of adipose tissue Shu et al. recommend a protocol based on the as a CPA for a broad range of downstream bioanalytical applications (Shu et al. 2015). As a further example, slow freezing of umbilical cord tissue explants using in 10% DMSO has recently been reviewed by Arutyunyan et al. (Arutyunyan et al. 2018).

Carnevale et al. have optimized a protocol for slow freezing of whole trabecular fragments enclosing the bone marrow through utilization of human serum and DMSO (Carnevale et al. 2016). Using cryopreservation cocktail composed of 0.05 M glucose, 0.05 M sucrose and 1.5 M ethylene glycol for slow freezing of human dental follicle tissue Park et al. communicated >70% of cell survival rate after 3 months of tissue storage (Park et al. 2017). Cryopreservation of ready-to-use precision-cut tissue slices (PCTS) might become an indispensable tool for pharmaceutical applications. Fahy et al. demonstrated a varying degree of success of using slow freezing and vitrification for PCTS from different tissue sources (Fahy et al. 2013). In addition, vitrification of whole biopsy cancer tissues for the preparation of PCTS to evaluate anticancer drug responses both in vitro and in vivo has recently been carried out (Zhang et al. 2020). In an effort to obtain fully functional dissociated primary neuronal cultures from cryopreserved tissues Cano-Jaimez et al. described an experimental protocol (using slow cooling and 10% DMSO) for frozen storage of mouse hippocampi (Cano-Jaimez et al. 2020).

2.7.2 Preservation of Decellularized Tissues

Many reports acknowledge the successful preservation of decellularized tissue matrix from a range of tissues. The main goal here is to preserve the native ultrastructure, tissue-specific components of extracellular matrix (ECM) – such as growth factors (GF) – and its biophysical properties. After cryopreservation acellular ECM may be either used for in vitro pre-seeding with respective cells or for in vivo

repopulation. The dual slow freezing of decellularized human amniotic membrane (without any CPAs) has resulted in preserved mechanical stability and retained levels of growth factors for the ophthalmological applications (Pogozhykh et al. 2020). The research team of Urbani et al. provided evidence that slow freezing under protection of 10% DMSO maintained the architecture and biomechanical properties of decellularized esophagi for clinical applications (Urbani et al. 2017). Notably, due to consistent success documented in both in vitro and in vivo studies, vitrification and freeze drying are becoming increasingly prevalent in preservation of decellularized tissues. Zouhair et al. have discussed preservation strategies for decellularized pericardial scaffolds for their off-the-shelf availability (Zouhair et al. 2019). In this study vitrification using VS83 solution and freeze-drying using sucrose as a lyoprotectant have been shown to better preserve pericardial patches compared to standard slow freezing in 10% DMSO. Freeze-drying of decellularized allogeneic heart valves with 80% sucrose resulted in preserved functionality and repopulation with recipients' cells after implantation into the juvenile sheep (Goecke et al. 2018). In good agreement with previous studies, Biermann et al. demonstrated that vitrification of allogeneic heart valves was accompanied by improved long-term durability as well as reduced immunogenicity in the orthotopic sheep model (Biermann et al. 2018).

2.7.3 Cryopreservation of Tissue-Engineered Constructs

Cryopreservation of 3D tissue-engineered constructs (TECs), although not yet optimized, offers great potential for clinical translation of tissue engineering (Urbani et al. 2018). Within the tissue engineering workflow a TEC formation is initiated starting from cryopreserved material followed by cultivation in a bioreactor. In turn, engineered 3D structure may be frozen and stored for future use. Herein the term "TEC" we use in a broad sense to define engineered cellular sheets and aggregates, cells and tissue-like structures enclosed in hydrogels and cell-seeded scaffolds (see Fig. 6 for illustration).

Ready availability of engineered constructs for the ultimate patient benefits is a strong argument that favors establishment of uninterrupted cold chains for their biobanking (Pogozhykh et al. 2018). In this context, an elegant approach to prepare scaffolds harboring in their structure ice-nucleating or ice recrystallization inhibition

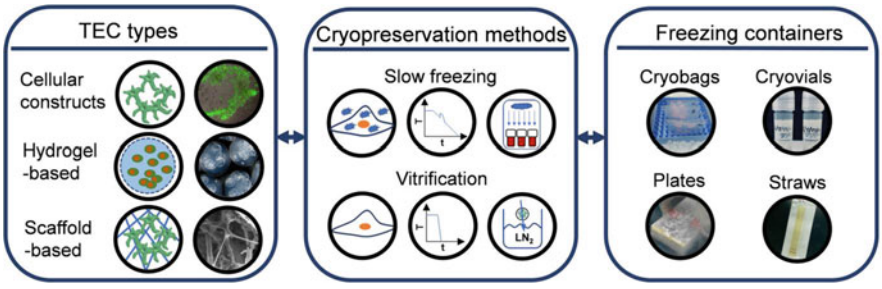

Fig. 6 Schematic illustration of current technological routes for TEC cryopreservation

agents to confer them cryoprotective properties at the stage of preparation has been recently endeavored (Weng et al. 2017; Nagao et al. 2018). The increasing number of publications related to TEC cryopreservation over the last decade, reflects growing research interest pertaining to this to matter (many papers are published tissue engineering-oriented journals) (summarized in the Table 4).

3 Best Practices in Applied Cryopreservation

3.1 Best Practice to Address Current Regulatory Concerns

3.1.1 Key Regulatory Aspects

Core issues that will be required for the suitability of preservation, storage and shipment of cell-based medicines will include sustained safety, consistent quality, and documented traceability.

Consistency of quality is assured by Good Manufacturing Practice (European Commission 2017; FDA 2020a; WHO 2014; ICH 2000) which provides a framework for addressing all aspects of the manufacturing process, including the manufacturing facility, environmental controls, staff training, raw materials, SOPs, quality control, storage, and shipment. All critical aspects of manufacturing will require some form of validation and this also applies to preservation methods, storage systems, and shipment equipment and procedures.

Traceability is key to robust risk assessment, demonstrates that all products have been manufactured in the prescribed way, and is crucial for investigations required following a serious adverse event or serious adverse reaction in patients.

Risk assessment is also a key element in delivery of safe and reliable cell based medicines, and it is important to apply this to all aspects of manufacture including the challenging risk assessment required to assure safety of raw materials and donor cells (Stacey et al. 2018; Petricciani et al. 2017).

The key elements of manufacturing consistency and traceability are delivered through implementation and careful maintenance of a quality management system according to GMP regulation and guidance (EMA 2019; FDA 2021). GMP is required for manufacture of all advance therapy medicinal products (ATMPs) (European Commission 2007) and also for product manufactured for clinical trials in the market authorization route to delivering regenerative medicines. However, certain cell-based medicines may be delivered under local authorization or "hospital exemption" arrangements which may vary between different regulatory jurisdictions, but in general are expected to meet the standards of GMP as is the storage of minimally manipulated cell and tissue preparations which do not fall formally under GMP but other regulations such as the EU Tissue and Cells Directive (European Commission 2004), its technical annexes (European Commission 2006a, b). Special guidance for cell-based medicines on topics such as microbial contamination and selection of raw materials may also be available for cell-based medicines (e.g., European Pharmacopoeia 2016; European Pharmacopoeia 2017).

Table 4 Summary of the technical aspects in the cryopreservation of TECs

Cryopreservation method	Overview	CPAs	Profile	Containers and supports	Outcome and comments	References
Scaffold-free cellular engineered constructs						
Slow freezing	Eteogenic matrix cell sheets	Cell Banker 1	1 °C/min from +4 °C to −80 °C; LN_2; water bath at 37 °C	Cryovials	No significant difference in cell viability between the fresh and slow-freezing groups; osteogenesis at bone defect sites	Kura et al. (2016)
	Engineered skeletal muscle tissue	10% DMSO	1 °C/min to −80 °C; water bath at 37 °C	Freezing tubes	Viability 93% for undifferentiated and 90% for differentiated muscle; a threefold increase in force production in the undifferentiated group as compared to unfrozen muscle	Grant et al. (2019)
	3D bovine colon organoids	Organoid-CPA	Gradient freezing to −80 °C, storage at −150 °C; transfer to −20 °C for 30 min, addition of warm culture medium and incubation on a heated stage at 3 °C for 3 min	Multi-well plates	Similar post-thaw growth rates and performance in cytotoxicity screenings at compared to unfrozen colonoids	Töpfer et al. (2019)

	3D MSCs-ECM complexes	10% DMSO/20% FBS	Placement into a −80 °C freezer; water bath at 37 °C	Cryovials	Preserved cell viability and successful bone regeneration after transplantation into rat calvarial defects	Motoike et al. (2018)
Vitrification	Chondrocyte sheets	20% DMSO, 20% EG, 0.5 M sucrose, and 10% carboxylated poly-L-lysine	Coating of a sample in minimum amount of vitrification solution; liquid nitrogen vapor; electric heating plate at 38 °C for 90 s	1. Stainless steel mesh; 2. Envelope of polyvinylidene chloride; 3. Culture dish	Viability (%) 1. 92 2. 87 3. 78	Maehara et al. (2013)
	Myoblast cell sheets	6.5 M EG, 0.7 M sucrose, and 10% carboxyl poly-L-lysine without serum	Holding above LN_2; a hot plate set at 37 °C with thermal insulation gel	Polypropylene mesh; packaging between thin polyethylene films	Viability and cardiac performance after cell sheet implantation in the chronic ligation rat model comparable with the fresh group	Ohkawara et al. (2018)
Hydrogel-based constructs						
Slow freezing	HepG2 cells in calcium alginate gel	Alginate dissolved in BioTool cryopreservation solution	−80 °C freezer; addition of culture medium at 37 °C	Covered PDMS mold in tissue-culture polystyrene plates	95% viability; formation of spheroid-like aggregates in long-term culture	Chen et al. (2018)
	MSCs-laden hyaluronic acid and polyethylene glycol-based hydrogels	10% DMSO	4 °C for 30 min, −20 °C for 60 min, −80 °C overnight	Microcentrifuge tube	~40–60% viability, preserved differentiation potential	Khetan and Corey (2019)
	MG63 cells in a wet-spun alginate fibers	10% DMSO; 10% glycerol; 10% DMSO/0.4 M trehalose; 10%	1 °C/min from +37 °C to −80 °C; LN_2; water bath at 37 °C	Cryovials	Viability 85, 71, 71 and 72%, respectively;	Cagol et al. (2017)

(continued)

Table 4 (continued)

Cryopreservation method	Overview	CPAs	Profile	Containers and supports	Outcome and comments	References
		glycerol/0.4 M trehalose			preserved viscoelastic properties	
	MSCs-laden alginate beads	7.5% DMSO; 30 min of loading	1 °C/min to −80 °C; transfer to −150 °C; water bath at 37 °C	Cryovials	Viability up to 70%	Gryshkov et al. (2015)
Vitrification	Porcine adipose-derived stem cell-laden alginate microcapsules	1 mol L^{-1} 1,2-propanediol, 1 mol L^{-1} EG, 10% (w/v) dextran T50 (≈0.002 mol L^{-1}), and 1 mol L^{-1} trehalose	Plunging into LN_2; water bath at 37 °C	Plastic straws	Viability higher than 70%	Zhao et al. (2017)
	MSCs-laden core-shell alginate hydrogel microfibers	0.75 mol L^{-1} 1 EG, 0.75 mol L^{-1} 1,2-propanediol, 1 mol L^{-1} trehalose and 10% (w/v) dextran T50 (≈0.002 mol L^{-1})	Plunging into LN_2; water bath at 37 °C	1. Cryovial; 2. Nylon mesh	Survival rates (%): 1. 10–13; 2. 84–89	Tian et al. (2019)
	Alginate encapsulated MSCs	10% DMSO, 20% EG, 20% 1,2-propanediol, 0.5 M sucrose	Plunging into LN_2; water bath at 40°C	Cryovial	Viability ~75%; preserved osteogenic and adipogenic differentiation potential	Trufanova et al. (2016)
Scaffold-based constructs						
Slow freezing	Optimaix™ porous collagen scaffolds seeded with MSCs	10% DMSO and 20% FBS; addition and removal of CPA using a bioreactor	1 °C/min from +4 °C to −80 °C; LN_2; water bath at 37 °C	Cryovials	Viability 59%	Petrenko et al. (2017a)

	3D collagen-hydroxyapatite scaffolds seeded with MSCs	Pretreatment with 0.1 M sucrose for 24 h; 10% DMSO with 0.3 M sucrose	1 °C/min to −80 °C; transfer to −150 °C; 1 min in water bath at 37 °C; addition of pre-warmed culture medium	Cryovials	Viability higher than 80%	Mutsenko et al. (2020)
	MSCs embedded in hydrogels of human platelet-poor blood plasma	Pretreatment with 0.1 M sucrose for 24 h, gel based on human platelet-poor blood plasma, 0.2 M sucrose and 1% DMSO	1 °C/min to −80 °C; LN_2; water bath at 37 °C	Cryovials	In vitro viability 73%; positive impact of MSCs in a full-thickness wound model in mice	Rogulska et al. (2019)
	MSCs augmented mesh scaffolds	10% DMSO/40% FBS	1 °C/min to −80 °C; LN_2; water bath at 37 °C for 45–60 s	Cryovials	No significant difference in population doubling time, multilineage differentiation potential and wound healing between frozen thawed and fresh bioconstructs	Bharti et al. (2020)
Vitrification	PCL-gelatin nanofibrous scaffolds by	40% EG 0.6 M sucrose; stepwise addition and removal of CPAs	Plunging into LN_2; water bath at 38–39 °C for 1 min	Pouch of ethylene vinyl oxide material	No significant differences between the vitrified and control TECs in cellular metabolic activity and proliferation	Wen et al. (2009)

LN_2 liquid nitrogen, *EG* ethylene glycol, *FBS* fetal bovine serum, *MSCs* mesenchymal stromal cells, *PCL* poly(lactic acid)

While each regional jurisdiction will maintain its own laws and regulatory guidance the International Conference on Harmonisation (https://www.ich.org/products/guidelines/quality/article/quality-guidelines.html) has established guidance documents which are adopted in multiple jurisdictions including the EU, Japan, and USA. Key among these for preservation and storage of regenerative medicines and cell therapies are ICH documents for risk assessment (ICH 2005), process validation (ICH 2009), product stability (ICH 1995), and demonstration of comparability following a process change (ICH 2004).

3.1.2 Importance of Regulatory Science

It is important to recognize that in any regulatory consideration of regenerative medicines, a fundamental requirement is to have a science-based understanding of the events and "signals" that may impact on product quality and safety. This is typically described as a regulatory science perspective, which has been defined by the US FDA as "the science of developing new tools, standards, and approaches to assess the safety, efficacy, quality, and performance of FDA-regulated products" (FDA 2011). Thus, establishing and maintaining a current knowledge of key scientific aspects of the systems involved in preservation, storage, and shipment of regenerative medicines is critical.

3.1.3 Principles for Operation of Storage Systems

All material stored for clinical use should be recorded in a robust inventory system which may be required to meet some specific regulatory requirements for data security/stability and protection of donor personal data, examples of which include the US Code of Federal Regulations 21, part 11 (FDA 2020c) and the EU General Data Protection Regulations (European Union 2018).

The storage environment must be secure and clean, although generally it is not required to provide the highest level of environmental air quality in storage areas, as the sterility of product is based on aseptic manufacture and product container integrity.

All critical storage procedures which could impact on the quality of the product will be controlled under GMP procedures and records, including staff training, storage access, cryogen filling regimes, inventory inspection/resolution, and picking/packaging/labeling for shipment. In addition, the integrity of cell and tissue containers must be assured to prevent contamination during storage, thus attention should be paid to avoiding direct contact of storage cryogen with cellular material (e.g., open straws, insecure closures, breakage-prone bags) and ensuring integrity of vial closures (e.g., internal screw-threads, gaskets, heat-sealable sleeves). In addition, stored material may need to be separated into isolated storage for new untested materials, in-process materials (those awaiting completion of quality control), released materials and quarantined materials that may be known to be contaminated.

Staff safety is clearly a priority for design and operation of storage systems and should include consideration of specific hazards associated with handling cryogens such as asphyxiation and frost-bite which will be hazards in the use of liquid nitrogen or carbon dioxide. In such cases, restricted and monitored personnel access, personal

protective equipment, environmental and personal monitors for atmospheric oxygen levels, and adequate ventilation are key issues to address.

Maintenance and storage stability are also crucial aspects requiring routine cleaning procedures, checks on liquid nitrogen delivery systems and storage vessel vacuum seals, and frequent monitoring of vessel temperatures to avoid temperature cycling in stored cell products. Contamination of storage vessels can become significant over time in use (Grout and Morris 2009; Fountain et al. 1997). Thus, while cleaning of liquid nitrogen vessels and electric freezers may prove challenging but approaches to reduce or otherwise manage buildup of contamination in storage vessels should be considered (Pessoa et al. 2014; Bajerski et al. 2020).

3.1.4 Choice of Low Temperature Storage Systems

Liquid nitrogen inventory vessels are probably the commonest means of mid- to long-term storage, but require careful backup in terms of assured liquid nitrogen supply. Safety issues may also be significant considerations for facility design and staff safety dealt with above.

Ultra-low electric freezer cabinets(−140 °C) can be an alternative to liquid nitrogen vessels, but require a highly robust and reliable power supply and/or local backup (e.g., uninterrupted power supply batteries, emergency generators). Other storage temperatures have been discussed elsewhere in this chapter. Such temperatures are generally not considered suitable for long-term storage of cryopreserved or vitrified materials, but may be used for shipment or short-term storage and only where the preservation method is suited for such storage, otherwise product failure may result.

Systems for sub-normothermic or hypothermic storage of cells for therapy will be subject to jurisdictional regulations, but different regulatory science considerations must be addressed regarding the mechanisms of degradation of cellular product (addressed elsewhere in this chapter) and growth of contamination in the unfrozen state.

3.1.5 Facility Design

There are a number of issues that need to be addressed for the selection and design of a storage facility as follows:

1) Location: A space is required which is not prone to high levels of microbial contamination and not below ground level to avoid the buildup of cryogen gas such as nitrogen.
2) Fabric considerations: structural considerations (e.g., floor weight baring limits, drains), finishes (e.g., floors, walls, ceilings) to facilitate cleaning, lighting and power.
3) Cryogen storage and supply: which may include the need for piped liquid cryogen or a liquid nitrogen production facility and areas for back up emergency filling vessels.
4) Environmental considerations: temperature, ventilation and air quality and contamination. NB. Ventilation is a crucial issue for assuring safe working to avoid

asphyxiation in areas where solid carbon dioxide or liquid nitrogen is used. In such areas sufficient forced ventilation must be available. Wall-mounted oxygen monitors should also be included and ideally will automatically trigger ventilation and a remote alarm.

