Stem Cell Biology and Regenerative Medicine

Dusko Ilic *Editor*

Stem Cell Banking

Humana Press

Stem Cell Biology and Regenerative Medicine

Series Editor
Kursad Turksen, Ph.D.
kursadturksen@gmail.com

For further volumes:
http://www.springer.com/series/7896

Dusko Ilic
Editor

Stem Cell Banking

Humana Press

Editor
Dusko Ilic
Division of Women's Health
King's College London School of Medicine
London, UK

ISSN 2196-8985 ISSN 2196-8993 (electronic)
ISBN 978-1-4939-0584-3 ISBN 978-1-4939-0585-0 (eBook)
DOI 10.1007/978-1-4939-0585-0
Springer New York Heidelberg Dordrecht London

Library of Congress Control Number: 2014935182

Printed on acid-free paper

Humana Press is a brand of Springer
Springer is part of Springer Science+Business Media (www.springer.com)

Preface

Stem cell banks are becoming essential public infrastructure assets. However, banking of stem cells is not just building a repository and storing samples. The planning, design, construction, and maintenance involve multiple skilled professionals. Stem cell banks are points where technology and medicine converge with ethics, laws, and regulations. They are established "to store, characterize, and supply ethically approved, quality-controlled stem cell lines for research and treatment." If properly designed and organized, their utilization will have a broad impact not only on the scientific community and medical professionals but also on the general public.

When I began thinking about the book, my first idea was to make two distinct sections. The first part would be focused on issues surrounding cord blood banking and the second one on issues around banking of non-cord blood stem cells, primarily mesenchymal and pluripotent stem cells. I realized very quickly that this would be impossible, as there were so many overlaps. Quality and regulatory standards in banking mesenchymal and pluripotent stem cells were actually built on experiences from cord blood banking. The issues that cord blood banks were facing are very similar to the issues that are or will trouble any stem cell banks worldwide.

The opening chapters, written by Jeremy Micah Crook and Glyn Stacey, are about setting standards for banking of pluripotent stem cells. These chapters reflect the overall aim of the book: bringing stem cell banking communities together to talk with each other and to move forward. The following chapter, in which Sergio Querol discusses global perspectives on cord blood stem cell banking, illustrates the importance of learning from experience. In the next three chapters, Rosario Isasi, James Lawford Davies, Sebastian Sethe, and Carlo Petrini provide a perspective on regulation and ethical issues in stem cell banking. Even though they are coming from different backgrounds, their concerns end up matching regardless of stem cell type or origin.

The second part of book is an overview of stem cell banking activities from all over the world. Anastasia Efthymiou and Mahendra Rao from the NIH Center for Regenerative Medicine and Justin Lowenthal from the NIH Department of Bioethics talk about efforts to develop pluripotent stem cell banks in the United States,

whereas Shinya Yamanaka and colleagues from the Center for iPS Cell Research and Application from Kyoto University describe the first steps toward banking of clinical grade iPS cells in Japan. Nahal Lalefar and Bert Lubin present the first and very successful sibling donor cord blood program initiated in 1998 with NIH support at Children's Hospital Oakland in California. Authors from the Anthony Nolan Cord Blood Program are discussing issues and strategies for cord blood banking from ethnic minority groups in the UK.

Systematic planning and continuous support are showing results everywhere in the world, not only in the most developed countries. In the chapter on banking of mesenchymal stem cells in India, Chandra Viswanathan and Prathibha Shetty demonstrate that not only governments but also companies can take on such a task and, at the end of the day, do a good job. A group of authors from the Royan Institute describe structures and regulations of stem cell banking for research and for clinical use in Iran.

Federal and local governments are usually keen to allocate budget funds for building public stem cell banks. However, very few are thinking about how to make these banks self-sustainable. What would happen when money from the government stops flowing in? Probably all of us in the stem cell banking business remember the enthusiasm that surrounded the Massachusetts Stem Cell Bank when it opened in 2008 with U.S. $8.6 million in public funds. Only four years later, when public funds run out, the bank was quietly closed down. Silvana Bardelli and Tiziano Moccetti examine the Swiss working model of investment return where core business funds research, which creates added value for comprehensive growth of the biobanking and cell therapy facility. A group of authors from Serbia, based on their experience, explain that flexibility in planning is absolutely essential for success when fluctuations in budget are unpredictable, and the regulatory framework is built almost from scratch alongside the public cord blood bank.

I hope that the readers of this collection of chapters on stem cell banking will take home a notion that to compete successfully in this business, one must be open to the world and work with others on common standards. Everything else is just a fine-tuning, which mainly depends on specific circumstances of political and economical setting.

London, UK Dusko Ilic

Acknowledgments

I thank each of the authors for devoting their time and sharing their expertise for the benefit of the community.

I owe my deepest gratitude to Andre Martey, Guy's and St Thomas' National Health Service Foundation Trust, London, UK. Without his invaluable help, this book would not have been possible.

Contents

Contributors

Hossein Baharvand Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, and Department of Developmental Biology, University of Science and Culture, Academic Center for Education, Culture & Research, Tehran, Iran

Silvana Bardelli Human Cardiac Stem Cell Unit, Cardiocentro Ticino Foundation, Lugano, Switzerland

Jeremy M. Crook Australian Institute of Innovative Materials, Illawarra Health and Medical Research Institute, and ARC Centre of Excellence for Electromaterials Science (ACES), Intelligent Polymer Research Institute, University of Wollongong, Wollongong, NSW, Australia

Department of Surgery, St. Vincent's Hospital, The University of Melbourne, Melbourne, VIC, Australia

James Lawford Davies Lawford Davies Denoon, London, UK

Marzieh Ebrahimi Department of Regenerative Medicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran

Anastasia G. Efthymiou Center for Regenerative Medicine, National Institutes of Health, Bethesda, MD, USA

Parvaneh Farzaneh Human and Animal Cell Bank, Iranian Biological Resource Center, Academic Center for Education, Culture & Research, Tehran, Iran

Susana G. Gomez Anthony Nolan Cord Blood Program, Nottingham, UK

Newsha Haghparast Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, Academic Center for Education, Culture & Research, Tehran, Iran

Vahid Hajihosseini Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, Academic Center for Education, Culture & Research, Tehran, Iran

Fatma Hatimy Anthony Nolan Cord Blood Program, Nottingham, UK

Rosario Isasi Centre of Genomics and Policy, Faculty of Medicine, Department of Human Genetics, McGill University, McGill University and Génome Québec Innovation Centre, Montreal, QC, Canada

Nahal Rose Lalefar Department of Hematology/Oncology, Children's Hospital and Research Center Oakland, Oakland, CA, USA

Emilija Lazic Mother and Child Health Care Institute of Serbia "Dr Vukan Čupić," Belgrade, Serbia

Justin Lowenthal Department of Bioethics, Clinical Center, National Institutes of Health, Bethesda, MD, USA

Bertram Lubin Department of Hematology/Oncology, Children's Hospital and Research Center Oakland, Oakland, CA, USA

Alejandro J. Madrigal Anthony Nolan Cord Blood Program, Nottingham, UK

Ayumi Matsunaga Medical Applications Promoting Office, Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan

Velibor Milovanovic Anthony Nolan Cord Blood Program, Nottingham, UK

Tiziano Moccetti Human Cardiac Stem Cell Unit and Cardiology Unit, Cardiocentro Ticino Foundation, Lugano, Switzerland

Linda Moss Anthony Nolan Cord Blood Program, Nottingham, UK

Monica Del Olmo Anthony Nolan Cord Blood Program, Nottingham, UK

Mohammad Pakzad Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, Academic Center for Education, Culture & Research, Tehran, Iran

Carlo Petrini Italian National Institute of Health, Rome, Italy

Sergio Querol Barcelona Cord Blood Bank, Programa Concordia, Banc Sang i Teixits, Barcelona, Spain

Anthony Nolan Cord Blood Program, Nottingham, UK

Cord Blood Working Group, World Marrow Donor Association, Leiden, The Netherlands

Mahendra Rao Center for Regenerative Medicine, National Institutes of Health, Bethesda, MD, USA

Megumu K. Saito Department of Clinical Application, Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan

Sebastian Sethe Lawford Davies Denoon, London, UK

Prathibha Shetty Regenerative Medicine Group, Reliance Life Sciences Pvt. Ltd., Navi Mumbai, India

Glyn N. Stacey UK Stem Cell Bank, National Institute for Biological Standards and Control, Hertfordshire, UK

Naoko Takasu Medical Applications Promoting Office, Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan

Mahdi Tondar Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, Academic Center for Education, Culture & Research, Tehran, Iran

Svetlana Vekic Bar Association of Serbia, Belgrade, Serbia

Chandra Viswanathan Regenerative Medicine Group, Reliance Life Sciences Pvt. Ltd., Navi Mumbai, India

Dragana Vujic Mother and Child Health Care Institute of Serbia "Dr Vukan Čupić," Belgrade, Serbia

Medical School, University of Belgrade, Belgrade, Serbia

Shinya Yamanaka Department of Reprogramming Science, Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan

Gladstone Institute of Cardiovascular Disease, San Francisco, CA, USA

Zeljko Zecevic Mother and Child Health Care Institute of Serbia "Dr Vukan Čupić," Belgrade, Serbia

Abbreviations

7-ADD	7-Aminoactinomycin D
AABB	American Association of Blood Banks
ACECR	Academic Center for Education, Culture and Research
AM	Amniotic membranes
ATCC	American Type Culture Collection
BBMR	British Bone Marrow Registry
BMDW	Bone Marrow Donors Worldwide
BMR	Batch manufacturing records
CAP	College of American Pathologists
CB	Cord blood
CBB	Cord Blood Bank
CBT	Cord blood transplantation
CBU	Cord blood units
CGH	Comparative genome hybridization
cGMP	Current good manufacturing practice
CiBK	Clinical iPS Cell Bank Kyoto
CIBMTR	Center for International Blood and Marrow Transplant Research
CiRA	Center for iPS Cell Research and Application
CIRM	California Institute for Regenerative Medicine
CLIA	Clinical Laboratory Improvement Amendments
CMV	Cytomegalovirus
CRM	Center for Regenerative Medicine
DMSO	Dimethyl sulfoxide
DUCBT	Double UCB transplants
EBMT	European Society for Blood and Marrow Transplantation
EBV	Epstein–Barr Virus
EGE	European Group on Ethics in Science and New Technologies
EWP	Ethics Working Party
FACT	Foundation for the Accreditation of Cellular Therapy
FCRE	Foundation for Cardiological Research and Education

FDA	Food and Drug Administration
FTE	Full-time equivalents
FY	Fiscal year
G-banding	Giemsa-banding
GCBP	Good Cell Banking Practice
GMP	Good Manufacturing Practice
GVHD	Graft-versus-host disease
GWAS	Genome-Wide Association Studies
HBV	Hepatitis B virus
HCCPU	High cellular common phenotype units
HCV	Hepatitis C virus
hESC	Human embryonic stem cells
HHV	Human herpes virus
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HORCSCT	Hematology-Oncology and Stem Cell Transplantation Research Center
HPC	Hematopoietic progenitor cell
HPV	Human papilloma virus
HQU	High quality units
HREC	Human Research Ethics Committees
HSC	Hematopoietic stem cell
HSCT	Hematopoietic stem cell transplantation
HSV	Herpes simplex virus
HTA	Human Tissue Authority
HTLV	Human T-lymphotropic virus
IBRC	Iranian Biological Resource Center
ICH	International Conference on Harmonization
ICMR	Indian Council of Medical Research
ICSCRT	Iranian Council of Stem Cell Research and Therapy
IC-SCRT	Institutional Ethics Committees for Stem Cell Research and Therapy
iPS	Induced pluripotent stem (cells)
IRB	Institutional Review Board
ISCBI	International Stem Cell Banking Initiative
ISCDP	Iranian Stem Cell Donor Program
ISCF	International Stem Cell Forum
ISCT	International Society for Cell Therapy
ISO	International Standards Organization
ISSCR	International Society of Stem Cell Research
IVF	In vitro fertilization
LULL	Limited use label license
MCB	Master Cell Bank
mESC	Mouse embryonic stem cells
MOA	Memorandum of understanding
MSC	Mesenchymal stem cells

MTA	Material transfer agreement
NABL	National Accreditation of Biological Laboratories
NAC-SCRT	National Apex Committee for Stem Cell Research and Therapy
NAS	National Academy of Sciences
NAT	Nucleic Acid-based Test
NHS	National Health Service
NHSBT	National Health Service Blood Transfusion
NIH	National Institute of Health
NMDP	National Marrow Donor Program
OECD	Organization for Economic Co-operation and Development
OI	Operational inventory
PCR	Polymerase chain reaction
PGD	Pre-implantation genetics diagnostics
PMDA	Pharmaceuticals and Medical Devices Agency
PSC	Pluripotent stem cells
QA	Quality assurance
QC	Quality control
RBC	Red blood cells
RC	Release criteria
RLS	Reliance Life Sciences
SCID	Severe combined immunodeficiency
SCU	Stem Cell Unit
SDCB	Sibling Donor Cord Blood
SIRM	Swiss Institute for Regenerative Medicine
SKY	Spectral Karyotyping
SNP	Single nucleotide polymorphism
SOHO V&S	Vigilance and surveillance of substances of human origin
SOP	Standard operation procedures
STR	Short tandem repeat
TC	Transplant Center
TCS	Token cell stock
TNC	Total nucleated cells
TPHA	*Treponema pallidum* hemagglutination assay
UCB	Umbilical cord blood
WCB	Working Cell Bank
WHO	World Health Organization

Part I
General Issues in Stem Cell Banking

Chapter 1
Setting Quality Standards for Stem Cell Banking, Research and Translation: The International Stem Cell Banking Initiative

Jeremy M. Crook and Glyn N. Stacey

1 Introduction

A major challenge of delivering human stem cells for research and medicine is access to quality-controlled and ethically sourced cell lines developed and banked according to globally acceptable standards. ISCBI has undertaken the first step to harmonizing stem cell resources by firstly producing a consensus-guidance for banking and supplying hES cells for research [1]. Although underscoring hES cells, the document is broadly applicable to all human stem cell lines including iPS cells. A second installment a propos clinical-grade stem cell lines will soon be completed for the clinical translation of cells. Both guidance are based on the knowledge and experience of stem cell banks from around the world, and leading academic and industry experts with extensive experience in the derivation, procurement, culture, storage, characterization, and distribution of human stem cells for research and clinical-use [2, 3]. Moreover, the documents were developed in consultation with the U.S. Food and Drug Administration (USFDA; www.fda.gov), World Health Organisation (WHO; www.who.int), ISCF Ethics Working Party, ISSCR and the International Society for Cell Therapy (ISCT; www.celltherapysociety.org), and

J.M. Crook (✉)
Australian Institute of Innovative Materials, Illawarra Health and Medical Research Institute, and ARC Centre of Excellence for Electromaterials Science (ACES), Intelligent Polymer Research Institute, University of Wollongong, Wollongong, NSW, Australia

Department of Surgery, St. Vincent's Hospital, The University of Melbourne, Melbourne, VIC, Australia
e-mail: jcrook@uow.edu.au.