5) Areas for separate storage vessels to allow segregation of materials of different levels of risk as discussed above.
6) Inventory systems: racking systems should be suitable for the materials and containers to be stored and must meet requirements of regulations such as the European Union Tissues and Cells Directive (EUTCD) (European Commission 2004, 2006a, b) and other best practice systems such as FACT (http://www.factwebsite.org) to assure security, stability, and backup.

3.2 Best Practices in Shipping and Packaging

The cold chain process, it is necessary to transport advanced therapies to sustain stability of products transported between clinics and manufacturing sites is crucial and typically relies on the use of reduced temperature (FACT 2018; Jesson et al. 2016; EMA 2020a; Stacey et al. 2017b; Swioklo and Connon 2017). Sometimes this may be a finished cell-based medicine, but it could also include the transport of starting materials such as biopsies and apheresis derived biologicals which form part of the source material for the manufacture of a therapy (Jesson et al. 2016).

The type of therapy and specific manufacturing conditions dictate whether this shipment must be at ultra-low temperatures, or if hypothermic, unfrozen, preservation is preferred, however the need for tight control, and risk of cellular damage, exists for both conditions (EMA 2001; FACT–JACIE 2018; FACT 2018; ISO 2020; WHO 2019).

UN3373 regulatory requirements will apply in all cases of shipping and packaging. Within the EU, there are also minimum good distribution practice (GDP) standards that a wholesale distributor must meet to ensure that the quality and integrity of medicines is maintained throughout the supply chain, in the case of advanced therapies (EMA 2001; ISO 2020; WHO 2019).

3.2.1 Unfrozen Shipment

In many situations cold, unfrozen shipment is permissible. With hematopoietic peripheral blood stem cells (PBSC) and bone marrow for example, fresh transport is preferable where possible as it avoids the cell loss associated with cryopreservation and subsequent thaw (Correia et al. 2016; Matsumoto et al. 2002). Unfrozen shipment also allows for a simpler cold chain, without the risks associated with the formation and melting of ice and cryoprotectants, as well as the practical challenges involved in controlled rate cooling and sensitive cryopreservation protocols. However, transport in the unfrozen state, typically at a hypothermic (0–10 °C) or subnormothermic (10–20 °C), is usually particularly time sensitive. Cells at these hypothermic temperatures maintain (albeit reduced) metabolic activity, and so consume oxygen and nutrients which often cannot be added during the transportation

step. Low temperatures themselves can also be damaging and so special buffers and/or diluents must be used to prevent damage (Correia et al. 2016; Swioklo and Connon 2017).

Many products, such as fresh cord blood or bone marrow, are transported at ambient or hypothermic temperatures, while other products such as PBSC should be transported under hypothermic or ideally cryopreserved temperatures only. This is particularly useful for therapies where a simple donor/recipient relationship exists and where minimal manipulation of the biological is required, and also for cell therapies where manufacture on demand is the current preferred delivery model.

Fresh products in this situation must be hand couriered and placed in a secondary sealed container to prevent leakage during transport (FACT 2018; Simione and Sharp 2017). It is also key to ensure that all relevant documentation is shipped with the product and not separately. The packaging must be validated against any impacts and shocks which may occur during transportation, for example it is recommended that for heat-sealed products, at least three heat-sealed areas are created to minimize the change of a leak. It is also desirable to avoid x-ray scanning – OBC (on board courier) shipping is the best way to avoid this (FACT–JACIE 2018; FACT 2018).

Reusable shipping containers should be validated and periodically requalified to ensure they hold temperature for the maximum time as specified by the manufacturer under both winter and summer environmental conditions. Additional validation studies may be necessary if shipping in regions of ambient temperature extremes (ISO 2020). Calibrated data loggers should be used to ensure that transport was carried out as per the validated protocol, and maximum shipping times should be determined prior to shipment. For local and short-term shipments, cold packs may be sufficient, but more robust cooling systems such as phase-change materials cooling may be needed for longer transport times or where there is a risk of delay in shipment (FACT–JACIE 2018; FACT 2018).

3.2.2 Cryopreserved Shipment

Cryopreserved shipments tend to be less time critical as long as the low temperatures can be assured, although the ultra-low temperatures required (usually around −135 °C or −150 °C for samples cryopreserved with DMSO (EMA 2020a, b) can be more difficult to achieve physically, and any deviations can be more damaging. In the case of cryopreserved shipments, the same stringent requirements as for hypothermic shipment must be followed, but with some additional considerations. For example, the primary container must not only be securely wrapped (FACT–JACIE 2018; ISO 2020), but the materials must not become brittle under the low temperatures and impacts or vibration which may occur during transportation.

An immediate consideration of low temperature transport is the risk of transient warming during transfer of the advanced therapy from its ultra-low temperature storage condition to the cold temperature shipper. In ambient air, cryopreserved therapies can warm extremely rapidly, and serious damage can occur to the cells within even when the advanced therapy appears frozen. Transfer between shipping

devices must be validated and must be rapid, with stringent precautions taken to reduce passive warming (Simione and Sharp 2017).

Tamper evident seals on the containment device during shipment must also be considered, and real-time GPS tracking is often used to track shipping progress, especially in the cases where patients must be prepared to receive a therapy soon after its arrival. Additional real-time monitoring such as temperature (often available in cryoshipper dataloggers and through specialized couriers), tilt, and shock monitoring can also ensure safe and effective delivery of a therapy and should be recorded for traceability of the cold chain (FACT–JACIE 2018).

The parameters of the cold-temperature chamber are also key – they must be validated to ensure they can maintain the required temperature and hold time (FACT–JACIE 2018; ISO 2020). These shipping devices must typically be stored upright as sideways storage will result in more rapid warming, and precautions to ensure correct handling and storage during transit must be taken. Re-useable shippers typically require decontamination between each shipment, and this should form part of a best-practice shipment (ISO 2020). Particular attention must be taken to decontaminate not just any potential leaks from a biological payload, but also contaminants from the air, LN_2, or from previously transported cells.

3.2.3 Receiving Shipments

Hospitals receiving shipments requiring ultra-low storage must be capable of receiving such a shipment and have processes in place to ensure that the advanced therapy is transferred effectively to local storage or thawed for use before any shipment container warms (FACT–JACIE 2018).

Receiving staff should receive appropriate training for the handling of cryogenic materials, including the use of personal protective equipment (FACT 2018).

As advanced therapies become more prevalent, requirements for local storage close to patient populations (and close to manufacturing) – especially for allogeneic therapies, must be considered.

3.2.4 Transport and Manipulation Within a Hospital

The point at which GMP manipulation ends in a given process must be considered.

Steps such as thawing cryopreserved therapies would normally be carried out under controlled conditions. Most advanced therapies are, and must be, infused directly after thawing, however those requiring a manipulation, including washing out of any cryoprotectants, could be considered part of the GMP manufacturing process depending on local regulations, and the clinic should carry out such manipulations in appropriate facilities (EMA 2001).

For therapies requiring no manipulations after shipment, except thawing, the manufacturer should have proven that a treatment is stable for a defined length of time under specific storage and shipment conditions, and if it can be verified that a therapy was shipped meeting these conditions. In this case a GMP manufacturing process would not normally be required post-shipment, and pharmacy controls would suffice as is the case for other temperature-sensitive medicines (including vaccines).

4 Conclusions

In this chapter we have considered the biopreservation approaches and methods that have facilitated the delivery of established cell-based medicines that have provided healthcare benefits for many decades. Furthermore, we have considered cell types now being developed for future therapies, such as pluripotent stem cells and organoids. Complex cell preparations including stem cells used in advanced therapies have brought significant challenges in terms of preserving mixtures of different cell populations and retention of key cell types and their functionality. Development of new cryoprotectants and cooling profiles may be needed to provide successful preservation of the ever more diverse cell therapy products. Vital to the development of new preservation technologies will be improved understanding of the biology of the therapeutic cell preparations and development of appropriate analytical approaches that will include the need for assays that will enable evaluation of functionality of cell-based therapeutic products. While there are existing regulatory frameworks for cell-based medicines in many countries, product developers may need to develop product specific approaches to the establishment of informative critical quality attributes and assays to evaluate them. Some products that are currently recalcitrant to cryopreservation will continue to require alternative non-frozen storage approaches that present potential challenges of instability, short shelf life, and growth of contaminating organisms. Thus, ongoing research and development into preservation technologies for therapeutic cell products will be vital to enable realization of the full potential of these new advanced medicines.

Acknowledgments Development of this chapter is facilitated by the UNESCO Chair in Cryobiology hosted by the Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine (http://www.cryo.in.ua) and supported by the Society for Low Temperature Biology Committee (https://www.sltb.info).

The authors thank the University Hospital Hradec Králové and the Charles University for financial support – supported by MH CZ–DRO (UHHK, 00179906) and Charles University grant projects SVV 260543/2020 and PROGRES Q40/06.

Dr. Olga Mykhailova is supported by a Post-doctoral Fellowship from the Canadian Blood Services.

References

Acker JP, Razatos A (2020) Whole blood and apheresis collection of blood components intended for transfusion. In: Cohn CS, Delaney M, Johnson ST, Katz LM (eds) Technical manual. AABB, Bethesda, pp 141–172

Acker JP, Marks DC, Sheffield WP (2016) Quality assessment of established and emerging blood components for transfusion. Journal of blood transfusion 2016:4860284. https://doi.org/10.1155/2016/4860284

Ahrens N, Tormin A, Paulus M, Roosterman D, Salama A, Krenn V, Neumann U, Scheding S (2004) Mesenchymal stem cell content of human vertebral bone marrow. Transplantation 78(6): 925–929. https://doi.org/10.1097/01.tp.0000133305.81823.2a

Akiyama Y, Shinose M, Watanabe H, Yamada S, Kanda Y (2019) Cryoprotectant-free cryopreservation of mammalian cells by superflash freezing. PNAS 116(16):7738–7743. https://doi.org/10.1073/pnas.1808645116

Akkök CA, Holte MR, Tangen JM, Ostenstad B, Bruserud O (2009) Hematopoietic engraftment of dimethyl sulfoxide-depleted autologous peripheral blood progenitor cells. Transfusion 49: 354–361. https://doi.org/10.1111/j.1537-2995.2008.01949.x

Alshalani A, Howell A, Acker JP (2018) Impact of blood manufacturing and donor characteristics on membrane water permeability and in vitro quality parameters during hypothermic storage of red blood cells. Cryobiology 80:30–37. https://doi.org/10.1016/j.cryobiol.2017.12.008

Amouzegar A, Dey BR, Spitzer TR (2019) Peripheral blood or bone marrow stem cells? Practical considerations in hematopoietic stem cell transplantation. Transfus Med Rev 33:43–50. https://doi.org/10.1016/j.tmrv.2018.11.003

Arav A, Patrizio P (2019) Techniques of cryopreservation for ovarian tissue and whole ovary. Clin Med Insights Reprod Health 13:1179558119884945. https://doi.org/10.1177/1179558119884945

Areman EM, Sacher RA, Deeg HJ (1990) Processing and storage of human bone marrow: a survey of current practices in North America. Bone Marrow Transplant 6:203–209

Armitage WJ (2009) Cryopreservation for corneal storage. Dev Ophthalmol 43:63–69. https://doi.org/10.1159/000223839

Armitage JO, Jagannath S, Spitzer G, Bierman P, Kessinger A, Kumar P, Cabanillas F, Zander A, Vellekoop L, Dicke KA (1986) High dose therapy and autologous marrow transplantation as salvage treatment for patients with diffuse large cell lymphoma. Eur J Cancer Clin Oncol 22: 871–877. https://doi.org/10.1016/0277-5379(86)90376-7

Arutyunyan I, Fatkhudinov T, Sukhikh G (2018) Umbilical cord tissue cryopreservation: a short review. Stem Cell Res Ther 9(1):236. https://doi.org/10.1186/s13287-018-0992-0

Awan M, Buriak I, Fleck R, Fuller B, Goltsev A, Kerby J, Lowdell M, Mericka P, Petrenko A, Petrenko Y, Rogulska O, Stolzing A, Stacey GN (2020) Dimethyl sulfoxide: a central player since the dawn of cryobiology, is efficacy balanced by toxicity? Regen Med 15(3):1463–1491. https://doi.org/10.2217/rme-2019-0145

Baek SK, Cho YS, Kim IS, Jeon SB, Moon DK, Hwangbo C, Choi JW, Kim TS, Lee JH (2019) A Rho-associated coiled-coil containing kinase inhibitor, Y-27632, improves viability of dissociated single cells, efficiency of colony formation and cryopreservation in porcine pluripotent stem cells. Cell Reprogram 21(1):37–50. https://doi.org/10.1089/cell.2018.0020

Baghbaderani BA, Syama A, Sivapatham R, Pei Y, Mukherjee O, Fellner T, Zeng X, Rao MS (2016) Detailed characterisation of human induced pluripotent stem cells manufactured for therapeutic applications. Stem Cell Rev Rep 12(4):394–420. https://doi.org/10.1007/s12015-016-9662-8

Bajerski F, Bürger A, Glasmacher B, Keller ERJ, Müller K, Mühldorfer K, Nagel M, Rüdel H, Müller T, Schenkel J, Overmann J (2020) Factors determining microbial colonization of liquid nitrogen storage tanks used for archiving biological samples. Appl Microbiol Biotechnol 104(1):131–144. https://doi.org/10.1007/s00253-019-10242-1

Barlogie B, Attal M, Crowley J, van Rhee F, Szymonifka J, Moreau P, Durie BG, Harousseau JL (2010) Long-term follow-up of autotransplantation trials for multiple myeloma: update of protocols conducted by the Intergroupe Francophone du Myelome, Southwest Oncology Group, and University of Arkansas for Medical Sciences. J Clin Oncol 287:1209–1214. https://doi.org/10.1200/JCO.2009.25.6081

Barnes DW, Loutit JF (1955) The radiation recovery factor: preservation by the Polge-Smith-Parkes technique. J Natl Cancer Inst 15:901–905

Barr RD, Whang-Peng J, Perry S (1975) Hemopoietic stem cells in human peripheral blood. Science 190:284–285. https://doi.org/10.1126/science.1179209

Baumert B, Kawa MP, Kotowski M, Grymuła K, Safranow K, Pabisiak K, Pius E, Peregud-Pogorzelski J, Walczak M, Ostrowski M, Machaliński B (2012) Bone marrow of multiorgan

donors underutilized: implications for improvement of accessibility of hematopoietic cells for transplantations. Transplantation 93(2):165–171. https://doi.org/10.1097/tp.0b013e31823e7ae2

Baust JM (2002) Molecular mechanisms of cellular demise associated with cryopreservation failure. Cell Preserv Technol 1(1):17–31. https://doi.org/10.1089/15383440260073266

Baust JM, Cosentino M, Meeks E, Baer J, Van Buskirk RG, Baust JG (2005) Apoptotic cell death contributes significantly to peripheral blood mononuclear cells cryopreservation failure. Cryobiology 51(4):354–355

Baust JM, Corwin W, Snyder KK, Van Buskirk R, Baust JG (2016) Cryopreservation: evolution of molecular based strategies. Adv Exp Med Biol 951:13–29. https://doi.org/10.1007/978-3-319-45457-3_2

Baust JM, Campbell LH, Harbell JW (2017a) Best practice for cryopreservation, thawing and recovering, and assessing cells. In Vitro Cell Dev Biol Anim 53(10):855–871. https://doi.org/10.1007/s11626-017-0201-y

Baust JG, Snyder KK, Van Buskirk R, Baust JM (2017b) Integrating molecular control to improve cryopreservation outcome. Biopreserv Biobank 15(2):134–141. https://doi.org/10.1089/bio.2016.0119

Beers J, Gulbranson DR, George N, Siniscalchi LI, Jones J, Thomson JA, Chen G (2012) Passaging and colony expansion of human pluripotent stem cells by enzyme-free dissociation in chemically defined culture conditions. Nat Protoc 7(11):2029–2040. https://doi.org/10.1038/nprot.2012.130

Berens C, Heine A, Müller J, Held SA, Mayer K, Brossart P, Oldenburg J, Pötzsch B, Wolf D, Rühl H (2016) Variable resistance to freezing and thawing of CD34-positive stem cells and lymphocyte subpopulations in leukapheresis products. Cytotherapy 18(10):1325–1331. https://doi.org/10.1016/j.jcyt.2016.06.014

Berz D, McCormack EM, Winer ES, Colvin GA, Quesenberry PJ (2007) Cryopreservation of hematopoietic stem cells. Am J Hematol 82(6):463–472. https://doi.org/10.1002/ajh.20707

Bharti MK, Bhat IA, Pandey S, Shabir U, Peer BA, Indu B, Bhat AR, Kumar GS, Amarpal CV, Sharma GT (2020) Effect of cryopreservation on therapeutic potential of canine bone marrow derived mesenchymal stem cells augmented mesh scaffold for wound healing in guinea pig. Biomed Pharmacother 121:109573. https://doi.org/10.1016/j.biopha.2019.109573

Bhattacharya N, Stubblefield P (eds) (2009) Frontiers of cord blood science. Springer, London, p 400. https://doi.org/10.1007/978-1-84800-167-1

Biermann AC, Marzi J, Brauchle E, Wichmann JL, Arendt CT, Puntmann V, Nagel E, Abdelaziz S, Winter AG, Brockbank KGM, Layland S, Schenke-Layland K, Stock UA (2018) Improved long-term durability of allogeneic heart valves in the orthotopic sheep model. Eur J Cardiothorac Surg 55:484–493. https://doi.org/10.1093/ejcts/ezy292

Bissoyi A, Nayak B, Pramanik K, Sarangi SK (2014) Targeting cryopreservation-induced cell death: a review. Biopreserv Biobank 12(1):23–34. https://doi.org/10.1089/bio.2013.0032

Bláha M, Maisnar V, Jebavý L, Měřička P, Rondiak J, Šulc K, Vaňásek J Jr (1990) Transplantation of blood progenitor cells collected from peripheral blood – first experience. Vojen Zdrav Listy LIX:218–223

Blaha M, Mericka P, Zak P, Stepanova V, Vavra L, Maly J, Tousovska K (2003) The risk of infection transmission from blood progenitor cell concentrates. J Hematother Stem Cell Res 12:161–164. https://doi.org/10.1089/152581603321628304

Blazar BR, Lasky LC, Perentesis JP, Watson KV, Steinberg SE, Filipovich AH, Orr HT, Ramsay NK (1986) Successful donor cell engraftment in a recipient of bone marrow from a cadaveric donor. Blood 67(6):1655–1660

Boonlayangoor P, Telischi M, Boonlayangoor S, Sinclair T, Millhouse E (1980) Cryopreservation of human granulocytes: study of granulocyte function and ultrastructure. Blood 56:237–245

Briard JG, Poisson JS, Turner TR, Capicciotti CJ, Acker JP, Ben RN (2016) Small molecule ice recrystallization inhibitors mitigate red blood cell lysis during freezing, transient warming and thawing. Sci Rep 6:23619. https://doi.org/10.1038/srep23619

Brunstein CG, Miller JS, Cao Q, McKenna DH, Hippen KL, Curtsinger J, Defor T, Levine BL, June CH, Rubinstein P, McGlave PB, Blazar BR, Wagner JE (2011) Infusion of ex vivo expanded T regulatory cells in adults transplanted with umbilical cord blood: safety profile and detection kinetics. Blood 117(3):1061–1070. https://doi.org/10.1182/blood-2010-07-293795

Bueno C, Montes R, Memendez P (2010) The ROCK inhibitor Y-27632 negatively affects the expansion/survival of both fresh and cryopreserved cord blood-derived CD34+ hematopoietic progenitor cells. Stem Cell Rev 6(2):215–223. https://doi.org/10.1007/s12015-010-9118-5

Bull M, Lee D, Stucky J, Chiu YL, Rubin A, Horton H, McElrath MJ (2007) Defining blood processing parameters for optimal detection of cryopreserved antigen-specific responses for HIV vaccine trials. J Immunol Methods 322(1–2):57–69. https://doi.org/10.1016/j.jim.2007.02.003

Cagol N, Bonani W, Maniglio D, Migliaresi C, Motta A (2017) Effect of cryopreservation on cell-laden hydrogels: comparison of different cryoprotectants. Tissue Eng Part C Methods 24:20–31. https://doi.org/10.1089/ten.tec.2017.0258

Canaani J, Beohou E, Labopin M, Ghavamzadeh A, Beelen D, Hamladji RM, Niederwieser D, Volin L, Markiewicz M, Arnold R, Mufti G, Ehninger G, Socié G, Kröger N, Mohty M, Nagler A (2019) Trends in patient outcome over the past two decades following allogeneic stem cell transplantation for acute myeloid leukaemia: an ALWP/EBMT analysis. J Intern Med 285:407–418. https://doi.org/10.1111/joim.12854

Cano-Jaimez M, Tagliatti E, Mendonca PRF, Nicholson E, Vivekananda U, Kullmann DM, Volynski KE (2020) Preparation of dissociated mouse primary neuronal cultures from long-term cryopreserved brain tissue. J Neurosci Methods 330:108452. https://doi.org/10.1016/j.jneumeth.2019.108452

Cap AP, Beckett A, Benov A, Borgman M, Chen J, Corley JB, Doughty H, Fisher A, Glassberg E, Gonzales R, Kane SF, Malloy WW, Nessen S, Perkins JG, Prat N, Quesada J, Reade M, Sailliol A, Spinella PC, Stockinger Z, Strandenes G, Taylor A, Yazer M, Bryant B, Gurney J (2018) Whole blood transfusion. Mil Med 183:44–51. https://doi.org/10.1093/milmed/usy120

Carnevale G, Pisciotta A, Riccio M, De Biasi S, Gibellini L, Ferrari A, La Sala GB, Bruzzesi G, Cossarizza A, de Pol A (2016) Optimized cryopreservation and banking of human bone-marrow fragments and stem cells. Biopreserv Biobank 14:138–148. https://doi.org/10.1089/bio.2015.0001

Carreras E, Dufour C, Mohty M, Kröger N (eds) (2019) The EBMT handbook: hematopoietic stem cell transplantation and cellular therapies [Internet], 7th edn. Springer, Cham. https://doi.org/10.1007/978-3-030-02278-5

Chabot D, Tremblay T, Paré I, Bazin R, Loubaki L (2017) Transient warming events occurring after freezing impairs umbilical cord-derived mesenchymal stromal cells functionality. Cytotherapy 19:978–989. https://doi.org/10.1016/j.jcyt.2017.04.005

Chakraborty N, Bilgrami S, Maness L, Guo C, Perez-Diez A, Mukherji B, Tutschka P (1999) Myeloablative chemotherapy with autologous peripheral blood stem cell transplantation for metastatic breast cancer: immunologic consequences affecting clinical outcome. Bone Marrow Transplant 24:837–843. https://doi.org/10.1038/sj.bmt.1701999

Chamberlain C, Ahsan G, James J, Gilmour KC (2019) 88 Validation of controlled rate freezing of T-cells for KYMRIAH® production. Arch Dis Child 104:A34. https://doi.org/10.1136/archdischild-2019-gosh.88

Chatterjee A, Saha D, Niemann H, Gryshkov O, Glasmacher B, Hofmann N (2017) Effects of cryopreservation on the epigenetic profile of cells. Cryobiology 74:1–7. https://doi.org/10.1016/j.cryobiol.2016.12.002

Chavez JC, Bachmeier C, Kharfan-Dabaja MA (2019) CAR T-cell therapy for B-cell lymphomas: clinical trial results of available products. Ther Adv Hematol 10:2040620719841581. https://doi.org/10.1177/2040620719841581

Chen B, Wright B, Sahoo R, Connon CJ (2013) A novel alternative to cryopreservation for the short-term storage of stem cells for use in cell therapy using alginate encapsulation. Tissue Eng Part C Methods 19(7):568–576. https://doi.org/10.1089/ten.TEC.2012.0489

Chen X, Zhang H, Mou W, Qi Z, Ren X, Wang G, Jiao H, Kong X, Gui J (2016) Flow cytometric analyses of the viability, surface marker expression and function of lymphocytes from children following cryopreservation. Mol Med Rep 14(5):4301–4308. https://doi.org/10.3892/mmr.2016.5780

Chen R, Wang B, Liu Y, Lin R, He J, Li D (2018) A study of cryogenic tissue-engineered liver slices in calcium alginate gel for drug testing. Cryobiology 82:1–7. https://doi.org/10.1016/j.cryobiol.2018.05.002

Chipman AM, Jenne C, Wu F, Kozar RA (2020) Contemporary resuscitation of hemorrhagic shock: what will the future hold? Am J Surg 220(3):580–588. https://doi.org/10.1016/j.amjsurg.2020.05.008

Christoffersson G, von Herrath M (2019) Regulatory immune mechanisms beyond regulatory T cells. Trends Immunol 40(6):482–491. https://doi.org/10.1016/j.it.2019.04.005

Cid J, Escolar G, Galan A, López-Vilchez I, Molina P, Díaz-Ricart M, Lozano M, Dumont LJ (2016) In vitro evaluation of the hemostatic effectiveness of cryopreserved platelets. Transfusion 56:580–586. https://doi.org/10.1111/trf.13371

Correia C, Koshkin A, Carido M, Espinha N, Šarić T, Lima PA, Serra M, Alves PM (2016) Effective hypothermic storage of human pluripotent stem cell-derived cardiomyocytes compatible with global distribution of cells for clinical applications and toxicology testing. Stem Cells Transl Med 5(5):658–669. https://doi.org/10.5966/sctm.2015-0238

Corwin WL, Baust JM, Baust JG, Van Buskirk RG (2014) Characterization and modulation of human mesenchymal stem cell stress pathway response following hypothermic storage. Cryobiology 68:215–226. https://doi.org/10.1016/j.cryobiol.2014.01.014

Cosentino L, Corwin W, Baust J, Diaz-Mayoral N, Cooley H, Shao W, Baust J (2007) Preliminary report: evaluation of storage conditions and cryococktails during peripheral blood mononuclear cell cryopreservation. Cell Preserv Technol 5(4):189–204. https://doi.org/10.1089/cpt.2007.9987

Costantini A, Mancini S, Giuliodoro S, Butini L, Regnery CM, Silvestri G, Montroni M (2003) Effects of cryopreservation on lymphocyte immunophenotype and function. J Immunol Methods 278:145–155. https://doi.org/10.1016/S0022-1759(03)00202-3

Cowan AJ, Baldomero H, Atsuta Y, Mikhael J, Aljurf M, Seber A, Greinix H, Koh M, Worel N, Libby EN, Pasquini M, Galeano S, Saber W, Iida M, Jaimovich G, Rolon JM, Kodera Y, Benakli M, Nosa BG, Elhaddad A, Szer J, Passweg J, Kroeger N, Weisdorf D, Niederwieser D (2020) The global state of hematopoietic cell transplantation for multiple myeloma: an analysis of the Worldwide Network of Blood and Marrow Transplantation Database and the Global Burden of Disease Study. Biol Blood Marrow Transplant 26:2372–2377. https://doi.org/10.1016/j.bbmt.2020.08.018

Cox MA, Kastrup J, Hrubisko M (2012) Historical perspectives and the future of adverse reactions associated with haemopoietic stem cells cryopreserved with dimethyl sulfoxide. Cell Tissue Bank 13:203–215. https://doi.org/10.1007/s10561-011-9248-2

Crook JM, Peura TT, Kravets L, Bosman AG, Buzzard JJ, Horne R, Hentze H, Dunn NR, Zweigerdt R, Chua F, Upshall A, Colman A (2007) The generation of six clinical-grade human embryonic stem cell lines. Cell Stem Cell 1(5):490–494. https://doi.org/10.1016/j.stem.2007.10.004

Czech Pharmacopoeia 2009 Supplement (2015a) Assessment of CD34/CD45+ cells in haematopoietic medicinal products. Grada Publishing, Prague, pp 300–301

Czech Pharmacopoeia 2009 Supplement (2015b) Assessment of the number of colony forming cells in human progenitor cells. Grada Publishing, Prague, pp 304–305

Czech Pharmacopoeia 2009 Supplement (2015c) Assessment of the number of nucleated cells and of their viability. Grada Publishing, Prague, pp 306–307

Daily MI, Whale TF, Partanen R, Harrison AD, Kilbride P, Lamb S, Morris GJ, Picton HM, Murray BJ (2020) Cryopreservation of primary cultures of mammalian somatic cells in 96-well plates benefits from control of ice nucleation. Cryobiology 93:62–69. https://doi.org/10.1016/j.cryobiol.2020.02.008

De Sousa PA, Downie JM, Tye BJ, Bruces K, Dand P, Dhanjal S, Serhal P, Harper J, Turner M, Bateman M (2016) Development and production of good manufacturing practice grade human embryonic stem cell lines as source material for clinical application. Stem Cell Res 17(2): 379–390. https://doi.org/10.1016/j.scr.2016.08.011

Disis ML, dela Rosa C, Goodell V, Kuan LY, Chang JC, Kuus-Reichel K, Clay TM, Kim Lyerly H, Bhatia S, Ghanekar SA, Maino VC, Maecker HT (2006) Maximizing the retention of antigen specific lymphocyte function after cryopreservation. J Immunol Methods 308(1–2):13–18. https://doi.org/10.1016/j.jim.2005.09.011

Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop DJ, Horwitz E (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 8: 315–317. https://doi.org/10.1080/14653240600855905

Dou R, Saunders RE, Mohamet L, Ward CM, Derby B (2015) High throughout cryopreservation of cells by rapid freezing of sub-μl drops using inkjet printing-cryoprinting. Lab Chip 15: 3503–3513. https://doi.org/10.1039/C5LC00674K

Douay L, Lopez M, Gorin NC (1986) A technical bias: differences in cooling rates prevent ampoules from being a reliable index of stem cell cryopreservation in large volumes. Cryobiology 23:296–301. https://doi.org/10.1016/0011-2240(86)90035-0

Dovgan B, Barlič A, Knežević M, Miklavčič D (2017) Cryopreservation of human adipose-derived stem cells in combination with trehalose and reversible electroporation. J Membr Biol 250:1–9. https://doi.org/10.1007/s00232-016-9916-z

Dumont LJ, Cancelas JA, Dumont DF, Siegel AH, Szczepiorkowski ZM, Rugg N, Pratt PG, Worsham DN, Hartman EL, Dunn SK, O'Leary M, Ransom JH, Michael RA, MacDonald VW (2013) A randomized controlled trial evaluating recovery and survival of 6% dimethyl sulfoxide-frozen autologous platelets in healthy volunteers. Transfusion 53:128–137. https://doi.org/10.1111/j.1537-2995.2012.03735.x

Eggenhuizen PJ, Ng BH, Ooi JD (2020) Treg enhancing therapies to treat autoimmune diseases. Int J Mol Sci 21(19):7015. https://doi.org/10.3390/ijms21197015

Esmaeilzadeh A, Tahmasebi S, Athari SS (2020) Chimeric antigen receptor-T cell therapy: applications and challenges in treatment of allergy and asthma. Biomed Pharmacother 123:109685. https://doi.org/10.1016/j.biopha.2019.109685

European Commission (2004) Directive 2004/23/EC of the European Parliament and of the Council of 31 March 2004 on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells. OJ L 102: 48–58. https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:102:0048:0058:en:PDF. Accessed 30 Jan 2021

European Commission (2006a) Commission Directive 2006/17/EC of 8 February 2006 implementing Directive 2004/23/EC of the European Parliament and of the Council as regards certain technical requirements for the donation, procurement and testing of human tissues and cells. OJ L 38:40–52. https://eur-lex.europa.eu/legal-content/EN/AUTO/?uri=celex:32006L0017

European Commission (2006b) Commission Directive 2006/86/EC of 24 October 2006 implementing Directive 2004/23/EC of the European Parliament and of the Council as regards traceability requirements, notification of serious adverse reactions and events and certain technical requirements for the coding, processing, preservation, storage and distribution of human tissues and cells. OJ L 294:32–50. https://eur-lex.europa.eu/legal-content/EN/AUTO/?uri=celex:32006L0086

European Commission (2007) Regulation (EC) no. 1394/2007 of the European Parliament and of the Council of 13 November 2007 on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) no. 726/2004 (text with EEA relevance). OJ L 324: 121–137. https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:324:0121:0137:en:PDF. Accessed 30 Jan 2021

European Commission (2017) Guidelines on good manufacturing practice specific to advanced therapy medicinal products, Brussels. https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-4/2017_11_22_guidelines_gmp_for_atmps.pdf. Accessed 30 Jan 2021

European Directorate for the Quality of Medicines & Health Care (EDQM) (2017) Guide to the preparation, use and quality assurance of blood components. Recommendation no. R(95) 15, 19th edn. Council of Europe, Strasbourg. http://www.ipst.pt/files/IPST/INFORMACAO_DOCUMENTACAO/EDQM_Blood_transfusion_guide_19ed_2017_pub_PUBSD-89.pdf

European Directorate for the Quality of Medicines & Health Care (EDQM) (2019) Guide to the quality and safety of tissues and cells for human application, 4th edn. Council of Europe, Strasbourg. https://www.edqm.eu/sites/default/files/medias/fichiers/Transplantation/transplantation_leaflet_guide_quality_safety_tissues_and_cells_human_application_4th_edition_2019.pdf

European Medicines Agency (EMA) (2001) Good distribution practice. European Medicines Agency (EMA), Amsterdam. https://www.ema.europa.eu/en/human-regulatory/post-authorisation/compliance/good-distribution-practice

European Medicines Agency (EMA) (2019) Guidelines relevant for advanced therapy medicinal products. EMA, Amsterdam. https://www.ema.europa.eu/en/human-regulatory/research-development/advanced-therapies/guidelines-relevant-advanced-therapy-medicinal-products. Accessed 30 Jan 2021

European Medicines Agency (EMA) (2020a) Kymriah: EPAR – product information. Annex 1 – summary of product characteristics. European Medicines Agency (EMA), Amsterdam. https://ec.europa.eu/transparency/regdoc/rep/3/2018/EN/C-2018-5717-F1-EN-ANNEX-1-PART-1.PDF

European Medicines Agency (EMA) (2020b) Yeskarta: EPAR – product information. Annex 1 – summary of product characteristics. European Medicines Agency (EMA), Amsterdam. https://www.ema.europa.eu/en/documents/product-information/yescarta-epar-product-information_en.pdf

European Pharmacopoeia (2016) Chapter 2.6.27. Microbiological examination of cell-based preparations. EDQM, Council of Europe, Strasbourg

European Pharmacopoeia (2017) European Pharmacopoeia 9.0, section 5.2.12. Raw materials for production of cell-based gene therapy products. EDQM, Council of Europe, Strasbourg, pp 627–630

European Union (2018) Regulation (EU) 2018/1725 of the European Parliament and of the Council of 23 October 2018 on the protection of natural persons with regard to the processing of personal data by the Union institutions, bodies, offices and agencies and on the free movement of such data, and repealing Regulation (EC) no. 45/2001 and Decision no. 1247/2002/EC (text with EEA relevance). OJ L 295:39–98. https://eur-lex.europa.eu/legal-content/en/ALL/?uri=CELEX%3A32018R1725

Eyles JE, Vessillier S, Jones A, Stacey G, Schneider CK, Price J (2019) Cell therapy products: focus on issues with manufacturing and quality control of chimeric antigen receptor T-cell therapies. J Chem Technol Biotechnol 94:1008–1016. https://doi.org/10.1002/jctb.5829

Fahy GM, Guan N, de Graaf IA, Tan Y, Griffin L, Groothuis GM (2013) Cryopreservation of precision-cut tissue slices. Xenobiotica 43:113–132. https://doi.org/10.3109/00498254.2012.728300

Fales I, Rahmatova S, Kobylka P, Matejckova S, Zahlavova L, Hruba A (2005) Cord Blood Bank of the Czech Republic, six month check. Bone Marrow Transplant 35:S125

Fernandes S, Khan N, Kale V, Limaye L (2019) Catalase incorporation in freezing mixture leads to improved recovery of cryopreserved iPSC lines. Cryobiology 90:21–29. https://doi.org/10.1016/j.cryobiol.2019.09.003

Ferrebee JW, Atkins L, Lochte HL Jr, McFarland RB, Jones AR, Dammin GJ, Thomas ED (1959) The collection, storage and preparation of viable cadaver marrow for intravenous use. Blood 14(2):140–147