G.N. Stacey
UK Stem Cell Bank, National Institute for Biological Standards and Control, South Mimms, Blanche Lane, Potters Bar, EN6 3QG Hertfordshire, UK

D. Ilic (ed.), *Stem Cell Banking*, Stem Cell Biology and Regenerative Medicine,
DOI 10.1007/978-1-4939-0585-0_1,

seek to transcend national boundaries where incongruence between different sets of standards or disregard for standards risks repeating the mistakes of other fields such as cord blood banking [4]. Surprisingly, for cord blood transplantation, quality-related issues are not uncommon, resulting in less than optimal cell viability, cord blood manufacturing, and graft potency. Other examples of commonly reported "serious events" include mislabeling of products, mishaps during transport, including thawing of units and breakage of bags. A major contributor is variable/unstandardized quality systems between cord banks giving rise to a heterogeneous international inventory [4]. Importantly, recognition of the need for improved information management systems, quality assurance, and stringent regulatory compliance is driving new solutions for standardizing and maximizing quality and minimizing errors. Needless to say there is much to be learned from the cord blood experience with quality and regulatory compliance standards for hES and iPS cells need to be met by establishing procedural protocols, and monitoring production, banking, and distribution processes. Here, we coin the term Good Cell Banking Practice (GCBP) to describe the standards which can be broadly applied to formalized cell banking in general.

2 Establishment and Promulgation of Standards

In accordance with ISCBI's first consensus guidance [1], standardized principles of quality assurance for GCBP broadly apply to the following:

- Ethics of donor consent for cell line derivation, procurement, and use.
- Authorization and governance of operation.
- Good cell banking procedure.

A fourth key requirement not included in the ISCBI guidance is the standardized naming of stem cell lines [5].

2.1 Ethics

International ethical standards of donor consent for cell line derivation, procurement, and use have been disseminated by the ISSCR, ISCF, and USNAS. Briefly, the provenance of a cell line should include evidence of a donors' voluntary informed consent for deriving a cell line from primary tissue, and use of the line for a wide range of ethically approved research, genetic testing, and, where relevant, product development for clinical application and/or commercialisation. Regulation of the use of cells should extend to the prohibition of third party users unless authorized by the bank.

An "Ethics+" approach is recommended that where possible an ethics body provides ethical oversight independent of management of a banks day-to-day activity [6].

While large-scale entities may require their own ethics body, institutional Human Research Ethics Committees (HRECs) may adequately deal with oversight of ethical considerations of small-scale institutional banks.

2.2 Authorization and Governance

The activities of a stem cell bank should be authorized or accredited by an appropriate local and/or international authority, and in accordance with local, state, or national rules/laws. For example, a bank can be registered or licensed by a statutory authority. There are few examples worldwide of legislative intervention for biobanking. The UK Human Tissue Act 2004 [7] codifies a licensing scheme for dealings with human tissue, including biobanks, under the EU Tissue and Cells Directive 2004 [8].

Governance involves oversight of overall bank performance including operational, technical, security, and legal matters. A standard of good governance should ensure consistent management of day-to-day activities, cohesive policies on bank operation, and accountability to stakeholders [9].

2.3 Good Cell Banking Procedure

Procedural standards of good cell banking ensure the cells accepted into and offered by a bank are indisputably authentic and fit for purpose, and maintained for continuity and longevity. They can be summarized as follows:

- First and foremost, it is recommended that a bank use recognized quality management principles defined by the International Standards Organization (ISO 9000 standards). The standards provide a framework for managing and operating any service such as cell line supply, to which other quality assurance procedures such as operation and maintenance of facilities, equipment, and staff training can be integrated.
- Good documentation practice is vital for any quality assurance (QA) system. Among other things good documentation provides a record of all key aspects of deposited cells including provenance, procurement, in-house processing (including characterization) and release. Correct, complete, and current record keeping provides traceability as an essential requirement for GCBP and all aspects of Good Manufacturing Practice (GMP).
- Implementation of a tiered system of cell banking and off-site storage of backup material minimizes risk factors such as microbial contamination, loss of stem cell characteristics, genetic drift, cross contamination of cell lines, and loss of cell line stocks. A tiered banking system includes preparation of a Token Cell Stock (TCS) or pre-master stock, Master Cell Bank (MCB; at the lowest practi-

cable passage number), and Working Cell Bank (WCB). The TCS is prepared by expanding seed cells under quarantine conditions. Once tested negative for microbial contaminants, the TCS is used to generate MCBs from which WCBs are generated for release and distribution. Back-up material can be generated from either the TCS or MCBs.

- As indicated above, a first task upon receipt of cells into a facility is to test their sterility for freedom from detectable levels of contaminating bacterial and fungal pathogens. Testing should be performed on antibiotic-free cultures. Since the growth of fungi, certain bacteria and mycoplasma can be slow, they may not be visible. It is therefore recommended that testing be performed on antibiotic-free cultures, frequently. While some assays may exceed the capabilities of some laboratories, they can be outsourced to certified service providers.Sterility testing should also include viral pathogens such as HBV, HCV, HIV, HTLV I/II, EBV, and CMV. Depending on the intended use of cell lines, screening may be extended to cover other blood-born pathogens such as HPV, HSV, and HHV. While screening should occur sooner rather than later, it is most commonly performed on a MCB. Importantly, while the potential for a virological contamination occurring is low, a bank should determine a best response to managing an event.
- A standard approach to cell line characterization is vital to assessing the status of cells of a line from initial receipt of seed stock through to an MCB and WCB. For hES and iPS cells, characterization should include confirmation of identity (minimally performed on TCS and WCB), self-renewal and pluripotency, and stability (minimally performed on TCS, MCB, WCB).

  Cell line authentication by identity testing is critical to verifying the source of a cell line and demonstrating that it is free of contamination by another cell line. The importance of cell line identity is highlighted by Nature mandating Short Tandem Repeat (STR) profiling (DNA fingerprinting) for papers reporting new hES cell lines [10]. STR is a standard genotyping technique that is both quick and inexpensive to perform by a service provider. The American Type Culture Collection (ATCC; www.attc.org) has coordinated a consensus standard ASN-0002 for identifying and authenticating human cell lines using STR profiling [11]. The standard was submitted to the American National Standards Institute (ANSI; www.ansi.org) and officially published in February 2012.

  As a hallmark characteristic of hES and iPS cells, pluripotency must be verified on a regular basis. While teratoma formation in vivo using SCID mice is the recommended standard, other less stringent and complementary methods can be used, including in vitro formation of embryoid bodies, differentiation to specific somatic cell lineages, profiling pluripotency gene expression, and immunocytochemistry of pluripotency markers [12].

  Monitoring cell line stability should include recording cell culture appearance and plating efficiency from passage to passage, and doubling rate and karyology every 10–20 passages. Giemsa-banding (G-banding) is most often employed to assess chromosomal stability. However, efficient and cost-effective methods for higher-resolution genotyping (enabling micro-amplifications and -deletions) are

also available, including spectral karyotyping (SKY), comparative genome hyrbridization (CGH), and microarray-based-single nucleotide polymorphism (SNP) analysis. In accordance with the ISCBI guidance, when applying G-banding, chromosome counting and analysis of banding patterns should be performed for 20 and 8 metaphases respectively.

- By operating in accordance with the above-mentioned procedural standards a bank will be able to assure users that the stem cells provided satisfy key criteria to be fit-for-purpose and suitable for release. While the specific release criteria (RC; i.e., the specific measures that must be met to enable the bank to release cells for use) will depend on the intended application of a cell line, for healthy, disease-free hES and iPS cells, the RC should at least include documented evidence of a single uncontaminated and stable human pluripotent cell line of known identity.

2.4 Standardized Nomenclature

A recent call and proposal for standardizing the naming of hES and iPS cell lines highlights the risks and confusion arising from an unregulated system where thousands of lines have been generated in hundreds of laboratories world-wide, with almost as many different approaches to naming [5]. Similar consideration should and could be given to the naming of other (non-pluripotent) stem cell types. Field experts devised the nomenclature, which follows an intuitive naming strategy and includes a unique identifier. More specifically, the standard allows for a description of the source reference (e.g. laboratory or institution), a numerical cell line identifier, and other specific characteristics such as disease, reporter genes, clone number, or patient/donor number. The nomenclature has been endorsed by the steering committee of ISCI. Banking facilities are encouraged to apply the nomenclature to stem cell lines that they derive and promote its use to the wider stem cell community.

3 Adoption and Implementation of Standards

The promulgation of standards is important, although not the whole solution. Once devised, the greater challenge will be to have the standards adopted and implemented by the field. While likely requiring in many instances a significant shift in the mindset of users, in establishing standards that comply with relevant guidelines and regulations for laboratory testing under Good Laboratory Practice [13, 14], GMP, Good Cell Culture Practice [15], the procurement and handling of human cells [16, 17], cell therapies, and more specifically human stem cell research and medicine [18, 19], ISCBI avoided “gold-plating” and therefore the risk of the first guidance being too onerous so as to be impracticable because of high pecuniary costs and time required to implement. Although the supplementary guidance for

clinical-grade pluripotent stem cell lines will proffer more rigorous standards, the same approach is being taken to offer a workable and most cost-effective way to gaining regulatory approval for the production of clinically-compliant biologics.

ISCBI's approach to facilitate the adoption of the standards outlined in the guidances extends to developing a questionnaire for operators to appraise themselves [20]. By performing what is essentially a gap analysis to determine the extent to which a bank or laboratory complies with the procedures and practices endorsed by ISCBI, it is anticipated that they will be encouraged to address any shortfalls and enhance standardization.

Besides ISCBI's efforts, there is an important role for institutional governance to support and promote best practice by researchers, resource managers, and distribution centers, perhaps as a requirement under policy. Similarly, other influential bodies such as granting agencies and publishing houses could require universal compliance with standards to gain access to funding and publish in journals [20].

4 Conclusion

The establishment and implementation of common standards for providing stem cells for research and translation is a global imperative. While many aspects of delivery from the point of harvesting primary cells to providing stem cells suitable for commercial/clinical use are continually evolving (e.g. somatic cell reprogramming for deriving iPS cells, and hES/iPS cell culture and cryopreservation) many processing steps are sufficiently developed beyond proof-of-concept and able to be selected for standard application. This is not to say that standards cannot be changed but rather a process or procedure is deemed optimal, reproducible, and fit-for-purpose, with the potential to upgrade or update if necessary.

The standards setting group of ISCBI has taken the lead in defining procedural guidelines that are essential to the provision of quality-controlled human pluripotent stem cells, and applicable to human stem cells in general. It is hoped that addressing current variation in transnational standards in stem cell banking and research will facilitate best practice in the procurement and maintenance of existing and future stem cell resources and international collaboration. Importantly, the guidelines together with other relevant standards recommended in this chapter and elsewhere are intended to advance rather than restrict stem cell research and development. It is clear that by establishing and implementing standards, now the field will more quickly and cost-effectively translate efforts to develop stem cells for medicine and other applications dependent on quality assurance, regulatory approval, and public acceptance.