Filbert H, Attig S, Bidmon N, Renard BY, Janetzki S, Sahin U, Welters MJ, Ottensmeier C, van der Burg SH, Gouttefangeas C, Britten CM (2013) Serum-free freezing media support high cell

quality and excellent ELISPOT assay performance across a wide variety of different assay protocols. Cancer Immunol Immunother 62(4):615–627. https://doi.org/10.1007/s00262-012-1359-5

Fisher V, Khuu H, David-Ocampo V, Byrne K, Pavletic S, Bishop M, Fowler DH, Barrett AJ, Stroncek DF (2014) Analysis of the recovery of cryopreserved and thawed CD34+ and CD3+ cells collected for hematopoietic transplantation. Transfusion 54(4):1088–1092. https://doi.org/10.1111/trf.12428

Fliedner TM, Körbling M, Calvo W, Bruch C, Herbst E (1977) Cryopreservation of blood mononuclear leukocytes and stem cells suspended in a large fluid volume. A preclinical model for a blood stem cell bank. Blut 35:195–202. https://doi.org/10.1007/bf00999460

Florek M, Schneidawind D, Pierini A, Baker J, Armstrong R, Pan Y, Leveson-Gower D, Negrin R, Meyer E (2015) Freeze and thaw of CD4+CD25+Foxp3+ regulatory T cells results in loss of CD62L expression and a reduced capacity to protect against graft-versus-host disease. PLoS One 10(12):e0145763. https://doi.org/10.1371/journal.pone.0145763

Focosi D, Amabile G (2018) Induced pluripotent stem cell-derived red blood cells and platelet concentrates: from bench to bedside. Cells 7:2. https://doi.org/10.3390/cells7010002

Foundation for the Accreditation of Cellular Therapy (FACT) (2018) Standards for immune effector cells. Foundation for the Accreditation of Cellular Therapy. http://www.factwebsite.org/iecstandards

Foundation for the Accreditation of Cellular Therapy (FACT) and the Joint Accreditation Committee ISCT and EBMT (JACIE) (2018) International standards for hematopoietic cellular therapy product collection, processing, and administration, 7th edn. FACT–JACIE. https://www.ebmt.org/sites/default/files/2018-06/FACT-JACIE%207th%20Edition%20Standards.pdf

Fountain D, Ralston M, Higgins N, Gorlin JB, Uhl L, Wheeler C, Antin JH, Churchill WH, Benjamin RJ (1997) Liquid nitrogen freezers: a potential source of microbial contamination of hematopoietic stem cell components. Transfusion 37(6):585–591. https://doi.org/10.1046/j.1537-2995.1997.37697335152.x

Fox JM, Wiggins RC, Moore JWJ, Brewer C, Hunter B, Andrew AC, Martin F (2017) Methodology for reliable and reproducible cryopreservation of human cervical tissue. Cryobiology 77:14–18. https://doi.org/10.1016/j.cryobiol.2017.06.004

Fraser H, Safinia N, Grageda N, Thirkell S, Lowe K, Fry LJ, Scottá C, Hope A, Fisher C, Hilton R, Game D, Harden P, Bushell A, Wood K, Lechler RI, Lombardi G (2018) A rapamycin-based GMP-compatible process for the isolation and expansion of regulatory T cells for clinical trials. Mol Ther Methods Clin Dev 8:198–209. https://doi.org/10.1016/j.omtm.2018.01.006

Freedman J (2014) Transfusion – whence and why. Transfus Apher Sci 50:5–9. https://doi.org/10.1016/j.transci.2013.12.003

Freimark D, Sehl C, Weber C, Hudel K, Czermak P, Hofmann N, Spindler R, Glasmacher B (2011) Systematic parameter optimization of a Me(2)SO- and serum-free cryopreservation protocol for human mesenchymal stem cells. Cryobiology 63:67–75. https://doi.org/10.1016/j.cryobiol.2011.05.002

Fry LJ, Querol S, Gomez SG, McArdle S, Rees R, Madrigal JA (2015) Assessing the toxic effects of DMSO on cord blood to determine exposure time limits and the optimum concentration for cryopreservation. Vox Sang 109:181–190. https://doi.org/10.1111/vox.12267

Fujimi A, Matsunaga T, Kobune M, Kawano Y, Nagaya T, Tanaka I, Iyama S, Hayashi T, Sato T, Miyanishi K, Sagawa T, Sato Y, Takimoto R, Takayama T, Kato J, Gasa S, Sakai H, Tsuchida E, Ikebuchi K, Hamada H, Niitsu Y (2008) Ex vivo large-scale generation of human red blood cells from cord blood CD34+ cells by co-culturing with macrophages. Int J Hematol 87:339–350. https://doi.org/10.1007/s12185-008-0062-y

Fuller BJ, Petrenko AY, Rodriguez JV, Somov AY, Balaban CL, Guibert EE (2013) Biopreservation of hepatocytes: current concepts on hypothermic preservation, cryopreservation, and vitrification. Cryo Letters 34:432–452

Galkina E, Tanousis K, Preece G, Tolaini M, Kioussis D, Florey O, Haskard DO, Tedder TF, Ager A (2003) L-selectin shedding does not regulate constitutive T cell trafficking but controls the

migration pathways of antigen-activated T lymphocytes. J Exp Med 198(9):1323–1335. https://doi.org/10.1084/jem.20030485

Gálvez P, Clares B, Bermejo M, Hmadcha A, Soria B (2014) Standard requirement of a microbiological quality control program for the manufacture of human mesenchymal stem cells for clinical use. Stem Cells Dev 23:1074–1083. https://doi.org/10.1089/scd.2013.0625

Gao L, Nath SC, Jiao X, Zhou R, Nishikawa S, Krawetz R, Li X, Rancourt DE (2019) Post-passage rock inhibition induces cytoskeletal aberrations and apoptosis in human embryonic stem cells. Stem Cell Res 41:101641. https://doi.org/10.1016/j.scr.2019.101641

Garfall AL, Maus MV, Hwang W-T, Lacey SF, Mahnke YD, Melenhorst JJ, Weiss BM (2015) Chimeric antigen receptor T cells against CD19 for multiple myeloma. N Engl J Med 373(11): 1040–1047. https://doi.org/10.1056/NEJMoa1504542

Garritsen H, Gabrysiak T, Sputtek A, Biermann B, Woermann B (2009) Successful mobilisation and transplantation of hematopoietic stem cells (HSC) using Plerixafor plus G-CSF steady state mobilisation in a patient who previously failed twice to mobilise hematopoietic stem cells using G-CSF plus chemotherapy. Tissue Antigens 73:440–440

Gea-Banacloche J (2017) Granulocyte transfusions: a concise review for practitioners. Cytotherapy 19:1256–1269. https://doi.org/10.1016/j.jcyt.2017.08.012

Germain M, Grégoire Y, Vassallo RR, Acker JP, Cardigan R, de Korte D, Irving DO, Bégué S (2017) Quality control of apheresis platelets: a multicentre study to evaluate factors that can influence pH measurement. Vox Sang 112(4):318–325. https://doi.org/10.1111/vox.12505

Gianni AM, Siena S, Bregni M, Tarella C, Stern AC, Pileri A, Bonadonna G (1989) Granulocyte-macrophage colony-stimulating factor to harvest circulating haemopoietic stem cells for auto-transplantation. Lancet 2:580–585. https://doi.org/10.1016/s0140-6736(89)90711-3

Giarratana MC, Rouard H, Dumont A, Kiger L, Safeukui I, Le Pennec PY, François S, Trugnan G, Peyrard T, Marie T, Jolly S, Hebert N, Mazurier C, Mario N, Harmand L, Lapillonne H, Devaux JY, Douay L (2011) Proof of principle for transfusion of in vitro-generated red blood cells. Blood 118:5071–5079. https://doi.org/10.1182/blood-2011-06-362038

Ginis I, Grinblat B, Shirvan MH (2012) Evaluation of bone marrow-derived mesenchymal stem cells after cryopreservation and hypothermic storage in clinically safe medium. Tissue Eng Part C Methods 18:453–463. https://doi.org/10.1089/ten.TEC.2011.0395

Gluckman E, Broxmeyer HA, Auerbach AD, Friedman HS, Douglas GW, Devergie A, Esperou H, Thierry D, Socie G, Lehn P, Cooper S, English D, Kurtzberg J, Bard J, Boyse EA (1989) Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical-cord blood from an HLA-identical sibling. N Engl J Med 321:1174–1178. https://doi.org/10.1056/NEJM198910263211707

Goecke T, Theodoridis K, Tudorache I, Ciubotaru A, Cebutari S, Ramm R, Hoeffler K, Sarikouch S, Vásquez Rivera A, Haverich A, Wolkers WF, Hilfiker A (2018) In vivo performance of freeze-dried decellularized pulmonary heart valve allo- and xenografts orthotopically implanted into juvenile sheep. Acta Biomater 68:41–52. https://doi.org/10.1016/j.actbio.2017.11.041

Golab K, Leveson-Gower D, Wang XJ, Grzanka J, Marek-Trzonkowska N, Krzystyniak A, Millis JM, Trzonkowski P, Witkowski P (2013) Challenges in cryopreservation of regulatory T cells (Tregs) for clinical therapeutic applications. Int Immunopharmacol 16(3):371–375. https://doi.org/10.1016/j.intimp.2013.02.001

Gołąb K, Grose R, Placencia V, Wickrema A, Solomina J, Tibudan M, Konsur E, Ciepły K, Marek-Trzonkowska N, Trzonkowski P, Millis JM, Fung J, Witkowski P (2018) Cell banking for regulatory T cell-based therapy: strategies to overcome the impact of cryopreservation on the Treg viability and phenotype. Oncotarget 9(11):9728–9740. https://doi.org/10.18632/oncotarget.23887

Goltsev A, Dubrava T, Yampolska K, Lutsenko O, Gaevska Y, Babenko N, Bondarovych M (2019) The substantiation of adoptive transfer of tolerogenic dendritic cells for treatment of rheumatoid arthritis in mice. Cell Organ Transplantol 7(2):125–131. https://doi.org/10.22494/cot.v7i2.99

Grant L, Raman R, Cvetkovic C, Ferrall-Fairbanks MC, Pagan-Diaz GJ, Hadley P, Ko E, Platt MO, Bashir R (2019) Long-term cryopreservation and revival of tissue-engineered skeletal muscle. Tissue Eng Part A 25:1023–1036. https://doi.org/10.1089/ten.tea.2018.0202

Grigor EJM, Fergusson D, Kekre N, Montroy J, Atkins H, Seftel MD, Daugaard M, Presseau J, Thavorn K, Hutton B, Holt RA, Lalu MM (2019) Risks and benefits of chimeric antigen receptor T-cell (CAR-T) therapy in cancer: a systematic review and meta-analysis. Transfus Med Rev 33(2):98–110. https://doi.org/10.1016/j.tmrv.2019.01.005

Grout BW, Morris G (2009) Contaminated liquid nitrogen vapour as a risk factor in pathogen transfer. Theriogenology 71(7):1079–1082. https://doi.org/10.1016/j.theriogenology.2008.12.011

Gryshkov O, Hofmann N, Lauterboeck L, Pogozhykh D, Mueller T, Glasmacher B (2015) Multipotent stromal cells derived from common marmoset *Callithrix jacchus* within alginate 3D environment: effect of cryopreservation procedures. Cryobiology 71:103–111. https://doi.org/10.1016/j.cryobiol.2015.05.001

Guibert EE, Petrenko AY, Balaban CL, Somov AY, Rodriguez JV, Fuller BJ (2011) Organ preservation: current concepts and new strategies for the next decade. Transfus Med Hemother 38:125–142. https://doi.org/10.1159/000327033

Gulliksson H, Nordahl-Källman AS (2014) Effect of transient warming of red blood cells for up to 24 h: in vitro characteristics in CPD/saline-adenine-glucose-mannitol environment. Vox Sang 106:61–67. https://doi.org/10.1111/vox.12079

Guo H, Zhang H, Lu L, Ezzelarab MB, Thomson AW (2015) Generation, cryopreservation, function and in vivo persistence of ex vivo expanded cynomolgus monkey regulatory T cells. Cell Immunol 295(1):19–28. https://doi.org/10.1016/j.cellimm.2015.02.006

Han S-H, Shim S, Kin M-J, Shin H-Y, Jang W-S, Lee S-J, Jin Y-W, Lee S-S, Lee SB, Park S (2017) Long-term culture-induced phenotypic differences and efficient cryopreservation of small intestinal organoids by treatment with Rho kinase inhibitor. World J Gastroenterol 23:964–975. https://doi.org/10.3748/wjg.v23.i6.964

Hattori Y, Kato H, Nitta M, Takamoto S (2001) Decrease of L-selectin expression on human CD34+ cells on freeze-thawing and rapid recovery with short-term incubation. Exp Hematol 29(1):114–122. https://doi.org/10.1016/s0301-472x(00)00615-9

Heng BC (2009) Effect of Rho-associated kinase (ROCK) inhibitor Y-27632 on the viability of cryopreserved human bone marrow-derived mesenchymal stem cells. Tissue Cell 41:376–380. https://doi.org/10.1016/j.tice.2009.01.004

Heo YJ, Son CH, Chung JS, Park YS, Son JH (2009) The cryopreservation of high concentrated PBMC for dendritic cell (DC)-based cancer immunotherapy. Cryobiology 58(2):203–209. https://doi.org/10.1016/j.cryobiol.2008.12.006

Heshusius S, Heideveld E, Burger P, Thiel-Valkhof M, Sellink E, Varga E, Ovchynnikova E, Visser A, Martens JHA, von Lindern M, van den Akker E (2019) Large-scale in vitro production of red blood cells from human peripheral blood mononuclear cells. Blood Adv 3:3337–3350. https://doi.org/10.1182/bloodadvances.2019000689

Hess JR, Biomedical Excellence for Safer Transfusion (BEST) Collaborative (2012) Scientific problems in the regulation of red blood cell products. Transfusion 52:1827–1835. https://doi.org/10.1111/j.1537-2995.2011.03511.x

Hess JR, Thomas MJG (2003) Blood use in war and disaster: lessons from the past century. Transfusion 43:1622–1633. https://doi.org/10.1046/j.1537-2995.2003.00576.x

Horacek JM, Jebavy L, Jakl M, Zak P, Mericka P, Maly J (2009) Cardiovascular changes associated with infusion of hematopoietic cell grafts in oncohematological patients – impact of cryopreservation with dimethylsulfoxide. Exp Oncol 31:121–122

Huang H, Zhao G, Zhang Y, Xu J, Toth TL, He X (2017) Predehydration and ice seeding in the presence of trehalose enabled cell cryopreservation. ACS Biomater Sci Eng 3:1758–1768. https://doi.org/10.1021/acsbiomaterials.7b00201

Huang H, Rey-Bedón C, Yarmush ML, Berk UO (2020) Deep-supercooling for extended preservation of adipose-derived stem cells. Cryobiology 92:67–75. https://doi.org/10.1016/j.cryobiol.2019.11.004

Hughes SM, Ferre AL, Yandura SE, Shetler C, Baker CAR, Calienes F, Levy CN, Astronomo RD, Shu Z, Lentz GM, Fialkow M, Kirby AC, McElrath MJ, Sinclair E, Rohan LC, Anderson PL, Shacklett BL, Dezzutti CS, Gao D, Hladik F (2018) Cryopreservation of human mucosal tissues. PLoS One 13:e0200653. https://doi.org/10.1371/journal.pone.0200653

Hunt CJ (2011) Cryopreservation of human stem cells for clinical application: a review. Transfus Med Hemother 38(2):107–123. https://doi.org/10.1159/000326623

Hunt CJ (2017) Cryopreservation: vitrification and controlled rate cooling. Methods Mol Biol 1590: 41–77. https://doi.org/10.1007/978-1-4939-6921-0_5

Hunt CJ (2019) Technical considerations in the freezing, low-temperature storage and thawing of stem cells for cellular therapies. Transfus Med Hemother 46(3):134–149. https://doi.org/10.1159/000497289

Hunt CJ, Pegg DE (1996) Improved temperature stability in gas phase nitrogen refrigerators: use of a copper heat shunt. Cryobiology 33:544–551. https://doi.org/10.1006/cryo.1996.0058

Hunt CJ, Timmons PM (2007) Cryopreservation of human embryonic stem cell lines. Methods Mol Biol 368:261–270. https://doi.org/10.1007/978-1-59745-362-2_18

Ikematsu W, Teshima T, Kondo S, Makino S, Okamura S, Inaba S, Harada M, Niho Y (1992) Circulating CD34+ hematopoietic progenitors in the harvesting peripheral blood stem cells; enhancement by recombinant human granulocyte colony-stimulating factor. Biotherapy 5: 131–136. https://doi.org/10.1007/BF02171698

Imaizumi K, Nishishta N, Muramatsu M, Yamamoto T, Takenaka C, Kawamata S, Kobayashi K, Nishikawa S, Akuta T (2014) A simple and highly effective method for slow-freezing human pluripotent stem cells using dimethyl sulfoxide, hydroxyethyl starch and ethylene glycol. PLoS One 9(2):e88696. https://doi.org/10.1371/journal.pone.0088696

International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) (1995) ICH harmonised tripartite guideline. Quality of biotechnological products: stability testing of biotechnological/biological products Q5C. ICH. https://database.ich.org/sites/default/files/Q5C%20Guideline.pdf

International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) (2000) Good manufacturing practice guide for active pharmaceutical ingredients Q7. ICH. https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q7/Step4/Q7_Guideline.pdf

International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) (2004) ICH harmonised tripartite guideline. Comparability of biotechnological/biological products subject to changes in their manufacturing process Q5E. ICH. https://database.ich.org/sites/default/files/Q5E%20Guideline.pdf

International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) (2005) ICH harmonised tripartite guideline. Quality risk management Q9. ICH. https://database.ich.org/sites/default/files/Q9%20Guideline.pdf

International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) (2009) ICH harmonized tripartite guideline. Pharmaceutical development Q8 (R2). ICH. https://database.ich.org/sites/default/files/Q8_R2_Guideline.pdf

International Organization for Standardization (ISO) (2020) Biotechnology – general requirements for transportation of cells for therapeutic use (ISO standard no. 21973:2020, part 6). https://www.iso.org/standard/72326.html

Isachenko V, Lapidus I, Isachenko E, Krivokharchenko A, Kreienberg R, Woriedh M, Bader M (2009) Human ovarian tissue vitrification versus conventional freezing: morphological, endocrinological, and molecular biological evaluation. Reproduction 138:319–327. https://doi.org/10.1530/rep-09-0039

Iwatani M, Ikegami K, Kremenska Y, Hattori N, Tanaka S, Yagi S, Shiota K (2006) Dimethyl sulfoxide has an impact on epigenetic profile in mouse embryoid bodies. Stem Cells 24(11): 2549–2556. https://doi.org/10.1634/stemcells.2005-0427

Iyer RK, Bowles PA, Kim H, Dulgar-Tulloch A (2018) A industrializing autologous adoptive immunotherapies: manufacturing advances and challenges. Front Med 5:150. https://doi.org/10.3389/fmed.2018.00150

Jandová M, Šponer P, Vokurková D, Bauer PO, Filipová A, Filip S, Měřička P (2020) New cryopreservation technology of hMSCs: first preclinical results using DMSO-containing medium. Cryo Letters 41:50–56

Jesson HE, Robinson NJ, Medcalf N, Coopman K (2016) Chapter 11. Storage and delivery of stem cells for cellular therapies. In: Cabral JMS, Lobato de Silva C, Chase LG, Diogo MM (eds) Stem cell manufacturing. Elsevier, Amsterdam, pp 233–264. https://doi.org/10.1016/B978-0-444-63265-4.00011-X

Jiang B, Yan L, Miao Z, Li E, Wong KH, Xu R-E (2017) Spheroidal formation preserves human stem cells for prolonged time under ambient conditions for facile storage and transportation. Biomaterials 133:275–286. https://doi.org/10.1016/j.biomaterials.2017.03.050

Jomha NM, Elliott JA, Law GK, Maghdoori B, Forbes JF, Abazari A, Adesida AB, Laouar L, Zhou X, McGann LE (2012) Vitrification of intact human articular cartilage. Biomaterials 33: 6061–6068. https://doi.org/10.1016/j.biomaterials.2012.05.007

Jones SA, Jones JM, Leung V, Nakashima AK, Oakeson KF, Smith AR, Hunter R, Kim JJ, Cumming M, McHale E, Young PP, Fridey JL, Kelley WE, Stramer SL, Wagner SJ, West FB, Herron R, Snyder E, Hendrickson JE, Peaper DR, Gundlapalli AV, Langelier C, Miller S, Nambiar A, Moayeri M, Kamm J, Moulton-Meissner H, Annambhotla P, Gable P, McAllister GA, Breaker E, Sula E, Halpin AL, Basavaraju SV (2019) Sepsis attributed to bacterial contamination of platelets associated with a potential common source – multiple states, 2018. MMWR Morb Mortal Wkly Rep 68:519–523. https://doi.org/10.15585/mmwr.mm6823a2

Kaindl J, Meiser I, Majer J, Sommer A, Krach F, Katsen-Globa A, Winkler J, Zimmermann H, Neubauer JC, Winner B (2019) Zooming in on cryopreservation of hiPSCs and neural derivatives: a dual-center study using adherent vitrification. Stem Cells Transl Med 8(3):247–259. https://doi.org/10.1002/sctm.18-0121

Kashuba CM, Benson JD, Critser JK (2014) Rationally optimized cryopreservation of multiple mouse embryonic stem cell lines 1 – comparative fundamental cryobiology of multiple mouse embryonic stem cell lines and the implications for embryonic stem cell cryopreservation protocols. Cryobiology 68(2):166–175. https://doi.org/10.1016/j.cryobiol.2013.12.007

Katkov II, Kim MS, Bajpai R, Altman YS, Mercola M, Loring JF, Terskikh AV, Snyder EY, Levine F (2006) Cryopreservation by slow cooling with DMSO diminished production of Oct-4 pluripotency marker in human embryonic stem cells. Cryobiology 53:194–205. https://doi.org/10.1016/j.cryobiol.2006.05.005

Katkov II, Kan NG, Cimadamore F, Nelson B, Snyder EY, Terskikh AV (2011) DMSO-free programmed cryopreservation of fully dissociated and adherent human induced pluripotent stem cells. Stem Cells Int 2011:981606. https://doi.org/10.4061/2011/981606

Kebriaei P, Singh H, Huls MH, Figliola MJ, Bassett R, Olivares S, Jena B, Dawson MJ, Kumaresan PR, Su S, Maiti S, Dai J, Moriarity B, Forget MA, Senyukov V, Orozco A, Liu T, McCarty J, Jackson RN, Moyes JS, Rondon G, Qazilbash M, Ciurea S, Alousi A, Nieto Y, Rezvani K, Marin D, Popat U, Hosing C, Shpall EJ, Kantarjian H, Keating M, Wierda W, Do KA, Largaespada DA, Lee DA, Hackett PB, Champlin RE, Cooper LJ (2016) Phase I trials using Sleeping Beauty to generate CD19-specific CAR T cells. J Clin Invest 126(9):3363–3376. https://doi.org/10.1172/jci86721

Kenyon NS, Xu XM, Garcia-Serra A, Ricordi C (1995) Effect of depletion of class II bright cells on the immunogenicity and stem cell content of human vertebral body bone marrow. Transplant Proc 27(6):3419

Kessinger A, Armitage JO, Smith DM, Landmark JD, Bierman PJ, Weisenburger DD (1989) High-dose therapy and autologous peripheral blood stem cell transplantation for patients with lymphoma. Blood 74:1260–1265

Keung YK, Cobos E, Morgan D, Park M, Dixon S, Wu K, Park CH (1996) High cellular concentration of peripheral blood progenitor cells during cryopreservation adversely affects CFU-GM but not hematopoietic recovery. J Hematother 5:73–77. https://doi.org/10.1089/scd.1.1996.5.73

Khetan S, Corey O (2019) Maintenance of stem cell viability and differentiation potential following cryopreservation within 3-dimensional hyaluronic acid hydrogels. Cryobiology 90:83–88. https://doi.org/10.1016/j.cryobiol.2019.08.001

Kim J, Koo B-K, Knoblich JA (2020) Human organoids: model systems for human biology and medicine. Nat Rev Mol Cell Biol 7:1–14. https://doi.org/10.1038/s41580-020-0259-3

Kindwall-Keller TL, Ballen KK (2020) Umbilical cord blood: the promise and the uncertainty. Stem Cells Transl Med 9:1153–1162. https://doi.org/10.1002/sctm.19-0288

Knight SC, Farrant J, Morris GJ (1972) Separation of populations of human lymphocytes by freezing and thawing. Nat New Biol 239(90):88–89. https://doi.org/10.1038/newbio239088a0

Ko DW, Yoon JK, Ahn JI, Lee M, Yang WS, Ahn JY, Lim JM (2018) The importance of post-thaw subculture for standardizing cellular activity of fresh or cryopreserved mouse embryonic stem cells. Asian Australas J Anim Sci 31(3):335–343. https://doi.org/10.5713/ajas.17.0294

Kobold S, Guhr A, Mah N, Bultjer N, Seltmann S, Seiler Wulczyn AEM, Stacey G, Jie H, Liu W, Löser P, Kurtz A (2020) A manually curated database on clinical studies involving cell products derived from human pluripotent stem cells. Stem Cell Rep 15(2):546–555. https://doi.org/10.1016/j.stemcr.2020.06.014

Kodama A, Tanaka R, Saito M, Ansari AA, Tanaka Y (2013) A novel and simple method for generation of human dendritic cells from unfractionated peripheral blood mononuclear cells within 2 days: its application for induction of HIV-1-reactive CD4+ T cells in the hu-PBL SCID mice. Front Microbiol 4:292. https://doi.org/10.3389/fmicb.2013.00292

Kögler G, Critser P, Trapp T, Yoder M (2009) Future of cord blood for non-oncology uses. Bone Marrow Transplant 44:683–697. https://doi.org/10.1038/bmt.2009.287

Kojayan GG, Alexander M, Imagawa DK, Lakey JRT (2018) Systematic review of islet cryopreservation. Islets 10:40–49. https://doi.org/10.1080/19382014.2017.1405202

Körbling M, Fliedner TM, Pflieger H (1980) Collection of large quantities of granulocyte/macrophage progenitor cells (CFUc) in man by means of continuous-flow leukapheresis. Scand J Haematol 24:22–28. https://doi.org/10.1111/j.1600-0609.1980.tb01313.x

Korniski B, Darr TB, Hubel A (1999) Sub-zero osmotic characteristics of intact and disaggregated hepatocyte spheroids. Cryobiology 38:339–352

Koval GK, Lutsenko OD, Grisha IG, Sokil LV, Bondarovych MO, Ostankov MV, Yampolskaya KY, Ostankova LV, Goltsev AM (2019) Impact of lyophilisation on integrity of structural and functional characteristics of human cord blood leukoconcentrate. Probl Cryobiol Cryomed 29(4):332–343. https://doi.org/10.15407/cryo29.04.332

Kreher CR, Dittrich MT, Guerkov R, Boehm BO, Tary-Lehmann M (2003) CD4+ and CD8+ cells in cryopreserved human PBMC maintain full functionality in cytokine ELISPOT assays. J Immunol Methods 278(1–2):79–93. https://doi.org/10.1016/s0022-1759(03)00226-6

Kuleshova LL, Tan FCK, Magalhães R, Gouk SS, Lee KH, Dawe GS (2009) Effective cryopreservation of neural stem or progenitor cells without serum or proteins by vitrification. Cell Transplant 18:135–144

Kunova M, Matulka K, Eiselleova L, Salykin A, Kubikova I, Kyrylenko S, Hampl A, Dvorak P (2013) Adaptation to robust monolayer expansion produces human pluripotent stem cells with improved viability. Stem Cells Transl Med 2(4):246–254. https://doi.org/10.5966/sctm.2012-0081

Kura T, Akahane M, Shimizu T, Uchihara Y, Tohma Y, Morita Y, Koizumi M, Kawate K, Tanaka Y (2016) Use of cryopreserved osteogenic matrix cell sheets for bone reconstruction. Stem Cell Discov 6:13–23. https://doi.org/10.4236/scd.2016.61002

Kurosawa H (2012) Application of Rho-associated protein kinase (ROCK) inhibitor to human pluripotent stem cells. J Biosci Bioeng 114:577–581. https://doi.org/10.1016/j.jbiosc.2012.07.013

Lancaster MA, Knoblich JA (2014) Organogenesis in a dish: modelling development and disease using organoid technologies. Science 345:1247125. https://doi.org/10.1126/science.1247125

Lee JY, Lee JE, Kim DK, Yoon TK, Chung HM, Lee DR (2010) High concentration of synthetic serum, stepwise equilibration and slow cooling as an efficient technique for large-scale cryopreservation of human embryonic stem cells. Fertil Steril 93:976–985. https://doi.org/10.1016/j.fertnstert.2008.10.017

Levine BL, Miskin J, Wonnacott K, Keir C (2017) Global manufacturing of CAR T cell therapy. Mol Ther Methods Clin Dev 4:92–101. https://doi.org/10.1016/j.omtm.2016.12.006

Li T, Ma T (2012) Bioprocessing of cryopreservation for large-scale banking of human pluripotent stem cells. Biores Open Access 1:205–214. https://doi.org/10.1089/biores.2012.0224

Li X, Meng G, Krawetz R, Liu S, Rancourt DE (2008) The ROCK inhibitor Y-27632 enhances the survival rate of human embryonic stem cells following cryopreservation. Stem Cells Dev 17:1079–1086. https://doi.org/10.1089/scd.2007.0247

Li T, Mai Q, Gao J, Zhou C (2010a) Cryopreservation of human embryonic stem cells with a new bulk vitrification method. Biol Reprod 82:848–853. https://doi.org/10.1095/biolreprod.109.080713

Li Y, Tan J, Li L (2010b) Comparison of three methods for cryopreservation of human embryonic stem cells. Fertil Steril 93:999–1005. https://doi.org/10.1016/j.fertnstert.2008.10.052

Li XY, Liang ZH, Han C, Wei WJ, Song CL, Zhou LN, Liu Y, Li Y, Ji XF, Liu J (2017) Transplantation of autologous peripheral blood mononuclear cells in the subarachnoid space for amyotrophic lateral sclerosis: a safety analysis of 14 patients. Neural Regen Res 12(3):493–498. https://doi.org/10.4103/1673-5374.202918

Li R, Yu G, Azarin SM, Hubel A (2018) Freezing responses in DMSO-based cryopreservation of human iPS cells: aggregates versus single cells. Tissue Eng Part C Methods 24:289–299. https://doi.org/10.1089/ten.tec.2017.0531

Li R, Johnson R, Yu G, McKenna DH, Hubel A (2019) Preservation of cell-based immunotherapies for clinical trials. Cytotherapy 21(9):943–957. https://doi.org/10.1016/j.jcyt.2019.07.004

Li R, Hornberger K, Dutton JR, Hubel A (2020a) Cryopreservation of human iPS cell aggregates in a DMSO-free solution – an optimization and comparative study. Front Bioeng Biotechnol 8:1. https://doi.org/10.3389/fbioe.2020.00001

Lioznov M, Dellbrügger C, Sputtek A, Fehse B, Kröger N, Zander AR (2008) Transportation and cryopreservation may impair haematopoietic stem cell function and engraftment of allogeneic PBSCs, but not BM. Bone Marrow Transplant 42:121–128. https://doi.org/10.1038/bmt.2008.93

Liseth K, Abrahamsen JF, Bjørsvik S, Grøttebø K, Bruserud Ø (2005) The viability of cryopreserved PBPC depends on the DMSO concentration and the concentration of nucleated cells in the graft. Cytotherapy 7:328–333. https://doi.org/10.1080/14653240500238251

Liu Y, Xu X, Ma XH, Liu J, Cui ZF (2011) Effect of various freezing solutions on cryopreservation of mesenchymal stem cells from different animal species. Cryo Letters 32:425–435

Liu X, Zhao G, Chen Z, Panhwar F, He X (2018) Dual suppression effect of magnetic induction heating and microencapsulation of ice crystallisation enables low-cryoprotectant vitrification of stem cell-alginate hydrogel constructs. ACS Appl Mater Interfaces 10:16822–16835. https://doi.org/10.1021/acsami.8b04496

Liu W, Deng C, Godoy-Parejo C, Zhang Y, Chen G (2019) Developments in cell culture systems for human pluripotent stem cells. World J Stem Cells 11:968–981. https://doi.org/10.4252/wjsc.v11.i11.968

Lu TL, Pugach O, Somerville R, Rosenberg SA, Kochenderfer JN, Better M, Feldman SA (2016) A rapid cell expansion process for production of engineered autologous CAR-T cell therapies. Hum Gene Ther Methods 27(6):209–218. https://doi.org/10.1089/hgtb.2016.120

Lu Y-C, Fu D-J, An D, Chiu A, Schwartz R, Nikitin AY, Ma M (2017) Scalable production and cryostorage of organoids using core-shell decoupled hydrogel capsules. Adv Biosyst 1: 1700165. https://doi.org/10.1002/adbi.201700165

Lunyak VV, Amaro-Ortiz A, Gaur M (2017) Mesenchymal stem cells secretory responses: senescence messaging secretome and immunomodulation perspective. Front Genet 8:220. https://doi.org/10.3389/fgene.2017.00220

Ma X-E, Shi Y, Hou Y, Liu Y, Zhang L, Fan W-X, Ge D, Liu T-Q, Cui Z-F (2010) Slow-freezing cryopreservation of neural stem cell spheres with different diameters. Cryobiology 60:184–191. https://doi.org/10.1016/j.cryobiol.2009.10.013

Ma S, Li X, Wang X, Cheng L, Li Z, Zhang C, Ye Z, Qian Q (2019) Current progress in CAR-T cell therapy for solid tumors. Int J Biol Sci 15(12):2548–2560. https://doi.org/10.7150/ijbs.34213

Maehara M, Sato M, Watanabe M, Matsunari H, Kokubo M, Kanai T, Sato M, Matsumura K, Hyon SH, Yokoyama M, Mochida J, Nagashima H (2013) Development of a novel vitrification method for chondrocyte sheets. BMC Biotechnol 13:58. https://doi.org/10.1186/1472-6750-13-58

Makino S, Harada M, Akashi K, Taniguchi S, Shibuya T, Inaba S, Niho Y (1991) A simplified method for cryopreservation of peripheral blood stem cells at −80 degrees C without rate-controlled freezing. Bone Marrow Transplant 8:239–244

Malinin TI, Pegg DE, Perry VP, Brodine CE (1970) Long-term storage of bone marrow cells at liquid nitrogen and dry ice temperatures. Cryobiology 7:65–69. https://doi.org/10.1016/0011-2240(70)90049-0

Marquez-Curtis LA, Janowska-Wieczorek A, McGann LE, Elliott JA (2015) Mesenchymal stromal cells derived from various tissues: biological, clinical and cryopreservation aspects. Cryobiology 71:181–197. https://doi.org/10.1016/j.cryobiol.2015.07.003

Martin-Ibañez R, Unger C, Stromberg A, Baker D, Canals JM, Hovatta O (2008) Novel cryopreservation method for dissociated human embryonic stem cells in the presence of a ROCK inhibitor. Hum Reprod 23:2744–2754. https://doi.org/10.1093/humrep/den316

Massie I, Selden C, Hodgson H, Fuller B, Gibbons S, Morris GJ (2014) GMP cryopreservation of large volumes of cells for regenerative medicine: active control of the freezing process. Tissue Eng Part C Methods 20:693–702. https://doi.org/10.1089/ten.tec.2013.0571

Matsumoto N, Yoshizawa H, Kagamu H, Abe T, Fujita N, Watanabe S, Kuriyama H, Ishiguro T, Tanaka J, Suzuki E, Kobayashi K, Gemma A, Kudoh S, Gejyo F (2002) Successful liquid storage of peripheral blood stem cells at subzero non-freezing temperature. Bone Marrow Transplant 30(11):777–784. https://doi.org/10.1038/sj.bmt.1703692

Matsumura K, Bae JY, Kim HH, Hyon SH (2011) Effective vitrification of human induced pluripotent stem cells using carboxylated ε-poly-l-lysine. Cryobiology 63:76–83. https://doi.org/10.1016/j.cryobiol.2011.05.003

Matsumura K, Hayashi F, Nagashima T, Hyon SH (2013) Long-term cryopreservation of human mesenchymal stem cells using carboxylated poly-l-lysine without the addition of proteins or dimethyl sulfoxide. J Biomater Sci Polym Ed 24:1484–1497. https://doi.org/10.1080/09205063.2013.771318

Maude SL, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, Myers GD (2018) Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. N Engl J Med 378(5):439–448. https://doi.org/10.1056/NEJMoa1709866