References

1. ISCBI. Consensus guidance for banking and supply of human embryonic stem cell lines for research purposes. Stem Cell Rev. 2009;5:301–14.
2. Crook JM, Hei D, Stacey G. The International Stem Cell Banking Initiative (ISCBI): raising standards to bank on. In Vitro Cell Dev Biol Anim. 2010;46:169–72.
3. Crook JM, Peura TT, Kravets L, Bosman AG, Buzzard JJ, Horne R, Hentze H, Dunn NR, Zweigerdt R, Chua F, Upshall A, Colman A. The generation of six clinical-grade human embryonic stem cell lines. Cell Stem Cell. 2007;1:490–4.
4. Querol S, Gomez SG, Pagliuca A, Torrabadella M, Madrigal JA. Quality rather than quantity: the cord blood bank dilemma. Bone Marrow Transplant. 2010;45:970–8.
5. Luong MX, Auerbach J, Crook JM, Daheron L, Hei D, Lomax G, et al. A call for standardized naming and reporting of human ESC and iPSC lines. Cell Stem Cell. 2011;8:357–9.
6. Laurie G, Bruce A, Lyall C. The roles of values and interests in the governance of the life sciences: learning lessons from the 'Ethics+' approach of UK Biobank. In: Lyall C, Papaioannou T, Smith J, editors. The limits to governance: the challenge of policy-making for the new life sciences. Aldershot: Ashgate; 2009. p. 51–77.
7. Human Tissue Act. 2004. http://www.legislation.gov.uk/ukpga/2004/30/contents
8. EU tissue and cells directive. 2004. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:102:0048:0058:en:PDF
9. Isasi R, Knoppers BM. From banking to international governance: fostering innovation in stem cell research. Stem Cells Int. 2011;2011:498132.
10. Chatterjee R. Cell biology. Cases of mistaken identity. Science. 2007;315:928–31.
11. ATCC. Cell line misidentification: the beginning of the end. Nat Rev Cancer. 2010;10:441–8.
12. Buta C, David R, Dressel R, Emgard M, Fuchs C, Gross U, et al. Reconsidering pluripotency tests: do we still need teratoma assays? Stem Cell Res. 2013;11:552–62.
13. OECD. Organisation for Economic Co-operation and Development Directive 2004/10/EC, principles of good laboratory practice (GLP). J Eur Union. 2004;50:44–59.
14. USFDA. Code of Federal Regulations (CFR) 21, Part 58: good laboratory practice for nonclinical laboratory studies. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?CFRPart=58
15. Coecke S, Balls M, Bowe G, Davis J, Gstraunthaler G, Hartung T, et al. Guidance on good cell culture practice. a report of the second ECVAM task force on good cell culture practice. Altern Lab Anim. 2005;33:261–87.
16. OECD. Organisation for Economic Co-operation and Development Directive 2004/23/EC, Standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells. J Eur Union. 2004;2102:48–58.
17. OECD. Organisation for Economic Co-operation and Development Directive 2004/17/EC, technical requirements for the donation, procurement and testing of human tissues and cells. J Eur Union. 2006;38:40–52.
18. Hug K. Banks, repositories and registries of stem cell lines in Europe: regulatory and ethical aspects. Stem Cell Rev. 2009;5:18–35.
19. Liddell K, Wallace S. Emerging regulatory issues for human stem cell medicine. Genomics Soc Policy. 2005;1:54–73.
20. Stacey G, Crook JM, Hei D, Ludwig T. Banking human induced pluripotent stem cells: lessons learned from embryonic stem cells? Cell Stem Cell. 2013;13:385–8.

Chapter 2
The Challenge of Standardization in Stem Cell Research and Development

Glyn N. Stacey

1 Introduction

Pluripotent stem cell lines provide an unprecedented opportunity to generate large numbers of cells capable of differentiating into cell types of value in research, therapy, and product testing. However, they also show a degree of variability in the phenotype of their undifferentiated state as they are passaged in vitro, are subject to variable levels of the so-called spontaneous differentiation and also show different responses between cell lines when subjected to the same differentiation protocols [1, 2]. In addition, there continues to be very rapid development in our knowledge of their cell biology and new technology for their growth and characterization. As a consequence, few would argue against the assertion that standardization in stem cell research and the development of cell therapies is a good thing. However, there is the possibility for conflict to arise in approaches to standardization between basic research and product development, which could cause confusion and delay in smooth translation from research discoveries to clinical and industrial applications. The word "standard" can be used to refer to a document written to provide guidance or requirements under certain formal quality standards but may also refer to a physical preparation of a material to enable comparison of the properties of similar materials in different laboratories or in the same laboratory at different times [3]. In this chapter I will try to convey in summary, the key differences in the way in which "standardization" is applied in these two different settings, but focusing on the early stages of standardization for the development of appropriate seed stocks of pluripotent stem cell lines that may be used for protocol development and final manufacturing of cell therapies.

G.N. Stacey (✉)
UK Stem Cell Bank, National Institute for Biological Standards and Control,
South Mimms, Blanche Lane, Potters Bar, Hertfordshire EN6 3QG, UK
e-mail: Glyn.Stacey@nibsc.org

D. Ilic (ed.), *Stem Cell Banking*, Stem Cell Biology and Regenerative Medicine,
DOI 10.1007/978-1-4939-0585-0_2,

2 Phases of Standardization

Standardization for cell-based or cell-derived products, in general, has a number of distinct phases, which include:

1. Confirming the key attributes of selected candidate cell sources and using a range of analytical techniques to compare them to enable final selection of the cell source of preference.
2. Establishment of standardized scale-up and analytical systems for the bioprocessing of the cells to make the therapeutic product including stable expansion of undifferentiated cells.
3. Establishment of standardized assays and reference materials to assess the key safety and efficacy properties of the final therapeutic product.

Each stage involves a process of setting definitions or specifications for the cells and conditions and procedures to handle and grow them, and then establishing relevant controls to ascertain how each culture adheres to these specifications. Some of the terms used for important attributes of cells during the first stage are nominally the same as those used in later stages of standardization as will be outlined later. However, it is important to recognize that these same terms can mean very different things for the definition and control of starting material versus final product (see below).

Standardized characterization of human induced pluripotent cell (hiPSC) and human embryonic stem cell (hESC) lines for research purposes, has been used to establish the key generic properties of each of these cell types. Examples of such studies include those performed by the International Stem Cell Initiative (www.stem-cell-forum.net/), which carried out multi-center characterization of both hESCs and hiPSCs [4, 5]. The first of the studies showed that hESC in general, have common characteristics in terms of expression of certain self-renewal genes and surface markers [4] and the study achieved this by the use of common protocols for culture, sampling, and characterization. The generation of a consistent or standardized cell bank involves a very different perspective on standardization which aims to assure that each individual vial, on recovery, will yield a culture of cells that have the generic features of the cell line and ideally express these within certain measured and predictable limits.

3 Early Evaluation of Stem Cell Lines

Early evaluation and selection of cell lines for research, product manufacture, diagnostics, and cell therapies are all based upon certain fundamental attributes as follows:

- Purity (absence of adventitious agents and cells of different origin)
- Identity (authentic genotype and phenotype as originally described)

- Performance (functional response or expression)
- Stability in vitro (degree of phenotypic and genotypic variation that occurs on passage or use in different culture environments)

In addition to these, where cells are intended for cell therapy a further key characteristic will be their ability to form or cause tumors i.e., tumorigenicity and oncogenicity.

It has been observed that much of the research using hESC lines has been performed on relatively few individual cell lines [6]. Whilst this should facilitate a high degree of standardization to enable direct comparisons of data from different labs, it also raises certain scientific concerns. Firstly, literature will record the specific traits enshrined within the original donated cells and passage of the cells between labs may ultimately result in changes in the properties of the cells, inadvertent switching of the cell lines with others, or microbial contamination, clearly each event could alters the characteristics of the cells. Secondly, it may be that the characteristics of the selected cells are not necessarily completely representative of the cell type they are used to represent and thus misleading conclusions may be drawn from research performed on a very limited number of cells lines. In the development of cell culture-based research, it is best practice to begin any new research project by comparing results from a number of cell lines to identify the one which gives the best in vitro model of in vivo response. This process of selection involves the early stage evaluation already mentioned which requires completion of generic quality control criteria and also specific characterization.

4 Early Stage Evaluation and Characterization of Stem Cell Lines

4.1 Purity

It is important to check all cell lines for the common laboratory contaminants. General bacterial and fungal contamination will often be apparent due to dramatic changes in pH of the culture medium, its turbidity or appearance of microbial colonies. In addition another common lab contaminant, mycoplasma, can cause dramatic physiological and genetic changes in cell cultures. However, these organisms are often not detected in screening for bacterial contamination and special testing should be applied on a routine basis [7]. A variety of other organisms may arise such as *Achromobacter* spp. and *Mycobacteria avium* and *Leptospira* spp. may also, on rare occasions, establish persistent, yet difficult to diagnose, contamination and in such cases routine microscopic inspection of culture may be the only means to identify them.

4.2 Identity

One of the understated challenges for research based on cell lines is the incidence of inadvertent switching or cross-contamination of cell lines, a problem that has dogged the field of cancer research for decades and could also have significant effects on stem cell research [8]. It is readily resolved by performing DNA profiling (typically by STR genotyping) that will give a "bar code" specific to the donor of origin. Publication of such data should be shared amongst researchers and stem cell resource centers but open publication will need to be considered carefully to avoid potential exposure of donor identity [9].

A study already referred to [4] resolved the typical phenotypic features of hESCs including expression of five self-renewal related molecules, and it has been widely demonstrated that hiPSCs have the same characteristics. However, the markers commonly used (e.g., SSEA-3/4, TRA-1-60, TRA-1-81) are not necessarily specific for pluripotent stem cell lines and an overall picture of the characteristics and functionality of these cells is necessary to provide an unequivocal identification of the cell type.

4.3 Performance

This aspect of stem cell characterization has proved especially challenging. The key feature to be investigated is the pluripotency of the cell culture but this can prove difficult to replicate for different cell lines using a defined set of protocols to direct cells into the three germline lineages required to generate all cells of the human body. It may even prove difficult to replicate the same results from the same differentiation protocol used in different labs. Moreover, it can be difficult to elucidate whether these differences arise in the specific technical procedures used or the cell lines themselves. The ability of pluripotent stem cell lines to form benign teratomas in immune-compromised mice has been the reference method for identifying the property of pluripotency in human cell lines. However, there are fundamental technical problems with this assay in terms of reproducibility [10]. Furthermore, confirmation that pluripotency is retained throughout experimental studies would prove prohibitively costly and would be ethically untenable [11].

Alternative in vitro assays such as formation of embryoid bodies containing cells representative of all three germ layers and differentiation directed by small molecules have become widely used routine, but are still time consuming and challenging to establish appropriate controls. More recently, concerns have been raised over the use of markers of self-renewal and undifferentiated state as surrogates for pluripotency (see the workshop report Requirements for Establishment of iPSC resources under the International Stem Cell Banking Initiative (ISCBI) at www.stem-cell-forum.net/). Epigenetic profiling using arrays for analysis of epigenetic status of many genes is also seen as potential indicator of pluripotency [12] and there are

proposed rapid in vitro methods which use micro-patterned surfaces to stimulate lineage commitment that can be rapidly measured as published by Nazareth et al. [13]. However, it seems that a conclusion has yet to be drawn on a simple and accurate predictor of potential pluripotency and it may be that a combination of molecular and in vitro tests will be needed to provide a screen that can be used to confirm potential pluripotency of cells used in routine experimental work.

The author and colleagues have already described elsewhere in detail the specific testing procedures involved in quality control, characterization, and selection of pluripotent stem cell lines [7, 14].

4.4 Stability

Genomic stability in pluripotent stem cell lines is a much debated issue. This is generally due to the potential for it to give rise to transformed and potentially tumorigenic cells, which might persist in preparations of stem cell lines intended for patient therapy. However, the veracity of research data and its relevance to in vivo cell biology, could also be significantly affected by use of cells which have undergone certain genetic changes. It is obvious that no cell culture is absolutely genetically stable and stem cell lines, both iPSC and hESC, show genetic change in vitro culture as do other cell cultures [15]. However, a critical issue is to be able to discriminate between (1) natural copy number variations that may be consistent with the donors genome, (2) neutral mutations arising in cell culture that have no obvious phenotypic effect, (3) mutations which appear to be associated with the adaptation of stem cell cultures to in vitro passage and (4) those arising during cell expansion which might be associated with tumor development. It is possible that there is "grey scale" between these different types although changes in some genes may be of greater significance regarding potential transformation events.

Low level, abnormal clones in stem cell lines are not unusual [16] and if they are bestowed with a growth advantage due to other genetic changes they may rapidly dominate and replace all the cells within a culture just within a few passages. Human pluripotent cell lines are also known to have instability in their epigenetic profiles during cell culture [17]. It is clearly not feasible to constantly check genetic stability and typical general indicators of genetic and epigenetic stability such as karyology or array CGH are used at key points in the passage history of the cell line such as frozen stocks or cell banks.

5 Bioprocessing and Management of Stem Cell Banks

As already mentioned the stem cell field is a very dynamic environment with new discoveries and technologies appearing regularly. Standardizing the actual process of culturing and expanding pluripotent stem cells and their differentiation are

extremely challenging. Even if standardized protocols are used, minor procedural deviations and variability of reagents create subtle yet potentially significant influences on cells that are yet to be fully understood. A common approach to manage some of this variability is the use of a common cell line in different experiments and between collaborators. The cell line 2102Ep has been proposed as a control cell line and whilst this line is nullipotent (i.e., cannot produce differentiated progeny) it stably expresses the key markers of pluripotent stem cell lines and can be used as a control for certain characterization methods. The clone D of this line was successfully used as a control line, distributed from central stocks established at the UK Stem Cell Bank, in the characterization work done in 17 different labs around the world as part of the International Stem Cell Initiative [4].