Meng Y, Ren Z, Xu F, Zhou X, Song C, Wang VY-F, Lui W, Lu L, Thompson JA, Chen G (2018) Nicotinamide promotes cell survival and differentiation as kinase inhibitor in human pluripotent cells. Stem Cell Rep 11:1347–1356. https://doi.org/10.1016/j.stemcr.2018.10.023

Měricka P, Schustr P, Vins M, Dudek A, Vávra L, Cervinka M, Rondiak J (1991) Containers for freezing and storage of bone marrow stem cells. Sb Ved Pr Lek Fak Karlovy Univerzity Hradci Kralove 34:367–387

Měřička P, Straková H, Filip S, Bláha M, Vávra L, Ondráček J, Ettlerová E, Rondiak J, Jílková B, Pecka M (1995) Cryopreservation of peripheral blood stem cells. In: Proceedings of the

International Congress of Refrigeration, Hague, Netherlands, 20–25 August 1995, vol 1. International Institute of Refrigeration, Paris, pp 60–67

Měřička P, Vávra L, Straková H, Štěpánová V, Drahošová M (2000). Contribution to safe keeping of cells and tissues at −80°C. In: 6th international cryogenics conference of the International Institute of Refrigeration, Prague, 10–13 October 2000, p 217

Měřička P, Straková H, Lánská M, Vokurková D, Pecka M, Bláha M, Žák P (2010) Application of the new European Union legislation leads to an increased liquid nitrogen consumption in a haematopoietic progenitor cell bank. In: Cryogenics 2010. Proceedings of the 11th IIR international conference, Bratislava, 26–29 April 2010, vol 3. Refrigeration Science and Technology, pp 142–144

Měřička P, Straková H, Lánská M, Vokurková D, Pecka M, Bláha M, Žák P, Jebavý L (2011) Testing of cryopreserved concentrates at infusion leads to standardization of engraftment in autologous blood progenitor cell transplantation. In: 23th IIR international congress of refrigeration Prague, 20–26 August 2011, vol 23. International Institute of Refrigeration, pp 3609–3613

Měřička P, Straková H, Lánská M, Belada D, Žák P, Štěrba L, Brandejs D, Honegrová B (2017) How long do we need to store cryopreserved cells and tissues. In: Cryogenics 2017. Proceedings of the 14th IIR international conference, Dresden, 15–19 May, 2017. https://doi.org/10.18462/iir.cryo.2017.0005

Měřička P, Strakova H, Honegrova B, Lanska M, Vokurkova D, Maisnar V, Brandejs D, Jandova M, Gregor J, Zak P, Sterba L (2019a) Retrospective analysis of dimethylsulphoxide load in autologous peripheral progenitor cell transplantation in multiple myeloma. Cryo Letters 40: 264–265

Měřička P, Straková H, Honegrová B, Lánská M, Vokurková D, Brandejs D, Jandová M, Gregor J, Šponer P, Filip S, Štěrba L (2019b) Own experience from the 22 years lasting process of harmonization of the European Union safety and quality requirements for harvesting, processing and distribution of human cells and tissues for clinical transplantation. In: Cryogenics 2019. Proceedings of the 15th IIR international conference, Dresden, 8–11 April 2019, pp 280–286

Měřička P, Gregor J, Jandová M, Lánská M, Vokurková D, Fátorová I, Paterová P, Radocha J, Štěrba L (2021a) Cryopreservation of allogeneic haematopoietic progenitor cells (HPC) concentrates before transplantation–temporary or perspective solution. Cryo Letters 42(6):372

Měřička P, Jandová M, Gregor J, Lánská M, Vokurková D, Fátorová I, Paterová P, Radocha J, Štěrba L (2021b) Cryopreservation of non-mobilized peripheral blood mononuclear cells (PBMC) current situation and future perspectives. Cryo Letters 42(6):376–377

Meryman HT, Hornblower M (1972) A method for freezing and washing red blood cells using a high glycerol concentration. Transfusion 12:145–156. https://doi.org/10.1111/j.1537-2995.1972.tb00001.x

Meyer EH, Laport G, Xie BJ, MacDonald K, Heydari K, Sahaf B, Tang SW, Baker J, Armstrong R, Tate K, Tadisco C, Arai S, Johnston L, Lowsky R, Muffly L, Rezvani AR, Shizuru J, Weng WK, Sheehan K, Miklos D, Negrin RS (2019) Transplantation of donor grafts with defined ratio of conventional and regulatory T cells in HLA-matched recipients. JCI Insight 4(10):e127244. https://doi.org/10.1172/jci.insight.127244

Milone G, Mercurio S, Strano A, Leotta S, Pinto V, Battiato K, Coppoletta S, Murgano P, Farsaci B, Privitera A, Giustolisi R (2007) Adverse events after infusions of cryopreserved hematopoietic stem cells depend on non-mononuclear cells in the infused suspension and patient age. Cytotherapy 9:348–355. https://doi.org/10.1080/14653240701326756

Milson TJ Jr, Keller R (1982) The variable effect of cryopreservation on peripheral blood mononuclear populations. J Clin Lab Immunol 7(3):205–213

Miranda CC, Fernandes TG, Diogo MM, Cabral JMS (2018) Towards multi-organoid systems for drug screening applications. Bioengineering 5:49. https://doi.org/10.3390/bioengineering5030049

Mollison PL, Engelfriet P (1999) Blood transfusion. Semin Hematol 36:48–58

Morgenstern DA, Ahsan G, Brocklesby M, Ings S, Balsa C, Veys P, Brock P, Anderson J, Amrolia P, Goulden N, Cale CM, Watts MJ (2016) Post-thaw viability of cryopreserved peripheral blood stem cells (PBSC) does not guarantee functional activity: important implications for quality assurance of stem cell transplant programmes. Br J Haematol 174(6):942–951. https://doi.org/10.1111/bjh.14160

Morris GJ, Acton E (2013) Controlled ice nucleation in cryopreservation – a review. Cryobiology 66(2):85–92. https://doi.org/10.1016/j.cryobiol.2012.11.007

Moss AC, Higgins AZ (2016) Investigating the potential for cryopreservation of human granulocytes with concentrated glycerol. Cryobiology 72:290–293. https://doi.org/10.1016/j.cryobiol.2016.05.004

Motoike S, Kajiya M, Komatsu N, Takewaki M, Horikoshi S, Matsuda S, Ouhara K, Iwata T, Takeda K, Fujita T, Kurihara H (2018) Cryopreserved clumps of mesenchymal stem cell/extracellular matrix complexes retain osteogenic capacity and induce bone regeneration. Stem Cell Res Ther 9:73. https://doi.org/10.1186/s13287-018-0826-0

Mullard A (2018) 2017 FDA drug approvals. Nat Rev Drug Discov 17:81–85. https://doi.org/10.1038/nrd.2018.4

Mutsenko V, Barlič A, Pezić T, Dermol-Černe J, Dovgan B, Sydykov B, Wolkers WF, Katkov II, Glasmacher B, Miklavčič D, Gryshkov O (2019) Me2SO- and serum-free cryopreservation of human umbilical cord mesenchymal stem cells using electroporation-assisted delivery of sugars. Cryobiology 91:104–114. https://doi.org/10.1016/j.cryobiol.2019.10.002

Mutsenko V, Knaack S, Lauterboeck L, Tarusin D, Sydykov B, Cabiscol R, Ivnev D, Belikan J, Beck A, Dipresa D, Lode A, El Khassawna T, Kampschulte M, Scharf R, Petrenko AY, Korossis S, Wolkers WF, Gelinsky M, Glasmacher B, Gryshkov O (2020) Effect of "in air" freezing on post-thaw recovery of *Callithrix jacchus* mesenchymal stromal cells and properties of 3D collagen-hydroxyapatite scaffolds. Cryobiology 92:215–230. https://doi.org/10.1016/j.cryobiol.2020.01.015

Naaldijk Y, Staude M, Fedorova V, Stolzing A (2012) Effect of different freezing rates during cryopreservation of rat mesenchymal stem cells using combinations of hydroxyethyl starch and dimethylsulfoxide. BMC Biotechnol 12:49. https://doi.org/10.1186/1472-6750-12-49

Nagao M, Sengupta J, Diaz-Dussan D, Adam M, Wu M, Acker J, Ben R, Ishihara K, Zeng H, Miura Y, Narain R (2018) Synthesis of highly biocompatible and temperature-responsive physical gels for cryopreservation and 3D cell culture. ACS Appl Bio Mater 1:356–366. https://doi.org/10.1021/acsabm.8b00096

Neubauer JC, Stracke F, Zimmermann H (2017) Sterile plate-based vitrification of adherent human pluripotent stem cells and their derivatives using the TWIST method. Methods Mol Biol 1568:231–241. https://doi.org/10.1007/978-1-4939-6828-2_17

Nishigaki T, Teramura Y, Nasu A, Takada K, Toguchida J, Iwata H (2011) Highly efficient cryopreservation of human induced pluripotent stem cells using a dimethyl sulfoxide-free solution. Int J Dev Biol 55:305–311. https://doi.org/10.1387/ijdb.103145tn

Noorman F, Van Dongen TTCF, Plat MCJ, Badloe JF, Hess JR, Hoencamp R (2016) Transfusion: −80°C frozen blood products are safe and effective in military casualty care. PLoS One 11(12):e0168401. https://doi.org/10.1371/journal.pone.0168401

Notman R, Noro M, O'Malley B, Anwar J (2006) Molecular basis for dimethylsulfoxide (DMSO) action on lipid membranes. J Am Chem Soc 128:13982–13983. https://doi.org/10.1021/ja063363t

Ohkawara H, Miyagawa S, Fukushima S, Yajima S, Saito A, Nagashima H, Sawa Y (2018) Development of a vitrification method for preserving human myoblast cell sheets for myocardial regeneration therapy. BMC Biotechnol 18:56. https://doi.org/10.1186/s12896-018-0467-5

Orellana MD, De Santis GC, Abraham KJ, Fontes AM, Magalhães DA, Oliveira Vde C, Costa Ede B, Palma PV, Covas DT (2015) Efficient recovery of undifferentiated human embryonic stem cell cryopreserved with hydroxyethyl starch, dimethyl sulphoxide and serum replacement. Cryobiology 71:151–160. https://doi.org/10.1016/j.cryobiol.2015.01.005

Pal R, Mamidi MK, Das AK, Bhonde R (2012) Diverse effects of dimethyl sulfoxide (DMSO) on the differentiation potential of human embryonic stem cells. Arch Toxicol 86:651–661. https://doi.org/10.1007/s00204-011-0782-2

Panch SR, Srivastava SK, Elavia N, McManus A, Liu S, Jin P, Highfill SL, Li X, Dagur P, Kochenderfer JN, Fry TJ, Mackall CL, Lee D, Shah NN, Stroncek DF (2019) Effect of cryopreservation on autologous chimeric antigen receptor T cell characteristics. Mol Ther 27(7):1275–1285. https://doi.org/10.1016/j.ymthe.2019.05.015

Pandy PR, Tomney A, Woon MT, Uth N, Shafighi F, Ngabo I, Vallabhaneni H, Levinson Y, Abraham E, Ben-Nun IF (2020) End-to end platform for human pluripotent stem cell manufacture. Int J Mol Sci 21:89. https://doi.org/10.3390/ijms21010089

Parekkadan B, Milwid JM (2010) Mesenchymal stem cells as therapeutics. Annu Rev Biomed Eng 12:87–117. https://doi.org/10.1146/annurev-bioeng-070909-105309

Park BW, Jang SJ, Byun JH, Kang YH, Choi MJ, Park WU, Lee WJ, Rho GJ (2017) Cryopreservation of human dental follicle tissue for use as a resource of autologous mesenchymal stem cells. J Tissue Eng Regen Med 11:489–500. https://doi.org/10.1002/term.1945

Passweg JR, Baldomero H, Bader P, Bonini C, Cesaro S, Dreger P, Duarte RF, Dufour C, Falkenburg JH, Farge-Bancel D, Gennery A, Kröger N, Lanza F, Nagler A, Sureda A, Mohty M, European Society for Blood and Marrow Transplantation (EBMT) (2015) Hematopoietic SCT in Europe 2013: recent trends in the use of alternative donors showing more haploidentical donors but fewer cord blood transplants. Bone Marrow Transplant 50:476–482. https://doi.org/10.1038/bmt.2014.312

Paviglianiti A, Xavier E, Ruggeri A, Ceballos P, Deconinck E, Cornelissen JJ, Nguyen-Quoc S, Maillard N, Sanz G, Rohrlich PS, Garderet L, Volt F, Rocha V, Kroeger N, Gluckman E, Fegueux N, Mohty M (2016) Outcomes of unrelated cord blood transplantation in patients with multiple myeloma: a survey on behalf of Eurocord, the Cord Blood Committee of Cellular Therapy and Immunobiology Working Party, and the Chronic Leukemia Working Party of the EBMT. Haematologica 101:1120–1127. https://doi.org/10.3324/haematol.2015.138917

Pegg DE (2020) The relevance of ice crystal formation for the cryopreservation of tissues and organs. Cryobiology 93:3–11. https://doi.org/10.1016/j.cryobiol.2020.01.005

Pegg DE, Trotman RE (1959) The preservation of human bone marrow at −79 degrees C. A temperature-controlled method of two-stage cooling. J Clin Pathol 12:477–482. https://doi.org/10.1136/jcp.12.5.477

Pennington N, Gu D, Labbe A, Sherry A (2019) Evaluation of a sterling cycle controlled rate freezing device for simultaneous cryopreservation of multiple units. Cryobiology 91:195. https://doi.org/10.1016/j.cryobiol.2019.10.186

Perotti CG, Del Fante C, Viarengo G, Papa P, Rocchi L, Bergamaschi P, Bellotti L, Marchesi A, Salvaneschi L (2004) A new automated cell washer device for thawed cord blood units. Transfusion 44:900–906. https://doi.org/10.1111/j.1537-2995.2004.03389.x

Pessoa GA, Rubin MIB, Silva CAM, da Rosa DC (2014) Decontamination of naturally contaminated liquid nitrogen storage tanks. Rev Bras Zootec 43(5):244–249. https://doi.org/10.1590/S1516-35982014000500004

Petrenko YA, Rogulska OY, Mutsenko VV, Petrenko AY (2014) A sugar pretreatment as a new approach to the Me2SO- and xeno-free cryopreservation of human mesenchymal stromal cells. Cryo Letters 35:239–246

Petrenko YA, Petrenko AY, Martin I, Wendt D (2017a) Perfusion bioreactor-based cryopreservation of 3D human mesenchymal stromal cell tissue grafts. Cryobiology 76:150–153. https://doi.org/10.1016/j.cryobiol.2017.04.001

Petrenko Y, Sykova E, Kubinova S (2017b) The therapeutic potential of three-dimensional multipotent mesenchymal stromal cell spheroids. Stem Cell Res Ther 8:94. https://doi.org/10.1186/s13287-017-0558-6

Petrenko Y, Chudickova M, Vackova I, Groh T, Kosnarova E, Cejkova J, Turnovcova K, Petrenko A, Sykova E, Kubinova S (2019) Clinically relevant solution for the hypothermic

storage and transportation of human multipotent mesenchymal stromal cells. Stem Cells Int 2019:1–11. https://doi.org/10.1155/2019/5909524

Petrenko Y, Vackova I, Kekulova K, Chudickova M, Koci Z, Turnovcova K, Kupcova Skalnikova H, Vodicka P, Kubinova S (2020) A comparative analysis of multipotent mesenchymal stromal cells derived from different sources, with a focus on neuroregenerative potential. Sci Rep 10:4290. https://doi.org/10.1038/s41598-020-61167-z

Petricciani J, Hayakawa T, Stacey G, Trouvin JH, Knezevic I (2017) Scientific considerations for the regulatory evaluation of cell therapy products. Biologicals 50:20–26. https://doi.org/10.1016/j.biologicals.2017.08.011

Playfair JH, Pegg DE (1962) An improved container for cells preserved by freezing. J Clin Pathol 15:591–592. https://doi.org/10.1136/jcp.15.6.591

Pogozhykh O, Prokopyuk V, Prokopyuk O, Kuleshova L, Goltsev A, Figueiredo C, Pogozhykh D (2018) Towards biobanking technologies for natural and bioengineered multicellular placental constructs. Biomaterials 185:39–50. https://doi.org/10.1016/j.biomaterials.2018.08.060

Pogozhykh O, Hofmann N, Gryshkov O, von Kaisenberg C, Mueller M, Glasmacher B, Pogozhykh D, Börgel M, Blasczyk R, Figueiredo C (2020) Repeated freezing procedures preserve structural and functional properties of amniotic membrane for application in ophthalmology. Int J Mol Sci 21:4029. https://doi.org/10.3390/ijms21114029

Pollock K, Budenske JW, McKenna DH, Dosa PI, Hubel A (2017) Algorithm-driven optimization of cryopreservation protocols for transfusion model cell types including Jurkat cells and mesenchymal stem cells. J Tissue Eng Regen Med 11:2806–2815. https://doi.org/10.1002/term.2175

Prince HE, Lee CD (1986) Cryopreservation and short-term storage of human lymphocytes for surface marker analysis. Comparison of three methods. J Immunol Methods 93(1):5–18. https://doi.org/10.1016/0022-1759(86)90427-8

Pudil R, Vojádek J, Filip S, Mericka P, St'ásek J, Straková H, Vokurková D, Solar M, Mokrý J, Dominik J, Malý J (2005) Transplantation of bone marrow derived progenitor cells in acute myocardial infarction. The first results. Acta Med (Hradec Kralove) 48:153–155

Radocha J, Maisnar V, Zavrelová A, Cermanová M, Lánská M, Kmonícek M, Jebavý L, Bláha M, Malý J, Zák P (2013) Fifteen years of single center experience with stem cell transplantation for multiple myeloma: a retrospective analysis. Acta Med (Hradec Kralove) 56:9–13. https://doi.org/10.14712/18059694.2014.31

Reade MC, Marks DC, Johnson L, Irving DO, Holley AD (2013) Frozen platelets for rural Australia: the CLIP trial. Anaesth Intensive Care 41:804–805

Reichman S, Slembrouck A, Gaguardi G, Chaffiol A, Terray A, Nanteau C, Potey A, Belle M, Rabesandratana O, Duebel J, Orieux G, Nandrot EF, Sahel J-A, Goureau O (2017) Generation of storable retinal organoids and retinal pigmented epithelium from adherent human iPS cells in xeno-free and feeder-free conditions. Stem Cells 35:1176–1188. https://doi.org/10.1002/stem.2586