Even though researchers may gravitate towards the use of a relatively limited number of growth media, the variables in culture protocols, feeder cells, and culture surfaces mean that this is still not an area likely to become completely standardized in the near future. The field is still resolving the best way to grow cells stably and differentiate them reliably and efficiently. Control lines are therefore also useful to reflect the impact of a particular culture or differentiation process. The ISCI project has used standardized protocols and the control hESC line H9 in comparisons of different serum- and feeder-free culture systems [18] and is currently using the same hESC H9 stock as a functional biological control in a multi-laboratory comparison of different assays of pluripotency (www.stem-cell-forum.net/). Of course potential variation between cell lines may mean that trends observed in use of H9 may not necessarily reflect the data obtained with other lines. Accordingly, the use of a range of cell lines, in addition to a common shared line, will be valuable.

Higher level standardization of banking procedure and governance is also beneficial to assure use of acceptable norms in stem cell research. The ISCBI coordinates activities and interests in stem cell banking in more than 20 countries. The ISCBI has developed a consensus on principles of best practice in the procurement, banking testing, storage, and shipment of human pluripotent stem cells and details of this activity are given in Chap. 1.

6 Later Stage Fundamental Issues for Standardization of Cell-Based Products

In order to assure the quality and safety of biological medicines the international community World Health Organization (WHO) has agreed certain ways of measuring the active properties of medicines, which are described by the three fundamental criteria of identity, purity, and potency [19]. But what could these mean for cell therapies? Identity may be interpreted as the key markers of the active therapeutic cell type, but this may not be clear as often the cell therapy may be composed of a mixed population of potentially therapeutic cell subtypes and may change with in vitro passage. Purity

may also be a complicated concept for cell therapies for the same reasons. Quantifying what represents and the acceptable level of purity or diversity of potentially therapeutic cell types may be difficult. Potency is probably the most complicated characteristic to specify and quantify as the biological activity and efficacy is more complicated in its interaction with the patient, and assays to predict efficacy will probably require complex functional cell culture assays. In addition, there may be apparent "contaminant" cells or impurities, which are not evidently part of the therapeutic population but could significantly influence the potency of the product in either a positive or negative way.

In more typical biological medicines such as vaccines and biotherapeutics, the characteristics of potency are tested for individual batches of products through the use of international reference materials to standardize the assays used to measure potency (www.nibsc.org). In the case of cell therapies, the exact need for reference materials has yet to be elucidated clearly. Preparations of cellular RNA may provide useful reference materials for molecular characterization of products expression profiles and fixed cells could provide useful controls for flow cytometry quantitation of cell composition of cell therapies. However, the reference materials that will be needed to control cellular function as opposed to markers of identity, will take some time and effort to consider and develop.

Clearly whilst the characteristics here are very similar to those involved in the early stages of cell line evaluation, the definitions are distinct and it is important for those researchers and clinicians involved in early translational research to be aware of these differences so they can make a smoother translation into generation of cell therapy products.

7 Key Issues for the Future

The scientific community is currently preparing to generate large hiPSC line resources of potentially thousands of lines to provide genotypes of value in research and "haplotype" banks for clinical therapy. In these endeavors it will be essential to develop cell culture automation systems and rapid methods for quality control and characterization. These systems will all require qualification against standard methodologies and new standardization approaches. In addition, the application of new molecular technologies such as next generation sequencing and digital PCR, will require careful standardization to ensure the data generated is meaningful, accurate, and reliable. The massive datasets that will be generated will also require careful management to ensure their quality and fitness for high quality research and clinical utility. In conclusion it is vital that standardization approaches are employed throughout the development of stem cell lines, however, researchers and clinicians should be aware of the key differences in approaches to standardization as cells are progressed toward therapeutic products.

References

1. Osafune K, Caron L, Borowiak M, Martinez RJ, Fitz-Gerald CS, Sato Y, Cowan CA, Chien KR, Melton DA. Marked differences in differentiation propensity among human embryonic stem cell lines. Nat Biotechnol. 2008;26:313–5.
2. Koyanagi-Aoi M, Ohnuki M, Takahashi K, Okita K, Noma H, Sawamura Y, et al. Differentiation-defective phenotypes revealed by large-scale analyses of human pluripotent stem cells. Proc Natl Acad Sci U S A. 2013;110:20569–74.
3. Sheridan B, Stacey G, Wilson A, Ginty P, Bravery C, Marshall D. Standards can help bring products to market. Bioprocess Int. 2012;10:18–20.
4. International Stem Cell Initiative, Adewumi O, Aflatoonian B, Ahrlund-Richter L, Amit M, Andrews PW, et al. Characterization of human embryonic stem cell lines by the International Stem Cell Initiative. Nat Biotechnol. 2007;25:803–16.
5. International Stem Cell Initiative, Amps K, Andrews PW, Anyfantis G, Armstrong L, Avery S, et al. Screening ethnically diverse human embryonic stem cells identifies a chromosome 20 minimal amplicon conferring growth advantage. Nat Biotechnol. 2011;29:1132–44.
6. Löser P, Schirm J, Guhr A, Wobus AM, Kurtz A. Human embryonic stem cell lines and their use in international research. Stem Cells. 2010;28:240–6.
7. Stacey GN. Quality control of human stem cell lines. In: Masters J, Palsson B, Thomson J, editors. Human embryonic stem cells, Human cell culture, vol. 6. New York: Springer; 2007. p. 1–22.
8. MacLeod RA, Dirks WG, Matsuo Y, Kaufmann M, Milch H, Drexler HG. Widespread intraspecies cross-contamination of human tumour cell lines arising at source. Int J Cancer. 1999;83:555–63.
9. Gymrek M, McGuire AL, Golan D, Halperin E, Erlich Y. Identifying personal genomes by surname inference. Science. 2013;339(6117):321–4.
10. Müller FJ, Goldmann J, Löser P, Loring JF. A call to standardize teratoma assays used to define human pluripotent cell lines. Cell Stem Cell. 2010;7(6):412–4.
11. Buta C, David R, Dressel R, Emgård M, Fuchs C, Gross U, et al. Reconsidering pluripotency tests: do we still need teratoma assays? Stem Cell Res. 2013;11:552–62.
12. Müller FJ, Schuldt BM, Williams R, Mason D, Altun G, Papapetrou EP, et al. A bioinformatic assay for pluripotency in human cells. Nat Methods. 2011;8:315–7.
13. Nazareth EJ, Ostblom JE, Lücker PB, Shukla S, Alvarez MM, Oh SK, Yin T, Zandstra PW. High-throughput fingerprinting of human pluripotent stem cell fate responses and lineage bias. Nat Methods. 2013;10:1225–31.
14. Stacey GN. Sourcing human embryonic stem cell lines. In: Sullivan S, Cowan C, Eggan K, editors. Human embryonic stem cells: a practical handbook. New Jersey: John Wiley & Sons; 2007. p. 11–24.
15. Liang G, Zhang Y. Genetic and epigenetic variations in iPSCs: potential causes and implications for application. Cell Stem Cell. 2013;13:149–59.
16. Peterson SE, Westra JW, Rehen SK, Young H, Bushman DM, Paczkowski CM, et al. Normal human pluripotent stem cell lines exhibit pervasive mosaic aneuploidy. PLoS One. 2011;6:e23018.
17. Tompkins JD, Hall C, Chen VC, Li AX, Wu X, Hsu D, Couture LA, Riggs AD. Epigenetic stability, adaptability, and reversibility in human embryonic stem cells. Proc Natl Acad Sci U S A. 2012;109:12544–9.
18. International Stem Cell Initiative Consortium, Akopian V, Andrews PW, Beil S, Benvenisty N, Brehm J, et al. Comparison of defined culture systems for feeder cell free propagation of human embryonic stem cells. In Vitro Cell Dev Biol Anim. 2010;46:247–58.
19. ICH Topic Q 6 B Specifications: Test Procedures and Acceptance Criteria for Biotechnological/ Biological Products. Notes for guidance on sopecifications : test procedures and acceptance criteria forbiotechnological/biological products. September 1999 CPMP/ICH/365/96

Chapter 3
Global Perspective on Cord Blood Stem Cell Banking

Sergio Querol

1 Historical Background

1.1 Public Versus Private Cord Blood Banks

Public cord blood banks (CBBs) aim to store highly qualified and diverse units to help patients needing bone marrow transplantation to cure their diseases and for whom no donors are available. These banks are usually not for profit entities, which brings an equitable access to the therapy. In addition to these, a large number of private CBB have also been established. Their aim is storing CB for autologous or family use exclusively for a potential future application. In this setting, cost of storage is covered by donors, making such banks for-profit organizations with more solid economical structures. In consequence, there are far more units stored worldwide for private use, in the region of millions, than for public banking, with almost 600,000 units available from publicly accessible lists of inventories.

A trend to associate these two activities that are different in nature because they share the same biological product and cell processing technology has generated controversy. But in contrast to public CBBs, the clinical benefit of private CBBs remains to be proven. Private CB collection is usually offered as a life insurance for baby's future needs, using a long list of potential (current) indications of haematopoietic stem cell transplantation. This can mislead parents since in most of the listed diseases an autologous transplantation may not be the right indication. Professional

S. Querol (✉)
Barcelona Cord Blood Bank, Programa Concordia, Banc Sang i Teixits, Passeig Taulat 116, Barcelona 08005, Spain

Anthony Nolan Cord Blood Program, Nottingham, UK

Cord Blood Working Group, World Marrow Donor Association, Leiden, The Netherlands
e-mail: squerol@bst.cat; sergio.querol@anthonynolan.org

D. Ilic (ed.), *Stem Cell Banking*, Stem Cell Biology and Regenerative Medicine,
DOI 10.1007/978-1-4939-0585-0_3,

organizations and ethical groups question the beneficence of this initiative. Private banks are criticized for the methods used in approaching and informing donors, and whether the respect for autonomy principle is upheld. Furthermore, the therapy being sold is far from being guaranteed as successful. If future stem cell therapy treatments are developed, there is no guarantee that current processing and storage methods will be directly applicable. For example, many privately stored units have very low cellular content making them inappropriate for current recommended therapy. Moreover, many banks operate without the defined quality criteria required by accreditation standards schemes.

Quality is an area of mutual interest. Common standards that address aspects of procurement, processing, testing, and banking for both public and private CBBs are available. Also, there is a common need to make efforts in intensifying research in order to improve current clinical outcomes but also to foresee other areas of application mainly in the so-called regenerative medicine field. Newly explored models of public–private partnership, the hybrid CB banks, are currently being proposed, but such partnerships are notably driven by the need to improve the finances of public CBBs. These initiatives should share benefits to help research on therapeutic use of CB cells. Undoubtedly [more formal], sibling (directed) banking for a family patient in need shall be regularly offered when there is a clear indication for hematopoietic stem cell transplantation as it usually is by public CBBs.

The text that follows will focus on the public banking activity that has allowed the transplantation of more than 30,000 patients in contrast to a few dozen units used from private CB banks.

1.2 Development of Public Cord Blood Banking

Supported by seminal biological work [1], the first successful CBT took place in 1988 in Paris [2]. Encouraged by early results in sibling CBT, CBBs have been created all over the world and are now part of the worldwide network of organizations that aim to provide unrelated donor grafts to transplant centers (TCs), regardless of their location.

Amongst the approximately 1,000,000 hematopoietic progenitor cell (HPC) transplantations done at the end of 2012, 30,000 have been done with CB, mainly from unrelated donors from the almost 600,000 Cord Blood Units (CBUs) listed in Bone marrow Donors Worldwide (BMDW; www.bmdw.org) [3]. CBT currently provides 20 % of unrelated allogeneic HPC transplants. There are more than 100 CBB, 40 of them currently accredited by the Foundation for the Accreditation of Cellular Therapy (FACT)-NetCord certification scheme (www.factwebsite.org).

From the last 25 years to the present day, CB has shown its specific advantages, offering a rapidly available HPC source, easily identified by its human leucocyte antigens (HLA) and biological characteristics and deliverable to any TC in a matter of days. Although initially used mainly for children, CB has been shown to be a suitable option for transplantation in adults also, while matching rules and selection criteria have been evolving due to large clinical studies [4–8]. CBT allows the use

of non-matched grafts that increase the efficacy of the inventories in providing donors. The minimum matching level recommended is 4 out of 6, considering HLA-A and -B and antigen level and -DRB1 at allele level. Currently CBUs are more frequently used for adults than for children.

In parallel to these clinical CBT milestones, FACT and the American Association of Blood Banks (AABB; www.aabb.org) have done considerable work to establish good standardized practice through the creation of specialized banking standards. Meanwhile, an increasing number of CBBs have gone through voluntary accreditation in order to align their practices to the best international requirements. Nowadays, in most countries, CBBs and CBUs need to be recognized by their national health authorities (i.e., EU registration at competent authorities, USFDA Biologics License Applications), making CB a cellular therapy product with very stringent quality requirements.