Reich-Slotky R, Colovai AI, Semidei-Pomales M, Patel N, Cairo M, Jhang J, Schwartz J (2008) Determining post-thaw CD34+ cell dose of cryopreserved haematopoietic progenitor cells demonstrates high recovery and confirms their integrity. Vox Sang 94:351–357. https://doi.org/10.1111/j.1423-0410.2007.001028.x

Reimann KA, Chernoff M, Wilkening CL, Nickerson CE, Landay AL (2000) Preservation of lymphocyte immunophenotype and proliferative responses in cryopreserved peripheral blood mononuclear cells from human immunodeficiency virus type 1-infected donors: implications for multicenter clinical trials. The ACTG Immunology Advanced Technology Laboratories. Clin Diagn Lab Immunol 7(3):352–359. https://doi.org/10.1128/cdli.7.3.352-359.2000

Reubinoff BE, Pera MF, Vajta G, Trounson AO (2001) Effective cryopreservation of human embryonic stem cells by the open pulled straw vitrification method. Hum Reprod 16:2187–2194. https://doi.org/10.1093/humrep/16.10.2187

Richards M, Fong CY, Tan S, Chan WK, Bongso A (2004) An efficient and safe xeno-free cryopreservation method for the storage of human embryonic stem cells. Stem Cells 22: 779–789. https://doi.org/10.1634/stemcells.22-5-779

Rivera T, Zhao Y, Ni Y, Wang J (2020) Human-induced pluripotent stem cell culture methods under cGMP conditions. Curr Protoc Stem Cell Biol 54:e117. https://doi.org/10.1002/cpsc.117

Roberts ZJ, Better M, Bot A, Roberts MR, Ribas A (2018) Axicabtagene ciloleucel, a first-in-class CAR T cell therapy for aggressive NHL. Leuk Lymphoma 59(8):1785–1796. https://doi.org/10.1080/10428194.2017.1387905

Robey P (2017) Mesenchymal stem cells: fact or fiction, and implications in their therapeutic use. F1000Res 6:F1000 Faculty Rev 524. https://doi.org/10.12688/f1000research.10955.1

Rogulska O, Petrenko Y, Petrenko A (2017) DMSO-free cryopreservation of adipose-derived mesenchymal stromal cells: expansion medium affects post-thaw survival. Cytotechnology 69:265–276. https://doi.org/10.1007/s10616-016-0055-2

Rogulska O, Tykhvynska O, Revenko O, Grischuk V, Mazur S, Volkova N, Vasyliev R, Petrenko A, Petrenko Y (2019) Novel cryopreservation approach providing off-the-shelf availability of human multipotent mesenchymal stromal cells for clinical applications. Stem Cells Int 2019: 4150690. https://doi.org/10.1155/2019/4150690

Rowe AW, Eyster E, Kellner A (1967) Survival of human blood frozen by the low glycerol-liquid nitrogen rapid freeze technique. Cryobiology 3:379–380. https://doi.org/10.1016/S0011-2240(67)80103-2

Rowe AW, Eyster E, Kellner A (1968) Liquid nitrogen preservation of red blood cells for transfusion. A low glycerol-rapid freeze procedure. Cryobiology 5:119–128. https://doi.org/10.1016/s0011-2240(68)80154-3

Rowe W, Sputtek A, Kuehnl P (2006) Long-term storage of peripheral blood progenitor cells (PBPC) at −80°C results in a pronounced decrease of membrane integrity and clonogenic potential compared to storage at −170°C. Vox Sang 91(S3):30–31

Rubinstein P, Dobrila L, Rosenfield RE, Adamson JW, Migliaccio G, Migliaccio AR, Taylor PE, Stevens CE (1995) Processing and cryopreservation of placental/umbilical cord blood for unrelated bone marrow reconstitution. Proc Natl Acad Sci U S A 92:10119–101122. https://doi.org/10.1073/pnas.92.22.10119

Rybka WB, Fontes PA, Rao AS, Winkelstein A, Ricordi C, Ball ED, Starzl TE (1995) Hematopoietic progenitor cell content of vertebral body marrow used for combined solid organ and bone marrow transplantation. Transplantation 59(6):871–874. https://doi.org/10.1097/00007890-199503270-00012

Sasaki DT, Dumas SE, Engleman EG (1987) Discrimination of viable and non-viable cells using propidium iodide in two color immunofluorescence. Cytometry 8:413–420. https://doi.org/10.1002/cyto.990080411

Sato T, Stange DE, Ferrante M, Vries RG, Van Es JH, Van den Brink S, Van Houdt WJ, Pronk A, Van Gorp J, Siersema PD, Clevers H (2011) Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. Gastroenterology 141 (5):1762–1772. https://doi.org/10.1053/j.gastro.2011.07.050

Scheiwe MW, Pusztai-Markos Z, Essers U, Seelis R, Rau G, Körber C, Stürner KH, Jung H, Liedtke B (1981) Cryopreservation of human lymphocytes and stem cells (CFU-c) in large units for cancer therapy – a report based on the data of more than 400 frozen units. Cryobiology 18: 344–356. https://doi.org/10.1016/0011-2240(81)90107-3

Schuster SJ, Bishop MR, Tam CS, Waller EK, Borchmann P, McGuirk JP, Westin JR (2019) Tisagenlecleucel in adult relapsed or refractory diffuse large B-cell lymphoma. N Engl J Med 380(1):45–56. https://doi.org/10.1056/nejmoa1804980

Scott KL, Lecak J, Acker JP (2005) Biopreservation of red blood cells: past, present, and future. Transfus Med Rev 19:127–142. https://doi.org/10.1016/j.tmrv.2004.11.004

Serke S, Säuberlich S, Huhn D (1991) Multiparameter flow-cytometrical quantitation of circulating CD34(+)-cells: correlation to the quantitation of circulating haemopoietic progenitor cells by

in vitro colony-assay. Br J Haematol 77:453–459. https://doi.org/10.1111/j.1365-2141.1991.tb08609.x

Shafa M, Walsh T, Panchalingam KM, Richardson T, Menendez L, Tian X, Babu SS, Dadgar S, Beller J, Yang F, Baghbaderani BA (2020) Long-term stability and differentiation potential of cryopreserved cGMP-compliant human induced pluripotent stem cells. Int J Mol Sci 21:108. https://doi.org/10.3390/ijms21010108

Shi M, Ling K, Yong KW, Li Y, Feng S, Zhang X, Pingguan-Murphy B, Lu TJ, Xu F (2015) High-throughput non-contact vitrification of cell-laden droplets based on cell printing. Sci Rep 5:17928. https://doi.org/10.1038/srep17928

Shivakumar SB, Bharti D, Subbarao RB, Jang SJ, Park JS, Ullah I, Park JK, Byun JH, Park BW, Rho GJ (2016) DMSO- and serum-free cryopreservation of Wharton's jelly tissue isolated from human umbilical cord. J Cell Biochem 117:2397–2412. https://doi.org/10.1002/jcb.25563

Shu Z, Kang X, Chen H, Zhou X, Purtteman J, Yadock D, Heimfeld S, Gao D (2010) Development of a reliable low-cost controlled cooling rate instrument for the cryopreservation of hematopoietic stem cells. Cytotherapy 12(2):161–169. https://doi.org/10.3109/14653240903377037

Shu Z, Gao D, Pu LL (2015) Update on cryopreservation of adipose tissue and adipose-derived stem cells. Clin Plast Surg 42:209–218. https://doi.org/10.1016/j.cps.2014.12.001

Simione F, Sharp T (2017) Best practices for storing and shipping cryopreserved cells. In Vitro Cell Dev Biol Anim 53(10):888–895. https://doi.org/10.1007/s11626-017-0214-6

Singh H, Figliola MJ, Dawson MJ, Olivares S, Zhang L, Yang G, Maiti S, Manuri P, Senyukov V, Jena B, Kebriaei P, Champlin RE, Huls H, Cooper LJ (2013) Manufacture of clinical-grade CD19-specific T cells stably expressing chimeric antigen receptor using Sleeping Beauty system and artificial antigen presenting cells. PLoS One 8(5):e64138. https://doi.org/10.1371/journal.pone.0064138

Slichter SJ, Dumont LJ, Cancelas JA, Jones ML, Gernsheimer TB, Szczepiorkowski ZM, Dunbar NM, Prakash G, Medlin S, Rugg N, Kinne B, Macdonald VW, Housler G, Valiyaveettil M, Hmel P, Ransom JH (2018) Safety and efficacy of cryopreserved platelets in bleeding patients with thrombocytopenia. Transfusion 58:2129–2138. https://doi.org/10.1111/trf.14780

Smith JG, Joseph HR, Green T, Field JA, Wooters M, Kaufhold RM, Antonello J, Caulfield MJ (2007) Establishing acceptance criteria for cell-mediated-immunity assays using frozen peripheral blood mononuclear cells stored under optimal and suboptimal conditions. Clin Vaccine Immunol 14(5):527–537. https://doi.org/10.1128/CVI.00435-06

Smythe J, Armitage S, McDonald D, Pamphilon D, Guttridge M, Brown J, Green A, Brown C, Warwick RM, Lankester A, Fehily D, Contreras M, Navarrete C, Watt SM (2007) Directed sibling cord blood banking for transplantation: the 10-year experience in the national blood service in England. Stem Cells 25:2087–2093. https://doi.org/10.1634/stemcells.2007-0063

So S, Lee YL, Choi J, Kang S, Lee J-Y, Hwang J, Shin J, Dutton JR, Seo E-J, Lee BH, Kin CJ, Mitalipov S, Oh SJ, Kang E (2020) The Rho-associated kinase inhibitor fasudil can replace Y-27632 for use in human pluripotent stem cell research. PLoS One 15:e0233057. https://doi.org/10.1371/journal.pone.0233057

Söderdahl G, Tammik C, Remberger M, Ringdén O (1998) Cadaveric bone marrow and spleen cells for transplantation. Bone Marrow Transplant 21(1):79–84. https://doi.org/10.1038/sj.bmt.1701039

Sparrow RL, Komodromou H, Tippett E, Georgakopoulos T, Xu W (2006) Apoptotic lymphocytes and CD34+ cells in cryopreserved cord blood detected by the fluorescent vital dye SYTO 16 and correlation with loss of L-selectin (CD62L) expression. Bone Marrow Transplant 38(1):61–67. https://doi.org/10.1038/sj.bmt.1705405

Spinella PC, Pidcoke HF, Strandenes G, Hervig T, Fisher A, Jenkins D, Yazer M, Stubbs J, Murdock A, Sailliol A, Ness PM, Cap AP (2016) Whole blood for hemostatic resuscitation of major bleeding. Transfusion 56:S190–S202. https://doi.org/10.1111/trf.13491

Šponer P, Kučera T, Brtková J, Urban K, Kočí Z, Měřička P, Bezrouk A, Konrádová Š, Filipová A, Filip S (2018) Comparative study on the application of mesenchymal stromal cells combined

with tricalcium phosphate scaffold into femoral bone defects. Cell Transplant 27:1459–1468. https://doi.org/10.1177/0963689718794918

Sputtek A (2008) Cryopreservation of autologous peripheral blood stem cells for patient treatment: theory and practice. Cryo Letters 29:73–74

Sputtek A, Rowe AW (2006) Human peripheral blood progenitor cells require temperatures lower than minus 80 degrees C for long term storage. Cytotherapy 8(Suppl 1):Meeting abstract 96

Sputtek A, Benndorf C, Rowe A, Kuehnl P (2002) Why frozen peripheral blood progenitor cell (PBPC) concentrates should be thawed at the patient's bedside. Transfusion 42:46S–46S

Sputtek A, Lioznov M, Kröger N, Rowe AW (2011) Bioequivalence comparison of a new freezing bag (CryoMACS(®)) with the Cryocyte(®) freezing bag for cryogenic storage of human hematopoietic progenitor cells. Cytotherapy 13:481–489. https://doi.org/10.3109/14653249.2010.529891

Stacey GN, Connon CJ, Coopman K, Dickson AJ, Fuller B, Hunt CJ, Kemp P, Kerby J, Man J, Matejtschuk P, Moore H, Morris J, Oreffo ROC, Slater N, Ward S, Wiggins C, Zimmermann H (2017a) Preservation and stability of cell therapy products: recommendations from an expert workshop. Regen Med 12:553–564. https://doi.org/10.2217/rme-2017-0073

Stacey GN, Healy L, Man J, Hunt CJ, Morris J (2017b) Fundamental points to consider in the cryopreservation and shipment of cells for human application. In: Connon CJ (ed) Bioprocessing for cell based therapies. Wiley, Hoboken, pp 167–185. https://doi.org/10.1002/9781118743362.ch6

Stacey GN, Andrews PW, Asante C, Barbaric I, Barry J, Braybrook J, Buckle R, Chandra A, Coffey P, Crouch S, Driver P, Evans A, Gardner J, Ginty P, Goldring C, Hay DC, Healy L, Hows A, Hutchinson C, Jesson H, Kalber T, Kimber S, Leathers R, Moyle S, Murray T, Neale M, Pan D, Park K, Rebolledo RE, Revolta MN, Ritchie A, Roos EJ, Saeb-Parsy E, Schröder B, Sebastien S, Thomas A, Thomas R, Turner M, Vallier L, Vitillo L, Webster A, Williams D (2018) Science-based assessment of source materials for cell-based medicines. Report of a stakeholders workshop. Regen Med 13(8):935–944. https://doi.org/10.2217/rme-2018-0120

Stiff PJ, Koester AR, Weidner MK, Dvorak K, Fisher RI (1987) Autologous bone marrow transplantation using unfractionated cells cryopreserved in dimethylsulfoxide and hydroxyethyl starch without controlled-rate freezing. Blood 70:974–978

Stockschläder M, Hassan HT, Krog C, Krüger W, Löliger C, Horstman M, Altnöder M, Clausen J, Grimm J, Kabisch H, Zander A (1997) Long-term follow-up of leukaemia patients after related cryopreserved allogeneic bone marrow transplantation. Br J Haematol 96:382–386. https://doi.org/10.1046/j.1365-2141.1997.d01-2032.x

Stubbs JR, Tran SA, Emery RL, Hammel SA, Haugen DAL, Zielinski MD, Zietlow SP, Jenkins D (2017) Cold platelets for trauma-associated bleeding: regulatory approval, accreditation approval, and practice implementation-just the "tip of the iceberg". Transfusion 57:2836–2844. https://doi.org/10.1111/trf.14303

Sumida S, Eto S, Morishige F (1984) Combination therapy of megadose chemotherapy and frozen autologous marrow transplantation of patients with advanced solid cancer. Low Temp Med 9:43–46

Swioklo S, Connon CJ (2017) Short-term storage of cells for application in cell-based therapies. In: Connon CJ (ed) Bioprocessing for cell based therapies. Wiley, Hoboken, pp 187–210. https://doi.org/10.1002/9781118743362.ch7

T'Joen V, Cornelissen R (2012) Xeno-free plant-derived hydrolysate-based freezing of human embryonic stem cells. Stem Cells Dev 21:1716–1725. https://doi.org/10.1089/scd.2011.0374

T'Joen V, De Grande L, Declercq H, Cornelissen M (2012) An efficient, economical slow-freezing method for large-scale human embryonic stem cell banking. Stem Cells Dev 21(5):721–728. https://doi.org/10.1089/scd.2011.0192

Tarusin ND, Petrenko AY, Semenchenko AO, Mutsenko VV, Zaikov VS, Petrenko AY (2015) Efficiency of the sucrose-based solution and UW solution for hypothermic storage of human

mesenchymal stromal cells in suspension or within alginate microspheres. Probl Cryobiol Cryomed 25(4):329–339. https://doi.org/10.15407/cryo25.04.329

Taylor MJ, Baicu S (2009) Review of vitreous islet cryopreservation. Organogenesis 5:155–166. https://doi.org/10.4161/org.5.3.9812

Tedder RS, Zuckerman MA, Brink NS, Goldstone AH, Fielding A, Blair S, Patterson KG, Hawkins AE, Gormon AM, Heptonstall J, Irwin D (1995) Hepatitis B transmission from contaminated cryopreservation tank. Lancet 346:137–140. https://doi.org/10.1016/s0140-6736(95)91207-x

Thirumala S, Goebel WS, Woods EJ (2013) Manufacturing and banking of mesenchymal stem cells. Expert Opin Biol Ther 3(5):673–691. https://doi.org/10.1517/14712598.2013.763925

Thomas ED, Storb R (1970) Technique for human marrow grafting. Blood 36:507–515

Thomas ED, Lochte HL Jr, Lu WC, Ferrebee JW (1957) Intravenous infusion of bone marrow in patients receiving radiation and chemotherapy. N Engl J Med 257(11):491–496. https://doi.org/10.1056/nejm195709122571102

Thompson O, von Meyenn F, Hewitt Z, Alexander J, Wood A, Weightman R, Gregory S, Krueger F, Andrews S, Barbaric I, Gokhale PJ, Moore HD, Reik W, Milo M, Nik-Zainal S, Yusa K, Andrews PW (2020) Low rates of mutation in clinical grade human pluripotent stem cells under different culture conditions. Nat Commun 11:1528. https://doi.org/10.1038/s41467-020-15271-3

Thomson AE, O'Connor TW (1971) Observations on cryopreservation of lymphocytes in chronic lymphocytic leukaemia and normal human lymphocytes. Scand J Haematol 8(6):425–438. https://doi.org/10.1111/j.1600-0609.1971.tb00895.x

Tian C, Zhang X, Zhao G (2019) Vitrification of stem cell-laden core-shell microfibers with unusually low concentrations of cryoprotective agents. Biomater Sci 7:889–900. https://doi.org/10.1039/C8BM01231H

Tinmouth A, Chin-Yee I (2001) The clinical consequences of the red cell storage lesion. Transfus Med Rev 15:91–107. https://doi.org/10.1053/tmrv.2001.22613

To LB, Dyson PG, Branford AL, Russell JA, Haylock DN, Ho JQ, Kimber RJ, Juttner CA (1987) Peripheral blood stem cells collected in very early remission produce rapid and sustained autologous haemopoietic reconstitution in acute non-lymphoblastic leukaemia. Bone Marrow Transplant 2:103–108

Töpfer E, Pasotti A, Telopoulou A, Italiani P, Boraschi D, Ewart MA, Wilde C (2019) Bovine colon organoids: from 3D bioprinting to cryopreserved multi-well screening platforms. Toxicol In Vitro 61:104606. https://doi.org/10.1016/j.tiv.2019.104606