2 Current Situation

Unrelated CBT activity started in 1993. Since the first cases were published in 1996 [9], CBT increased steadily first in pediatric and later also in adult patients. CBT in adult patients eventually surpassed CBT in pediatric patients in 2006. World unrelated transplantation in children remains constant since 2007 at the level of 1,250 CBT per year. Unrelated CBT in adult patients has continued growing until 2,750 procedures per year as reported by the World Marrow Donor Association (WMDA; www.worldmarrow.org) in 2010. This figure has remained constant since then. Last year, in spite of a significant decrease in CBT activity in Europe and US, the number was balanced by the particular growth in Japan (with more than 1,000 CBT/year), Korea, France, Mexico, and UK. Figure 3.1 shows CBT activity reported to the WMDA since 1995.

This activity is in contrast to the growth of the international inventory of units available for transplantation. Figure 3.2 shows the number of units registered in the international inventory of public CBUs (BMDW) where a continuous growth of units, surpassing those used, is registered. Average growth in the last 3 years when the CBTs are stabilized is 10 %.

In addition to this disproportionate growth, the units used by transplant center are not the same as those preferentially banked (Fig. 3.3).

3 Updating Current Inventory to Meet Clinical Needs

3.1 Off-the-Shelf High Quality Units

One of the most attractive concepts when referring to CB is that of being an "off-the-shelf" therapy. At the time the therapy is needed there are no uncertainties related to donor availability. In the case of adult donors, there are two major risk steps: one, ethics related to the donor regarding potential adverse events; the other, biological risk related to donor issues during collection that may compromise a quality harvest when patients are normally conditioned.

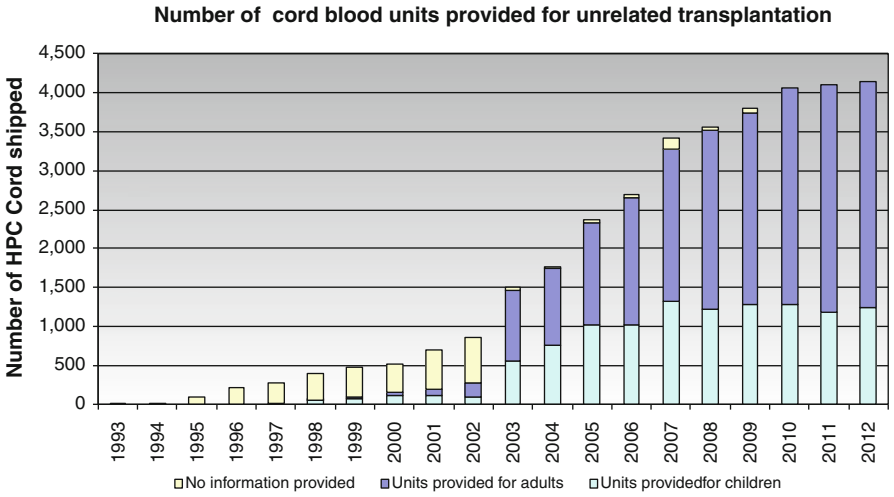


Fig. 3.1 CBT activity reported to the WMDA since 1995. Number of CBU provided for unrelated transplantation, courtesy of WMDA

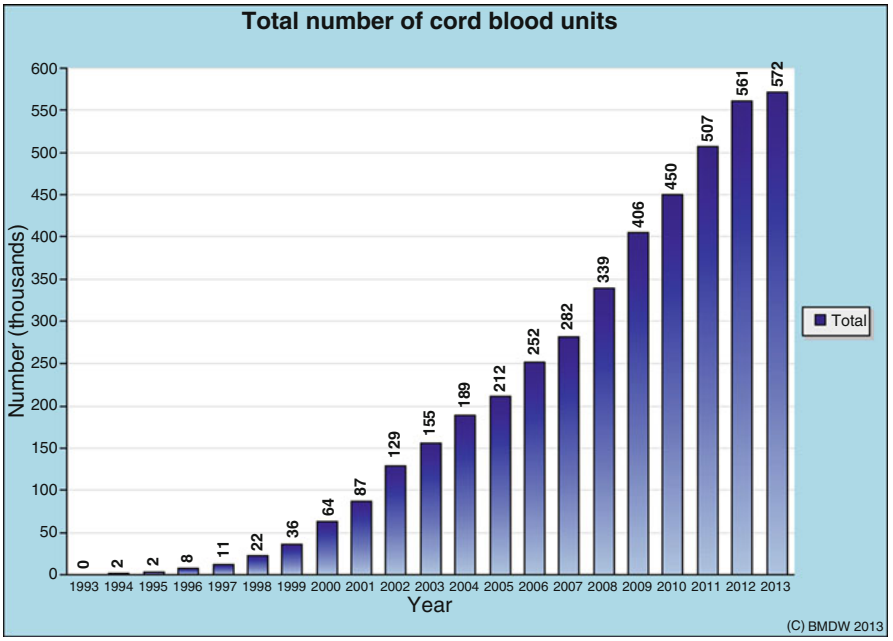


Fig. 3.2 Total number of UCBU registered at BMDW (from www.bmdw.org, May 2013)

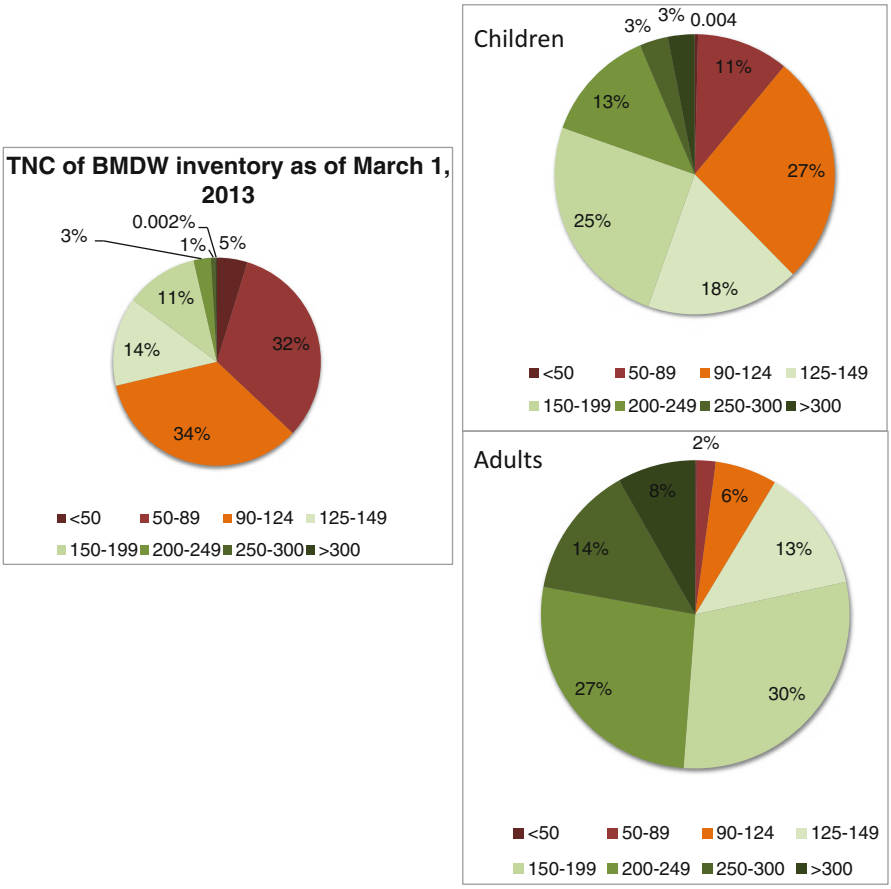


Fig. 3.3 Distribution of units available in the international public inventories and those used for transplantation. Figure shows 2012 data. *Left*: Total nucleated cell (TNC) of BMDW inventory as of January 01, 2013. *Right*: TNC of CBU products shipped in 2012 for children (*above*) and adults (*below*)

To achieve "off-the-shelf" goal a CBB must offer guaranteed, safe, and reliable products. These products need to be well qualified in front and immediately available. This means that more upfront investment is necessary and consequently the units produced must be those with highest probability of being used. These units are referred to as High Quality Units (HQU). Within these units of particularly high cell count (above 200×10^7 TNCs), are rapidly depleted from the inventories, and it is especially important replacing them constantly. These units need to be collected from groups of very common HLA phenotypes because they are practically used in urgent protocol where a 4 out of 6 match could be enough to obtain good transplantation outcomes (i.e., in sequential consolidation therapies in high risk leukemia). Therefore, it is necessary to specifically define size and characteristics of the High Cellular Common Phenotype Units (HCCPU) for fast-track 4 out of 6 matched CBTs.

3.2 Operational Inventory (OI)

Each CBB has needs according to their financial situation and the expected number of units for transplantation defines the size of their operational inventory. All units belonging to this operational inventory should have the following test performed upfront. Our current criteria defining a HQU are:

- Safety:
  - Eligibility of medical background, risk behaviors, and travel history.
  - Testing including infectious disease markers (HBV, HCV, HIV-1 and -2, HTLV-I and -II, CMV, EBV, and Toxoplasmosis), bacteria and fungi, genetic hemoglobin screening.
- Identity:
  - CB HLA -A, -B, -C (intermediate two digit and National Marrow Donor Program/NMDP/codes or better), and -DRB1 (high four digit). Maternal HLA-A,-B and -DRB1 by low resolution.
  - ABO and Gender.
  - Reference Samples: maternal/cord plasma and serum, and DNA samples.
  - Contiguous segment: one minimum to ship with the unit.
- Purity:
  - Cell dose and $CD34^+$ enumeration, and red blood cells (RBC) content post-processing.
- Potency:
  - Clonogenic efficiency (CLONE) of $CD34^+$ cells: >10 % (preferable pre-freeze or alternatively from a contiguous segment).
  - Viability of $CD34^+/CD45^+$double positive cells as determined with a dye 7-aminoactinomycin D (7-AAD) pre-freezing: >85 %.
  - Immediate viability ($CD34^+$) and cell yield post-thawing of a contiguous segment within normal range.

So, the foundations to generate an updated CB inventory are:

- Collecting highly diverse donations (shifting towards minorities).
- Upfront testing of new and banked HQUs.
- Promoting immediate releasing tests (for instance developing a functional flow cytometry).
- Simplifying the access to users (an adult volunteer donor center needs to protect donors from direct access by the users, which is not the case of CBU that are actually off-the-shelf cell therapy products). For that reason it is necessary to promote:
  - Directly web accessible inventories.
  - No cost associated with donor selection and extended testing (to facilitate selection of the best units).

– To facilitate the widespread use of double CBT, protocols promote the "costing per transplantation procedure" instead of "costing per unit" as it occurs with adult volunteer collections.

4 Sustainability

4.1 The Growth Paradox

The growth paradox can be formulated as following: After a lineal growth of units provided to transplantation over units added to the inventory (up to 10,000), there is a progressive slowing down of this efficiency probably due to the match of less predominant HLA phenotypes (Fig. 3.4). This means a significant decrease in the efficacy of the inventory once it has reached over 10,000 CBUs. Despite the total number of patients benefiting from this inventory increase, the cost per unit provided also increases but to a higher proportion making the growth unaffordable. According to this projection, the cost of a unit provided from an inventory of 50,000 is more than two-times higher than the one provided from an inventory of 10,000 after compensating for a higher capacity of exporting unit to other countries.

In the model presented here, a CBB bank of 10,000 offers cheaper units but has only a possibility of transplanting one third of local patients relying then in importing two thirds of patients. On the contrary, a CBB of 50,000 results in doubling the cost per unit offered but is able to provide local units for two thirds of the patients, relying only on one third from international transactions.

4.2 Confronting the Cost of CB Transplants

4.2.1 Value of the Inventoried Units

As shown in Table 3.1, and stratifying by frozen TNC dose, there are defined qualities (Q categories) that will carry different usage rates. Accordingly, CBUs with more than 200×10^7 TNC have a 9.01 % yearly likelihood of being used. By contrast, units below 90×10^7 have a probability of use of 0.04 % per year. Taken together, I propose the definition of operational inventory that with a likelihood of use above 1 % per year.

4.2.2 Production Costs

CBB is becoming increasingly expensive mainly due to the stringent regulation required to guarantee the production of very reliable products. Production costs are too high to maintain this activity in the long-term and there is a need to optimize resources in order to decrease these costs.

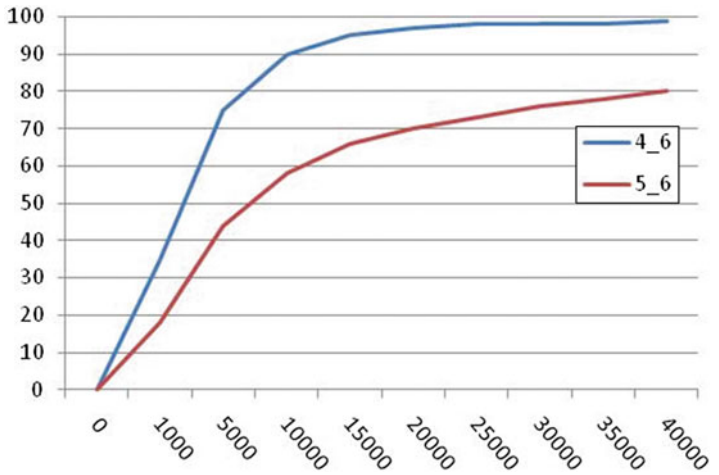


Fig. 3.4 Probability of finding at least one donor for each match level according to current criteria (match at antigen level for HLA-A and B and allele level for HLA-DRB1)

Table 3.1 Quality categories of the units in the inventory according to the yearly usage rate (data from WMDA corresponding to the year 2010)

Q category	Numbers	Usage (%)
<90	214,200	0.04
90–125	168,300	0.34
125–150	61,200	1.19
150–200	51,000	2.54
>200	15,300	9.01
Global	510,000	0.79

The table below (Table 3.2) shows the production cost at Barcelona CBB as a reference point.