Trufanova NA, Zaikov VS, Zinchenko AV, Petrenko AY, Petrenko YA (2016) Closed vitrification system as a platform for cryopreservation of tissue engineered constructs. Cryo Lett 37(6):440–447

Tsutsaeva AA, Popov NN, Voskoboinikova TN (1978) Vliyanie nizkikh temperatur i krioprotektora PEO-400 na morfofunkcional'nye svojstva kletok limfaticheskikh uzlov (Effect of low temperatures and cryoprotectant PEO-400 on the morphofunctional properties of lymph node cells). Probl Cryobiol 4:50–52

Turner RJ, Geraghty NJ, Williams JG, Ly D, Brungs D, Carolan MG, Guy TV, Watson D, de Leon JF, Sluyter R (2020) Comparison of peripheral blood mononuclear cell isolation techniques and the impact of cryopreservation on human lymphocytes expressing CD39 and CD73. Purinergic Signal 16(3):389–401. https://doi.org/10.1007/s11302-020-09714-1

Tyagarajan S, Schmitt D, Acker C, Rutjens E (2019) Autologous cryopreserved leukapheresis cellular material for chimeric antigen receptor-T cell manufacture. Cytotherapy 21(12):1198–1205. https://doi.org/10.1016/j.jcyt.2019.10.005

U.S. Food and Drug Administration (U.S. FDA) (2011) Strategic plan for regulatory science. FDA. https://www.fda.gov/science-research/advancing-regulatory-science/strategic-plan-regulatory-science#:~:text=Advancing%20Regulatory%20Science%20at%20FDA%3A%20A%20Strategic%20Plan%20(August%202011)&text=FDA's%20core%20responsibility%20is%20to,to%20review%20of%20product%20quality

U.S. Food and Drug Administration (U.S. FDA) (2019) Kymriah (tisagenlecleucel). FDA. https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/kymriah-tisagenlecleucel

U.S. Food and Drug Administration (U.S. FDA) (2020a) Current good manufacturing practice (cGMP) regulations. FDA. https://www.fda.gov/drugs/pharmaceutical-quality-resources/current-good-manufacturing-practice-cgmp-regulations

U.S. Food and Drug Administration (U.S. FDA) (2020b) Yescarta (axicabtagene ciloleucel). FDA. https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/yescarta-axicabtagene-ciloleucel

U.S. Food and Drug Administration (U.S. FDA) (2020c) 21 CFR part 211. Current good manufacturing practice for finished pharmaceuticals. FDA. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=211

U.S. Food and Drug Administration (U.S. FDA) (2021) Cellular & gene therapy guidances. FDA. https://www.fda.gov/vaccines-blood-biologics/biologics-guidances/cellular-gene-therapy-guidances

Urbani L, Maghsoudlou P, Milan A, Menikou M, Hagen CK, Totonelli G, Camilli C, Eaton S, Burns A, Olivo A, De Coppi P (2017) Long-term cryopreservation of decellularised oesophagi for tissue engineering clinical application. PLoS One 12:e0179341. https://doi.org/10.1371/journal.pone.0179341

Urbani L, Camilli C, Phylactopoulos DE, Crowley C, Natarajan D, Scottoni F, Maghsoudlou P, McCann CJ, Pellegata AF, Urciuolo A, Deguchi K, Khalaf S, Aruta SF, Signorelli MC, Kiely D, Hannon E, Trevisan M, Wong RR, Baradez MO, Moulding D, Virasami A, Gjinovci A, Loukogeorgakis S, Mantero S, Thapar N, Sebire N, Eaton S, Lowdell M, Cossu G, Bonfanti P, De Coppi P (2018) Multi-stage bioengineering of a layered oesophagus with in vitro expanded muscle and epithelial adult progenitors. Nat Commun 9:4286. https://doi.org/10.1038/s41467-018-06385-w

Valbuena D, Sanchez-Luengo S, Galan A, Sanchez E, Gomez E, Poo ME, Riuz V, Genbacev O, Krtolica A, Pellecier A, Moreno R, Simon C (2008) Efficient method for slow cryopreservation of human embryonic stem cells in xeno-free conditions. Reprod Biomed Online 17(1):127–135. https://doi.org/10.1016/s1472-6483(10)60302-1

Valeri CR (1973) Viability, function and rejuvenation of previously frozen washed red blood cells. In: Chaplin HJ, Jaffe ER, Lenfant C, Valeri CR (eds) Preservation of red blood cells. National Academy of Science, Washington, DC, pp 265–297. https://doi.org/10.17226/20416

Valeri CR, Zaroulis CG (1972) Rejuvenation and freezing of outdated stored human red cells. N Engl J Med 287:1307–1313. https://doi.org/10.1056/NEJM197212282872601

Valeri C, Feingold H, Marchionni L (1974) A simple method for freezing human platelets using 6 per cent dimethylsulfoxide and storage at −80 degrees C. Blood 43:131–136

Valeri CR, Ragno G, Khuri S (2005) Freezing human platelets with 6 percent dimethyl sulfoxide with removal of the supernatant solution before freezing and storage at −80°C without postthaw processing. Transfusion 45:1890–1898. https://doi.org/10.1111/j.1537-2995.2005.00647.x

van Gelder M, de Wreede LC, Bornhäuser M, Niederwieser D, Karas M, Anderson NS, Gramatzki M, Dreger P, Michallet M, Petersen E, Bunjes D, Potter M, Beelen D, Cornelissen JJ, Yakoub-Agha I, Russell NH, Finke J, Schoemans H, Vitek A, Urbano-Ispízua Á, Blaise D, Volin L, Chevallier P, Caballero D, Putter H, van Biezen A, Henseler A, Schönland S, Kröger N, Schetelig J (2017) Long-term survival of patients with CLL after allogeneic transplantation: a report from the European Society for Blood and Marrow Transplantation. Bone Marrow Transplant 52:372–380. https://doi.org/10.1038/bmt.2016.282

Vaňásek J, Filip S, Medková V, Bláha M, Mericka P, Volenec K (1998) Mobilization of peripheral blood progenitor cells (PBPC) through a combination of chemotherapy and G-CSF in breast cancer patients and a possibility of unprocessed whole blood collection. Bone Marrow Transplant 21:123–126. https://doi.org/10.1038/sj.bmt.1701058

Verheijen M, Lienhard M, Schrooders Y, Clayton O, Nudischer R, Boerno S, Timmermann B, Selevsek N, Schlapbach R, Gmuender H, Gotta S, Geraedts J, Herwig R, Kleinjans J, Caiment F

(2019) DMSO induces drastic changes in human cellular processes and epigenetic landscape in vitro. Sci Rep 9:4641. https://doi.org/10.1038/s41598-019-40660-0

Vermeulen M, Poels J, de Michele F, des Rieux A, Wyns C (2017) Restoring fertility with cryopreserved prepubertal testicular tissue: perspectives with hydrogel encapsulation, nanotechnology, and bioengineered scaffolds. Ann Biomed Eng 45:1770–1781. https://doi.org/10.1007/s10439-017-1789-5

von Tresckow B, Engert A (2011) The role of autologous transplantation in Hodgkin lymphoma. Curr Hematol Malig Rep 6:172–179. https://doi.org/10.1007/s11899-011-0091-0

Vormittag P, Gunn R, Ghorashian S, Veraitch FS (2018) A guide to manufacturing CAR T cell therapies. Curr Opin Biotechnol 53:164–181. https://doi.org/10.1016/j.copbio.2018.01.025

Wagh V, Meganathan K, Jagtap S, Gaspar JA, Winkler J, Spitkovsky D, Hescheler J, Sachinidis A (2011) Effects of cryopreservation on the transcriptome of human embryonic stem cells after thawing and culturing. Stem Cell Rev Rep 7(3):506–517. https://doi.org/10.1007/s12015-011-9230-1

Wagner E, Duval M, Dalle JH, Morin H, Bizier S, Champagne J, Champagne MA (2006) Assessment of cord blood unit characteristics on the day of transplant: comparison with data issued by cord blood banks. Transfusion 46:1190–1198. https://doi.org/10.1111/j.1537-2995.2006.00870.x

Ware CB, Baran SW (2007) A controlled-cooling protocol for cryopreservation of human and non-human primate embryonic stem cells. Methods Mol Biol 407:43–49. https://doi.org/10.1007/978-1-59745-536-7_4

Ware CB, Nelson AM, Blau CA (2005) Controlled-rate freezing of human ES cells. BioTechniques 38:879–883. https://doi.org/10.2144/05386ST01

Waters L, Cameron M, Padula MP, Marks DC, Johnson L (2018) Refrigeration, cryopreservation and pathogen inactivation: an updated perspective on platelet storage conditions. Vox Sang 113:317–328. https://doi.org/10.1111/vox.12640

Wats M, Ings S, Balsa C (2020) Failure of cryopreserved peripheral blood stem cells to ensure engraftment resolved by functional assays but not by post-thaw viability. Cryo Letters 41:158–159

Weinberg A, Zhang L, Brown D, Erice A, Polsky B, Hirsch MS, Owens S, Lamb K (2000) Viability and functional activity of cryopreserved mononuclear cells. Clin Diagn Lab Immunol 7(4):714–716. https://doi.org/10.1128/cdli.7.4.714-716.2000

Weinberg A, Louzao R, Mussi-Pinhata MM, Cruz ML, Pinto JA, Huff MF, de Castro AC, Sucupira MC, Denny TN (2007) Quality assurance program for peripheral blood mononuclear cell cryopreservation. Clin Vaccine Immunol 14(9):1242–1244. https://doi.org/10.1128/cvi.00187-07

Weinberg A, Song LY, Wilkening C, Sevin A, Blais B, Louzao R, Stein D, Defechereux P, Durand D, Riedel E, Raftery N, Jesser R, Brown B, Keller MF, Dickover R, McFarland E, Fenton T, Pediatric ACTG Cryopreservation Working Group (2009) Optimization and limitations of use of cryopreserved peripheral blood mononuclear cells for functional and phenotypic T-cell characterization. Clin Vaccine Immunol 16(8):1176–1186. https://doi.org/10.1128/cvi.00342-08

Weiner RS, Richman CM, Yankee RA (1977) Semicontinuous flow centrifugation for the pheresis of immunocompetent cells and stem cells. Blood 49:391–397

Weiner J, Duran-Struuck R, Zitsman J, Buhler L, Sondermeijer H, McMurchy AN, Levings MK, Sykes M, Griesemer A (2015) Restimulation after cryopreservation and thawing preserves the phenotype and function of expanded baboon regulatory T cells. Transplant Direct 1(1):1–7. https://doi.org/10.1097/TXD.0000000000000511

Wen F, Magalhães R, Gouk SS, Bhakta G, Lee KH, Hutmacher DW, Kuleshova LL (2009) Vitreous cryopreservation of nanofibrous tissue-engineered constructs generated using mesenchymal stromal cells. Tissue Eng Part C Methods 15:105–114. https://doi.org/10.1089/ten.tec.2008.0237

Weng L, Beauchesne PR (2020) Dimethyl sulfoxide-free cryopreservation for cell therapy: a review. Cryobiology 94:9–17. https://doi.org/10.1016/j.cryobiol.2020.03.012

Weng L, Tessier SN, Swei A, Stott SL, Toner M (2017) Controlled ice nucleation using freeze-dried *Pseudomonas* syringae encapsulated in alginate beads. Cryobiology 75:1–6. https://doi.org/10.1016/j.cryobiol.2017.03.006

Westbury G, Humble JG, Newton KA, Skinner ME, Pegg DE (1959) Disseminated malignant melanoma: response to treatment by massive dosage of a cytotoxic agent combined with autogenous marrow replacement. Lancet 1:968–969. https://doi.org/10.1016/s0140-6736(59)91082-7

Woods EJ, Sherry AM, Woods JR, Hardin JW, LaFontaine M, Brandacher G, Johnstone BH (2020) Ischemia considerations for the development of an organ and tissue donor derived bone marrow bank. J Transl Med 18(1):300. https://doi.org/10.1186/s12967-020-02470-1

World Health organization (WHO) (2014) WHO good manufacturing practices. WHO, Geneva. https://www.who.int/medicines/areas/quality_safety/quality_assurance/TRS986annex2.pdf?ua=1. Accessed 4 Feb 2021

World Health Organization (WHO) (2019) Guidance on regulations for the transport of infectious substances 2019–2020. World Health Organization, Geneva. https://apps.who.int/iris/bitstream/handle/10665/325884/WHO-WHE-CPI-2019.20-eng.pdf?ua=1. Accessed 2 Feb 2021

World Health Organization (WHO) (2020) Blood safety and availability. https://www.who.int/news-room/fact-sheets/detail/blood-safety-and-availability. Accessed 2 Feb 2021

Xu X, Cowley S, Flaim CJ, James W, Seymour L, Cui Z (2009) The roles of apoptotic pathways in the low recovery rate after cryopreservation of dissociated human embryonic stem cells. Biotechnol Prog 26:827–837. https://doi.org/10.1002/btpr.368

Xu Y, Zhang L, Xu J, Wei Y, Xu X (2014) Membrane permeability of the human pluripotent stem cells to Me2SO, glycerol and 12-propanediol. Arch Biochem Biophys 550–551:67–76. https://doi.org/10.1016/j.abb.2014.04.010

Xu H, Cao W, Huang L, Xiao M, Cao Y, Zhao L, Wang N, Zhou J (2018) Effects of cryopreservation on chimeric antigen receptor T cell functions. Cryobiology 83:40–47. https://doi.org/10.1016/j.cryobiol.2018.06.007

Yakoub-Agha I, Chabannon C, Bader P, Basak GW, Bonig H, Ciceri F, Corbacioglu S, Duarte RF, Einsele H, Hudecek M, Kersten MJ, Köhl U, Kuball J, Mielke S, Mohty M, Murray J, Nagler A, Robinson S, Saccardi R, Sanchez-Guijo F, Snowden JA, Srour M, Styczynski J, Urbano-Ispizua A, Hayden PJ, Kröger N (2020) Management of adults and children undergoing chimeric antigen receptor T-cell therapy: best practice recommendations of the European Society for Blood and Marrow Transplantation (EBMT) and the Joint Accreditation Committee of ISCT and EBMT (JACIE). Haematologica 105:297–316. https://doi.org/10.3324/haematol.2019.229781

Yan HHN, Siu HC, Law S, Ho SL, Yue SSK, Tsui WY, Chan D, Chan AS, Ma S, Lam KO, Bartfeld S, Man AHY, Lee BCH, Chan ASY, Wong JWH, Cheng PSW, Chan AKW, Zhang J, Shi J, Fan X, Kwong DLW, Mak TW, Yuen ST, Clevers H, Leung SY. A (2018) Comprehensive human gastric cancer organoid biobank captures tumor subtype heterogeneity and enables therapeutic screening. Cell Stem Cell 23(6):882–897.e11. https://doi.org/10.1016/j.stem.2018.09.016

Yang H, Acker JP, Cabuhat M, Letcher B, Larratt L, McGann LE (2005) Association of post-thaw viable CD34+ cells and CFU-GM with time to hematopoietic engraftment. Bone Marrow Transplant 35:881–887. https://doi.org/10.1038/sj.bmt.1704926

Yang PF, Hua TC, Wu J, Chang ZH, Tsung HC, Cao YL (2006) Cryopreservation of human embryonic stem cells: a protocol by programmed cooling. Cryo Letters 27:361–368

Yoshida T, Prudent M, D'Alessandro A (2019) Red blood cell storage lesion: causes and potential clinical consequences. Blood Transfus 17:27–52. https://doi.org/10.2450/2019.0217-18

Yoshihara S, Ikemoto J, Fujimori Y (2016) Update on granulocyte transfusions: accumulation of promising data, but still lack of decisive evidence. Curr Opin Hematol 23:55–60. https://doi.org/10.1097/MOH.0000000000000203

Yuan Y, Yang Y, Tian Y, Park J, Dai A, Roberts M, Liu Y, Han X (2016a) Efficient long-term cryopreservation of pluripotent stem cells at −80 °C. Sci Rep 6:34467. https://doi.org/10.1038/srep34476

Yuan Z, Lourenco Sda S, Sage EK, Kolluri KK, Lowdell MW, Janes SM (2016b) Cryopreservation of human mesenchymal stromal cells expressing trail for human anti-cancer therapy. Cytotherapy 18(7):860–869. https://doi.org/10.1016/j.jcyt.2016.04.005

Zeuner A, Martelli F, Vaglio S, Federici G, Whitsett C, Migliaccio AR (2012) Concise review: stem cell-derived erythrocytes as upcoming players in blood transfusion. Stem Cells 30:1587–1596. https://doi.org/10.1002/stem.1136

Zhang Y, Huang WJ, Yang QR, Zhang HD, Zhu XJ, Zeng M, Zhou X, Wang ZY, Li WJ, Jing HS, Zhang XB, Shi YP, Hu H, Yan HX, Li ZH, Zhai B (2020) Cryopreserved biopsy tissues of rectal cancer liver metastasis for assessment of anticancer drug response in vitro and in vivo. Oncol Rep 43:405–414. https://doi.org/10.3892/or.2019.7450

Zhao G, Liu X, Zhu K, He X (2017) Hydrogel encapsulation facilitates rapid-cooling cryopreservation of stem cell-laden core-shell microcapsules as cell-biomaterial constructs. Adv Healthc Mater 6(23):988. https://doi.org/10.1002/adhm.201700988

Zhao J, Song Y, Liu D (2019) Clinical trials of dual-target CAR T cells, donor-derived CAR T cells, and universal CAR T cells for acute lymphoid leukemia. J Hematol Oncol 12(1):17. https://doi.org/10.1186/s13045-019-0705-x

Zhou CQ, Mai QY, Li T, Zhuang GL (2004) Cryopreservation of human embryonic stem cells by vitrification. Chin Med J 117:1050–1055

Zouhair S, Aguiari P, Iop L, Vásquez Rivera A, Filippi A, Romanato F, Korossis S, Wolkers WF, Gerosa G (2019) Preservation strategies for decellularized pericardial scaffolds for off-the-shelf availability. Acta Biomater 84:208–221. https://doi.org/10.1016/j.actbio.2018.10.026

Web Links

FACT. http://www.factwebsite.org

Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine. http://www.cryo.org.ua

International Conference on Harmonisation. https://www.ich.org/products/guidelines/quality/article/quality-guidelines.html

Society for Low Temperature Biology Committee. https://www.sltb.info