Costs can be split by:

- *Collection program*. Cost per listed unit includes the corresponding cost of the 80 % of the units collected yet not finally banked due to a total cell dose below the required threshold (>120 TNC and $>4\times10^6$ $CD34^+$ cells). Thus, the cost of the donation program per unit listed is €310.
- *Production cost*. This results from the addition of processing costs (volume reduction and cryopreservation) and testing. Also, human resources used are added into this section. In our case a total of 8 full time equivalent posts per year are required to produce 1,000 CBUs. The overall cost is €200 for processing and €255 for testing and €333 for staffing. The total production cost per unit listed in the inventory is €788.
- *Management*. This concept may vary substantially between banks. In our case, the management cost per unit listed is €385, including a 10 % overhead.

Table 3.2 Production costs at Barcelona CBB for 12 full-time equivalents (FTEs) and 1,500 listed units per year (values in Euros)

Manufacturing cost	€	20 %
Collection	62	310
Disposable	12	
Transport	30	
HRs	20	
Processing		533
Volume reduction disposable	150	
Cryopreservation disposable	50	
HRs	333	
Testing		255
HLA (A, B, C, DRB1)	150	
Virology	35	
Sterility	15	
Hemoglobin	10	
Cell counting	5	
Flow cytometry	30	
CFU	10	
Management		385
Amortization (equipment/building)	200	
Equipment maintenance	50	
Overheads (10 %)	135	
Total	Euros	1,483

4.2.3 Procuring Costs and the Special Case of Double CBT

The storage of only operational units will result in a usage rate of 2.7 % per year. This proportion is the minimum amount acceptable to provide a procuring cost below €20,000 per unit if the producing cost of each banked units is €1,500 and a depreciation time of 5 years.

In summary, to maintain procuring costs below €20,000 per unit, we need to plan so that our bank will store only units belonging to a defined operational inventory (CBUs cryopreserved with more than 120×10^7 TNC). CBUs stored with cell dose above 120×10^7 TNC should be 10,000. Growing above this figure will increase procuring costs. In addition, to accomplish this goal, CB banks need to decrease production cost substantially, probably half the current production cost. Any other scenario will require external private or public funding to compensate the excess cost and to ensure the affordability of CBT as an alternative access to a curative therapy.

Finally, one particularly perturbing situation is the unaffordable cost for procedures requiring a double CBT protocol. Currently, a patient pays twice for the inability of CBU to produce a reliable engraftment. In my opinion, double CB transplantation was developed as a solution to improve safety of CBT and it appears reasonable to discount the cost of a CBU to correct this kind of "incomplete graft" that promotes successful engraftment. Initiatives taken by some banks which supply two units for the fee of one or sharing costs between banks and registers when these protocols are used need to be promoted.

5 Conclusion

Much effort has been taken worldwide to develop very large and highly qualified CB inventories but in the future there is a need to change strategy and to invest in quality units rather than quantity. This might facilitate the affordability of CBT and the sustainability of all initiatives. Building off-the-shelf inventories where the units are readily available will facilitate the use of CBU in fast-track protocols where consolidation on time is an advantage. Also, this will open design of new protocols especially for high-risk leukemia. On the other hand, the large amount of units failing to fulfill the strict criteria of clinical units requires the development of non-hematological applications for CB banking. Many researchers are proposing the use of CB cells for cell therapy, $CD34^+$ cells for large scale iPS generation, immune cells as naïve T regulatory cells and NK-derived CB cells for third-party, off-the-shelf cellular immunotherapy, and even cord serum or plasma as a mediator of immunomodulation in inflammation and regenerative medicine due to the unique protein profile of the composition. In this regard, CBBs are encouraged to develop ethically driven biobanks to offer these spare units to any ethically and scientifically sound research project that will return investment to make the CBBs more sustainable and finally benefit, if successful, many patients in need.

References

1. Broxmeyer HE, Douglas GW, Hangoc G, Cooper S, Bard J, English D, et al. Human umbilical cord blood as a potential source of transplantable hematopoietic stem/progenitor cells. Proc Natl Acad Sci U S A. 1989;86:3828–32.
2. Gluckman E, Broxmeyer HA, Auerbach AD, Friedman HS, Douglas GW, Devergie A, et al. Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical-cord blood from an HLA-identical sibling. N Engl J Med. 1989;321:1174–8.
3. Ballen KK, Gluckman E, Broxmeyer HE. Umbilical cord blood transplantation: the first 25 years and beyond. Blood. 2013;122:491–8.
4. Gluckman E, Rocha V, Boyer-Chammard A, Locatelli F, Arcese W, Pasquini R, et al. Outcome of cord blood transplantation from related and unrelated donors. N Engl J Med. 1997; 337:373–81.
5. Rubinstein P, Carrier C, Scaradavou A, Kurtzberg J, Adamson J, Migliaccio AR, et al. Outcomes among 562 recipients of placental blood transplant from unrelated donors. N Engl J Med. 1998;339:1565–77.
6. Wagner JE, Barker JN, DeFor TE, Baker KS, Blazar BR, Eide C, et al. Transplantation of unrelated donor umbilical cord blood in 102 patients with malignant and non-malignant diseases: influence of CD34 cell dose and HLA disparity on treatment-related mortality and survival. Blood. 2002;100:1611–8.
7. Gluckman E, Rocha V, Arcese W, Michel G, Sanz G, Chan KW, et al., on behalf of Eurocord Group. Factors associated with outcome of unrelated cord blood transplant: guidelines for donor choice. Exp Hematol. 2004;32:397–407.
8. Laughlin MJ, Eapen M, Rubinstein P, Wagner JE, Zhang MJ, Champlin RE, et al. Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. N Engl J Med. 2004;351:2265–75.
9. Kurtzberg J, Laughlin M, Graham ML, Smith C, Olson JF, Halperin EC, et al. Placental blood as a source of hematopoietic stem cells for transplantation into unrelated recipients. N Engl J Med. 1996;335:157–66.

Chapter 4
Stem Cell Research and Banking: Towards Policy on Disclosing Research Results and Incidental Findings

Rosario Isasi

1 Introduction

Scientific and policy debates on the issue of disclosing research results and incidental findings to participants have evolved over time. Scientific advances, including the continued refinement of whole genome and exome sequencing techniques, have helped this issue to maintain momentum. As a means of ensuring a robust informed consent process, a number of jurisdictions have adopted consent requirements that address disclosure of research findings in a variety of research contexts including genetic testing or genomic analysis [1–4]. Likewise, some institutions recommend or require disclosure of specific information regarding general and individual research results as well as incidental findings to participants [5–8]. However, policies remain scant and overall directed (if not conflated) to the general context of clinical genomics and genetic research. At the same time, across jurisdictions and studies, policies are often open to (conflicting) interpretations [9–11], particularly with respect to their implementation processes, thereby calling for greater guidance to facilitate a consistent approach [12].

All together, policies co-exist with a complex, dynamic, and polarized academic debate centered on the emergence of a context-specific "duty" to disclose qualified research findings to participants in genetic and genomic studies [13, 14]. As reflected in normative instruments and ethical guidelines, such duty is framed within the spectrum of liberal to restrictive approaches: this is, from a loose, discretional professional responsibility conferred to researchers and/or biorepositories, to an ethical

R. Isasi (✉)
Centre of Genomics and Policy, Department of Human Genetics, Faculty of Medicine, McGill University, McGill University and Génome Québec Innovation Centre, 740 Dr. Penfield, Suite 5206, Montreal, QC H3A 0G1, Canada
e-mail: Rosario.isasi@mcgill.ca

D. Ilic (ed.), *Stem Cell Banking*, Stem Cell Biology and Regenerative Medicine,
DOI 10.1007/978-1-4939-0585-0_4,

duty if not a (seldom) legal obligation [15–17]. Underpinning such ethical duty is the core bioethical principle of autonomy, beneficence, and non-maleficence; principles which must be carefully weighted in their implementation [14, 18, 19]. Equally important, such duty is based on the notions of reciprocity and solidarity [20–22], understood as a shared view of both providing equitable mechanisms for respecting participants' interests (including the ones from patient advocacy communities) and for promoting scientific progress by recognizing the intrinsic nature of the research enterprise, that is, as an endeavor directed at producing generalizable knowledge [23].

Seemingly, consensus exists on the need to clarify existing policies and to provide further guidance with respect to: (1) distilling the nature (i.e., professional or ethical duty, legal obligation), (2) length and (3) scope of responsibilities for disclosing (4) qualified findings (e.g., general, individual or incidental; Table 4.1) within (5) a context-specific setting [24] (e.g.. primary, secondary research, biobanking). However, such consensus (and apparent certainty) ends when attempting to develop detailed guidance surrounding such issues. In fact, even the essential conditions for disclosure (from analytical and clinical validity, clinical utility and actionability to personal utility; Table 4.2), and terminological and definitional issues (e.g., are there incidental, unanticipated or unexpected findings?; Primary or secondary variants?) remain contentious [7, 25–28].

Adding to the difficulty of finding guidance is the fact that there are only a small number of empirical studies assessing the views of stakeholders [29, 30]. These studies are often methodologically and contextually diverse, making the generalization of their conclusions questionable. Moreover, such studies are often directed to a selected group of stakeholders, leaving gaps with respect to the perspectives of other key stakeholders such as biobankers, funders, policy-makers and members of oversight bodies. In addition, there is a lack of studies systematically analyzing the uptake of disclosure policies and protocols, or on their impact after implementation (e.g., logistical and financial costs).

1.1 The Stem Cell Context

As stated above, scientific inquiry, policy debates, and normative activity in this area have focused primarily on the genetic/genomic research and clinical contexts, which only to a certain extent are suitable for extrapolation to the stem cell field. While aware of the risks of falling into an exceptionalistic view [31, 32], it is maintained that the particular complexities of stem cell research and banking warrant special consideration. To that end, recently adopted stem cell-specific policy guidance seeks to acknowledge that the vast range of pluripotent stem cell research related studies and the diversity of banking initiatives—in which pluripotent stem cell lines are continuously immortalized, transformed, and distributed [33–35]—are important factors to consider when drafting protocols for authenticating, disclosing, and managing research and incidental findings [23].

Table 4.1 Typology of findings

Typology	General definitional issues	Stem cell research specific issues
Feedback or baseline assessment	• Assessment tests/measurements prior to recruitment (e.g., Medical history and life-style questionnaires) communicated as immediate feedback	• Donor eligibility determination requires screening for risk factors (e.g., Infection and communicable diseases)
	• Results are not part of the research study per se and as such, are not considered as individual research findings	• Requirements vary according to national regulatory frameworks and institutional protocols.
	• In the general clinical research context, consensus exists over a duty to communicate abnormal measures and critical values (i.e., results indicating the presence of a life-threatening event for which urgent and immediate medical attention is necessary) discovered during a baseline assessment	• Requirements will also differ depending on the type of biological material being donated and the circumstances under which such material was initially collected (e.g., clinical care vs. research)
General research results	• Aggregate results are typically the central findings or general conclusions arising from a particular research study	• Protocols should also establish mechanisms for providing data on the usage of samples as well as lay summaries of study protocols accessing data and samples and their outcomes
	• Offering aggregate research results is an effective mechanism to show respect and gratitude to participants, as well as promoting trust in the research enterprise	• Various modalities of communication have been proposed, ranging from passive methods by means of websites or newsletters, to interactive communication tools
Incidental findings	• Unintended findings of potential health or reproductive importance to an individual discovered during the research process but beyond the aims of the study	• Genetic changes to cells during reprogramming/ derivation/culture processes may influence clinical validity and the significance of the data arising from a pluripotent stem cell line; thereby assumptions regarding data cannot be made unless confirmatory testing is performed on the source (primary sample from individual donor)
Individual research results	• A result of potential health or reproductive importance to an individual relating directly to the specific aims or focal variables of the particular study	

Summarized from [23]

Table 4.2 Disclosure criteria

Analytical validity	Clinical validity	Clinical utility	Personal utility	Actionability
Refers to a result that accurately and reliably identifies particular genetic characteristics or measures the genotype of interest	A test is clinically valid when it consistently and accurately detects or predicts the intermediate or final outcomes of interest	A result is clinically useful when it is both clinically valid and can significantly improve patient health outcomes	A finding is personally useful when the outcome has meaning for the individual	A finding is actionable when capable of being acted on. For instance, a finding is actionable if there are established therapeutic or preventive interventions or that have the potential to change the clinical course of a disease

Summarized from [14, 17]

As the stem cell field continues to grow, and particularly since the discovery of induced pluripotent stem cell lines from somatic tissues, there is a rejuvenated interest in biobanking. In general, biorepositories are considered vital research infrastructures providing primary material for stem cell research (i.e., collection sites for tissues for subsequent stem cell line derivation). Stem cell repositories specifically constitute a resource for access to authenticated, quality-controlled, and ethically sourced pluripotent stem cell lines. They "play an intermediary role promoting scientific utility and clinical safety while at the same time developing procedures to advance participants interests" [36].

Scientific advances, together with evidence (albeit anecdotal) of donor-participant support for a system of sustained interaction via the disclosure of qualified research findings [37, 38], are providing a compelling rationale for governments and scientific institutions to adopt prospective policies in the area of disclosure. In this chapter, we will provide an overview of stem cell-specific policy recommendations addressing the scientific, ethical, and legal implications of mandating the disclosure of individual research results, including incidental findings.

2 Stem Cell Research and Banking: Disclosing and Managing Results and Incidental Findings

Robust and effective infrastructures and processes to support a system for validating, managing, and disclosing (or justifying withholding) research and incidental findings are required. They are predicated in the establishment of prospective protocols

that take into account the context, type, duration, and nature of the relationship between the research participant and the researcher (or the biorepositories) [39–41]. Indeed, amongst many important factors, the specific research context is the one with greatest impact on establishing whether an ethical—or even a legal—duty could or should be established towards individuals and institutions situated at the different stages of the research cycle [42, 43]. Certainly, such systems are sustained within the framework of an equally robust informed consent process, as illustrated in policies and ethical guidelines adopted across jurisdictions [23, 44]. In the spirit of reciprocity, such policies urge primary stem cell researchers and cell repositories to adopt prospective protocols governing the management of information and its release back to donors as a way of addressing stakeholder expectations [23, 45].

As captured by guidelines issued by the International Society for Stem Cell Research [46], reciprocity towards research participants (and society in general) entails acknowledging their right to access information [47–49]. This is translated in the professional responsibility of researchers to communicate general research results so as to promote transparency and scientific and ethical integrity in the field [50]. For example, comprehensive research findings (whether negative, inconclusive, or positive) should be published in peer-reviewed publications and plain-language summaries should be made available to the public [7, 23].

Furthermore, any system supporting the disclosure (or justifying the withholding) of qualified research and incidental findings must establish clear thresholds that would inform the what, to whom, and when to provide such disclosure. Such systems must also acknowledge that the thresholds of clinical and analytical validity, clinical utility (and perhaps personal utility), together with actionability, entail evolving standards. As such, they require a mechanism for the ongoing monitoring of scientific progress. In the particular context of pluripotent stem cell research, systems also require the development of mechanisms to (a) ensure robust traceability [49, 51] and (b) enable confirmation of the direct relevance of cell line data with respect to the donor. In this order of ideas, the following sections provide an overview of three institutional policies that outline systems to enable the disclosure of research results and incidental findings arising in the context of pluripotent stem cell research and banking.

2.1 The International Stem Cell Forum and the Ethics Working Party

The International Stem Cell Forum (ISCF) (www.stem-cell-forum.net) was established under the auspices of the UK Medical Research Council with the objective of promoting global good practice and international collaboration to accelerate progress in stem cell research. The ISCF's membership consists of nineteen funders of stem cell research from around the world [52]. One of the ISCF's initiatives is the Ethics Working Party (EWP), an independent body mandated to prospectively identify, discuss, and analyze the ethical and policy issues arising in stem cell research.

Table 4.3 ISCF EWP policy statement recommendations

ISCF EWP recommendations for research findings	
Feedback or baseline assessment	In the absence of a compelling rationale, the duty to provide feedback at baseline assessment, beyond the communication of abnormal measures and critical values, should not be transposed to the stem cell research context
Return of general research findings	Where feasible, and provided appropriate donor consent has been sought, generalized information about the nature of stem cell line use by researchers and biorepositories in the form of aggregate research findings should be communicated to research participants via predefined mechanisms (e.g., websites, bulletins, letters, etc.) Protocols should clearly delineate the scope of the responsibilities (if any) imposed on primary or secondary researchers and biorepositories
Return of individual research results and incidental findings	For pluripotent stem cell lines, if the protocol foresees the return of individual research results and incidental findings, the EWP recommends that the following elements be considered in a return of results policy: (a) The donor has been offered and has consented to their return (b) The results or findings are analytically and clinically validated, have clinical utility and are actionable (c) Any genetic information derived from the stem cell line has been confirmed by analysis of a verifiable DNA sample from the original donor(s) (d) The protocol comprehensively and clearly describes the mechanisms and conditions for disclosure, including the scope of the responsibilities imposed on researchers (whether primary or secondary) and/or biorepositories and the health professional(s) charged with such disclosure (e) The protocol for disclosure has been approved by an oversight committee or by an independent ethics review committee Given the nature of human embryonic stem cell (hESC) lines and the circumstances of their derivation, the EWP cautions against any return of donor-specific results (whether research or incidental) to embryo and gamete donors

To that end, and considering the need for ethical deliberation and further policy guidance, in 2011 the ISCF EWP adopted a Policy Statement [23] on the disclosure and management of general, individual, and incidental research findings. The Policy Statement (Table 4.3) is narrowly tailored to address stem cell lines derived from human embryos and from somatic tissue via induced pluripotent stem (iPS) cell techniques from adult donors. It proposes a framework with criteria for future policy that is mindful of local ethical and legal constraints, while at the same time recognizes and respects the specific needs and interests of the particular donor population involved (i.e. children/minors, affected individuals, adults who lack the capacity to consent, healthy volunteers).

The ISCF EWP Policy Statement supports a context-specific system for communicating qualified research findings (as defined in Table 4.2) to participants and donors of human biological materials. Such a system should be founded in robust

and prospective donor informed consent that allows for the possibility of identifying and re-contacting donors while protecting their privacy. Moreover, the Policy Statement highlights the significant implications (e.g., financial, ethical, legal, scientific, etc.) of extending a duty to disclose certain research and incidental findings beyond primary researchers and cautions against this practice. Furthermore, it also cautions against any return of donor/participant-specific results (particularly with respect to human embryonic stem cell lines) when the genetic/genomic findings have not been validated by confirmatory testing.

In sum, the ISCF EWP only foresees the possible communication of individual and incidental findings for human pluripotent stem cell research (e.g., iPS cell and hES cell lines) in the terms described in Table 4.3.

2.2 The California Institute for Regenerative Medicine

In 2004, and following the passage of Proposition 71 [53], the California Institute for Regenerative Medicine (CIRM) (www.cirm.ca.gov) was created with the mission to support and advance stem cell research and regenerative medicine in California (United States). To achieve its core objectives, CIRM funds strategic stem cell research projects with the goal of "the discovery and development of cures, therapies, diagnostics, and research technologies to relieve human suffering from chronic disease and injury" [54]. To further fulfill its mandate, CIRM launched the iPS Cell Initiative designed to support disease modeling, target discovery as well as drug screening and development [7, 45] for prevalent, genetically complex diseases. The iPS Cell Initiative will support access to high quality human iPS cell lines through a biobanking resource model. To that end, it will recruit approximately 3,000 disease-specific donors for the derivation of an estimated similar number of new iPS cell lines [55].

After extended deliberation and analysis [7, 56, 57] by CIRM's Medical and Ethical Standards Working Group, prospective recommendations for the management of research findings arising in the context of the iPS Cell Initiative were developed. The guidance is directed at knowledge gained from the derivation, banking, and distribution of disease-specific iPS cell lines. Consistent with the ISCF-EWP Policy Statement cited above, CIRM proposes a system based on a robust and prospective consent process seeking specific approval to communicate relevant research findings to somatic cell donors.

CIRM's recommendations are designed to maintain an avenue of communication with donors without creating unrealistic expectations. In the case of genetically induced stem cell lines, thresholds for clinical and analytical validity, clinical utility, and actionability are indeterminate at this time, which limits the usefulness of disclosing individual research results. Several scientific and ethical considerations are mentioned [7], including:

- The nature and behavior of the genotypic and phenotypic data arising from an iPS cell line and its derivates, which does not necessarily correlate with the cell donor's native genotype at the time of sample collection.

- The absence of established and harmonized protocols for clinically validating results from research utilizing iPS cell lines (e.g., Clinical Laboratory Improvement Amendments—CLIA Act).
- The difficulty of interpreting findings for complex diseases and determining their actionability (e.g., mechanisms for quantifying relative risk and penetrance of complex diseases and undiagnosed complex conditions are yet to be validated).
- The risks of increasing therapeutic misconception.
- The conflation between research and clinical care, which in turn blurs the fundamentally different obligations of primary/secondary researchers and physicians.

To that end, CIRM recommends adopting a priori informed consent and research protocols that foresee the potential disclosure of:

- *General (aggregated) research results*: CIRM foresees disseminating aggregated non-identifiable results from iPS cell research via pre-established mechanisms mediated by the iPS cell repository. It further suggests the possibility of establishing mechanisms to "actively alert researchers or clinicians at the collection site to new findings without the need to associate the results with specific donors" [7]. Such alerts would allow the collection site teams to evaluate the potential clinical relevance of any particular finding and incorporate this knowledge into future (general) clinical care decisions.
- *Individual research results*: Collection sites recruiting patient cohorts should consider mechanisms for donor re-contact in the event qualified individual research results that emerge from future studies.
- *Incidental findings*: The disclosure of incidental genetic findings based on the analysis of genetically reprogrammed iPS cells is scientifically and ethically inappropriate at this time.

Altogether, CIRM's approach is designed to promote and protect the interest of patient donors by creating mechanisms to allow research findings to feedback to the patient-care environment. Ultimately, the efficacy of this approach will depend, to some degree, on the efficacy of iPS cell to model disease and inform the development of new therapeutic approaches.

2.3 The International Stem Cell Banking Initiative

The International Stem Cell Banking Initiative (ISCBI) [58]—established by the ISCF—is a global, interoperable network of stem cell banks, working jointly towards identifying and harmonizing best practices for banking, characterization, and testing of pluripotent stem cell lines [35, 59]. ISCBI's vision and mission is "to create a solid scientific and ethical framework for international stem cell banking and research" [60]. Important harmonization and standardization work has been carried out by ISCBI. In 2008, ISCBI adopted its first best practices: the "Consensus Guidance for Banking and Supply of Human Embryonic Stem Cell Lines for Research Purposes" [41]. The Guidance document seeks to be comprehensive in managing a wide-range of aspects involved in a bio-resource. A set of best practices

for clinical grade pluripotent stem cell lines is currently being developed by ISCBI [61]. The new guidelines, set to be published in mid 2014, will establish an international set of standards for stem cell lines destined to be used in clinical translation as well as clinical trials; and will cover procurement, characterization, testing, maintenance, and shipment. It is envisaged that ISCBI will have an important role to play in shaping research and its clinical translation by creating the foundations of stem cell banking [60].

With respect to the specific topic of disclosure of clinical significant information, ISCBI's policy adheres to the rationale and the recommendations developed both by the ISCF-EWP and CIRM. To that end, its new guidelines will recommend that biorepositories, in addition to determining the scope of donor's informed consent, also ascertain whether protocols for the disclosure of individual research results and/or incidental findings to somatic cell and gamete donors, have been adopted. Finally, ISCBI's guidance also cautions against such disclosure in the absence of validated assays enabling the determination of clinical significance or personally actionable information.

3 Conclusion

As this brief chapter illustrates, the debate surrounding the disclosure of individual, general, and incidental findings to participants in stem cell research studies has been stimulated by recent normative guidance on the subject. Solid scientific and ethical justifications have been provided in support of mechanisms that could allow for context-specific and qualified disclosures. Whether based on a doctrine of fiduciary duties, ancillary care or on the fundamental ethical principles of autonomy, beneficence, and non-maleficence [18, 62], such systems warrant a cautious approach cognizant of current scientific uncertainties. Moreover, to fully meet public expectations and to respect the interests of researchers, patients, and research participants alike, a transparent and balanced approach of the benefits and risks—as understood or considered by the different stakeholders—is required. In addition, given that any system for disclosure is necessarily predicated on obtaining voluntary, informed, and understanding informed consent, mechanisms to improve genetic/genomic and stem cell literacy amongst stakeholders are much needed. To that end, the research agenda remains complex and vast.

References

1. National Health and Medical Research Council. National statement on ethics conduct in human research. Australia: National Health and Medical Research Council; 2007.
2. Medical Research Council (MRC). Code of practice for the use of human stem cell lines. London: Medical Research Council; 2010.
3. European Group on Ethics in Science and New Technologies to the European Commission. "Recommendations on the ethical review of hESC FP7 research projects", opinion no. 22. 2007.

4. National Research Council and Institute of Medicine. 2008 Amendments to the National Academies' Guidelines for Human Embryonic Stem Cell Research (2005, amendments 2007, 2008, 2010). Washington, DC: The National Academies Press; 2008.
5. Office of Biorepositories and Biospecimen Research. National Cancer Institute, National Institutes of Health. Workshop on release of research results to participants in biospecimen studies, Bethesda, Maryland July 8–9, 2010. Report 2011 March 4.
6. Knoppers BM, Deschênes M, Zawati MH, Tassé AM. Population studies: return of research results and incidental findings policy statement. Eur J Hum Genet. 2013;21:245–7.
7. Lomax GP, Shepard KA. Return of results in translational iPS cell research: considerations for donor informed consent. Stem Cell Res Ther. 2013;4:1–5.
8. Abdul-Karim R, Berkman BE, Wendler D, Rid A, Khan J, Badgett T, Hull SC. Disclosure of incidental findings from next-generation sequencing in pediatric genomic research. Pediatrics. 2013;131:564–72.
9. Burke W, Matheny Antommaria AH, Bennett R, Botkin J, Clayton EW, Henderson GE, et al. Recommendations for returning genomic incidental findings? We need to talk! Genet Med. 2013;15:854–9.
10. McGuire AL, Joffe S, Koenig BA, Biesecker BB, McCullough LB, Blumenthal-Barby JS, et al. Point-counterpoint. Ethics and genomic incidental findings. Science. 2013;340:1047–8.
11. Wolf SM, Annas GJ, Elias S. Point-counterpoint. Patient autonomy and incidental findings in clinical genomics. Science. 2013;340:1049–50.
12. Fernandez CV, Strahlendorf C, Avard D, Knoppers BM, O'Connell C, Bouffet E, et al. Attitudes of Canadian researchers toward the return to participants of incidental and targeted genomics findings obtained in a pediatric research setting. Genet Med. 2013;15:558–64.
13. Jackson L, Goldsmith L, O'Connor A, Skirton H. Incidental findings in genetic research and clinical diagnostic tests: a systematic review. Am J Med Genet Part A. 2012;158A:3159–67.
14. Bredenoord AL, Onland-Moret NC, Van Delden JJM. Feedback of individual genetic results to research participants: in favor of a qualified disclosure policy. Hum Mutat. 2011;32:861–7.
15. Black L, Avard D, Zawati M, Knoppers B, Hébert J, Sauvageau G; on behalf of the Leucegene Project. Funding considerations for the disclosure of genetic incidental findings in biobank research. Clin Genet. 2013;84:397–406.
16. Clayton EW, Haga S, Kuszler P, Bane E, Shutske K, Burke W. Managing incidental genomic findings: legal obligations of clinicians. Genet Med. 2013;15:624–9.
17. Wolf SM, Crock BN, Van Ness B, Lawrenz F, Kahn JP, Beskow LM, et al. Managing incidental findings and research results in genomic research involving biobanks and archived data sets. Genet Med. 2012;14:361–84.
18. Affleck P. Is it ethical to deny genetic research participants individualised results? J Med Ethics. 2009;35:209–13.
19. Meltzer LA. Undesirable implications of disclosing individual genetic results to research participants. Am J Bioeth. 2006;6:28–30.
20. Quaid KA, Jessup NM, Meslin EM. Disclosure of genetic information obtained through research. Genet Test. 2004;8:347–55.
21. Murphy J, Scott J, Kaufman D, Geller G, LeRoy L, Hudson K. Public expectations for return of results from large-cohort genetic research. Am J Bioeth. 2008;8:36–43.
22. Edwards KF, Fullerton SM. Relationships with test-tubes: where's the reciprocity? Am J Bioeth. 2006;6:36–8.
23. International Stem Cell Forum Ethics Working Party (ISCF EWP). The disclosure and management of research findings in stem cell research and banking: policy statement. Regen Med. 2012;7:439–48.
24. Beskow LM, Burke W. Offering individual genetic research results: context matters. Sci Transl Med. 2010;2:38cm20.
25. Anastasova V, Mahalatchimy A, Rial-Sebbag E, Antó Boqué JM, Keil T, Sunyer J, et al. Communication of results and disclosure of incidental findings in longitudinal paediatric research. Pediatr Allergy Immunol. 2013;24:389–94.

26. Christenhusz GM, Devriendt K, Dierickx K. Secondary variants—in defense of a more fitting term in the incidental findings debate. Eur J Hum Genet. 2013;21:1331–4.
27. Knoppers BM, Dam A. Return of results: towards a lexicon? J Law Med Ethics. 2001;39:577–82.
28. Green RC, Berg JS, Grody WW, Kalia SS, Korf BR, Martin CL, et al. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. ACGM Policy Statement. Genet Med. 2013;15:565–73.
29. Klitzman R, Appelbaum PS, Fyer A, Martinez J, Buquez B, Wynn J, et al. Researchers' views on return of incidental genomic research results: qualitative and quantitative findings. Genet Med. 2013;15:888–95.
30. Daack-Hirsch S, Driessnack M, Hanish A, Johnson VA, Shah LL, Simon CM, et al. 'Information is information': a public perspective on incidental findings in clinical and research genome-based testing. Clin Genet. 2013;84:11–8.
31. Lomax G, Peckman S. Stem cell policy exceptionalism: proceed with caution. Stem Cell Rev. 2012;8:299–304.
32. Sage WM. Will embryonic stem cells change health policy? J Law Med Ethics. 2010;38:342–51.
33. International Stem Cell Initiative. Characterization of human embryonic stem cell lines by the International Stem Cell Initiative. Nat Biotechnol. 2007;25:803–16.
34. International Stem Cell Initiative. Screening ethnically diverse human embryonic stem cells identifies a chromosome 20 minimal amplicon conferring growth advantage. Nat Biotech. 2011;29:1132–44.
35. Crook JM, Hei D, Stacey G. The International Stem Cell Banking Initiative (ISCBI): raising standards to bank on. In Vitro Cell Dev Biol Anim. 2010;46:169–72.
36. Isasi R, Knoppers BM, Lomax G. Sustained interaction: the new normal for stem cell repositories? Regen Med. 2011;6:783–92.
37. Hempel C (presentation). PRIM&R advancing ethical research conference, 2010 December 7, Tuesday. http://addiandcassi.com/
38. Rothstein MA. Tiered disclosure options promote the autonomy and well-being of research subjects. Am J Bioeth. 2006;6:20–1.
39. Clayton EW, Ross LF. Implications of disclosing individual results of clinical research. JAMA. 2006;295:37; author reply 37–8.
40. Renegar G, Webster CJ, Stuerzebecher S, Harty L, Ide SE, Balkite B, et al. Returning genetic research results to individuals: points-to-consider. Bioethics. 2006;20:24–36.
41. International Stem Cell Banking Initiative (ISCBI). Consensus guidance for banking and supply of human embryonic stem cell lines for research purposes. Stem Cell Rev. 2009;5:301–14.
42. Wolf SM, Lawrenz FP, Nelson CA, Kahn JP, Cho MK, Clayton EW, et al. Managing incidental findings in human subjects research: analysis and recommendations. J Law Med Ethics. 2008;36:219–48.
43. Dixon-Woods M, Jackson C, Windridge KC, Kenyon S. Receiving a summary of the results of a trial: qualitative study of participants' views. BMJ. 2006;332:206–10.
44. Canadian Institutes of Health Research, Natural Sciences and Engineering Research Council of Canada, and Social Sciences and Humanities Research Council of Canada. Tri-council policy statement: ethical conduct for research involving humans. 2010.
45. RFA 12-04. CIRM human pluripotent stem cell (hPSC) repository award. http://www.cirm.ca.gov/sites/default/files/files/RFA12-04_hPSC_Repository.pdf
46. International Society for Stem Cell Research (ISSCR). Guidelines for the clinical translation of stem cells. 2008. www.isscr.org/clinical_trans/pdfs/ISSCRGLClinicalTrans.pdf
47. Bredenoord AL, Kroes HY, Cuppen E, Parker M, Van Delden JJM. Disclosure of individual genetic data to research participants: the debate reconsidered. Trend Genet. 2011;27:41–7.
48. Beskow LM. Considering the nature of individual research results. Am J Bioeth. 2006;6:38–40.

49. Knoppers BM, Isasi R. Stem cell banking: between traceability and identifiability. Genome Med. 2010;2:73.
50. Ormond KE. Disclosing genetic research results: examples from practice. Am J Bioeth. 2006;6:30–2.
51. Heaney C, Tindall G, Lucas J, Haga SB. Researcher practices on returning genetic research results. Genet Test Mol Biomarkers. 2010;14:821–7.
52. International Stem Cell Forum (ISCF). 2013. http://www.stem-cell-forum.net/about/
53. Proposition 71. http://www.cirm.ca.gov/sites/default/files/files/about_cirm/prop71.pdf
54. California Institute for Regenerative Medicine (CIRM). 2013. http://www.cirm.ca.gov/about-cirm/our-mission
55. California Institute for Regenerative Medicine (CIRM). 2013. http://www.cirm.ca.gov/our-funding/awards/cirm-human-pluripotent-stem-cell-biorepository-%E2%80%93-resource-safe-storage-and.
56. California Institute for Regenerative Medicine (CIRM). Summary and recommendations of the CIRM Human iPS Cell Banking workshop. 2010 Nov 17–18. http://www.cirm.ca.gov/sites/default/files/files/about_cirm/iPSC_Banking_Report.pdf
57. California Institute for Regenerative Medicine (CIRM). Ethical and policy considerations for a pluripotent stem cell resource center—2011 update. Summary of the April 29, 2011 annual meeting of the CIRM Medical Accountability Standards Working Group.
58. International Stem Cell Banking Initiative (ISCBI). 2013. http://www.stem-cell-forum.net/initiatives/international-stem-cell-banking-initiative/
59. Isasi R. Alliance, collaborations and consortia: the international stem cell forum and its role in shaping global governance and policy. World Stem Cell Report. Regen Med. 2012;7:84–8.
60. Stacey G, Healy L. Banking stem cell lines: an international perspective. World Stem Cell Report; 2010:113–6, Ed. Genetics Policy Institute.
61. International Stem Cell Banking Initiative (ISCBI). 2013. http://www.stem-cell-forum.net/initiatives/international-stem-cell-banking-initiative/iscbi-scoping-plans/
62. Kollek R, Petersen I. Disclosure of individual research results in clinico-genomic trials: challenges, classification and criteria for decision-making. J Med Ethics. 2011;37:271–5.

Chapter 5
The Legal Duties of Stem Cell Banks with Regard to Stem Cell Donors and Recipients

James Lawford Davies and Sebastian Sethe

1 Duties to the Stem Cell Donor

1.1 Business Law and Ownership

Many different types of stem cell biobank are feasible and are discussed elsewhere in this volume. For the purposes of a legal overview, we can usefully distinguish between 'research' and 'clinically' oriented stem cell biobanks, recognising that in practice these may also overlap. Many of the legal considerations also apply to either, albeit with different emphasis.[1]

Both research and clinical stem cell banks may be created for a variety of reasons and some of them may have a legal function or connection with a legal framework. Notably in human embryonic stem cell research, some stem cell banks have been established by law (for example, Spain) whereas others have a quasi-legal status as a consequence of a requirement to deposit any stem cell lines resulting from embryo research based not in statute, but arising through licensing and funding requirements (for example, the UK, the European hESCReg, France, Japan, and India) [2].

What, however, is the legal status of a stem cell bank? In Europe, the highest courts have had occasion to comment on the 'clinical' status of a cord blood stem cell bank and ruled that it was unlikely but not always impossible that such banks should be considered sufficiently close to clinical proceedings as to benefit from the same tax exemptions as a hospital [3]. In intellectual property law in the EU [4], the United States [5, 6], and internationally [7, 8], a database is defined as a collection

[1] For example, giving evidence to a 2009 UK House of Lords Science and Technology Committee inquiry into genomic medicine, Professor Andrew Morris (University of Dundee) stated that those carrying out genomic research in the UK needed to consider 43 relevant pieces of legislation, 12 sets of relevant standards, and eight professional codes of conduct (quoted in [1]).

J.L. Davies (✉) • S. Sethe
Lawford Davies Denoon, 14A Clerkenwell Green, London EC1R 0DP, UK
e-mail: james@lawforddaviesdenoon.com

D. Ilic (ed.), *Stem Cell Banking*, Stem Cell Biology and Regenerative Medicine,
DOI 10.1007/978-1-4939-0585-0_5,

of data or 'other materials' so it is therefore quite possible that the way in which a stem cell bank is organised, in conjunction with the actual collection, constitutes a protectable asset. We are not aware, however, of any cases where the applicability of database protection rights to biobanks has been tested in court.

Another approach when contemplating the status of a bank is to consider where property rights arise. Who owns the cells and cell lines? It has long been held that there can be 'no property rights in the human body'. The policy sensitivities are such that modern law continues to wrestle with this basic dictum, but a framework of property rights has emerged that can, after all, accrue in human biological material. In the UK, for example, an exception to the basic principle that there is no property in the human body is that tissue could become subject to property if it had been separated from the body and had been subject to the application of 'skill and care' by another. The UK Human Tissue Act 2004 also enshrined this dictum into law [9]. Recently, however, the UK courts rejected this distinction between the two types of tissue as 'not entirely logical'. If some rights exist for the donor, it has been argued that there is then no reason that tissue should not be 'property capable of passing from the donors to the donee'.[2]

Conversely, there is no reason why tissue donors or patient groups cannot enter into contracts with a stem cell bank that could include provision for patients' retention of certain property rights or a benefit-sharing arrangement. The bank's consent form, in conjunction with any participant information literature, will be an important document in this context.

However, this acknowledgement of a donor's rights has seldom been converted into a strong property right on the donor's part. In Europe, the European Court of Human Rights refused to recognise a university professor's right *not* to share data from research participants, even though he had twice assured the participants (and their parents) in writing that their data would remain confidential [10]. In the United States and elsewhere, there is a trend for the courts to deny that donors retain a right to share in the profits from products or tests developed from their samples.[3] In the context of research biobanking, the Catalona case is of particular interest [11]. In brief, Dr. Catalona established a biobank at Washington University, which also included some samples from patients of other surgeons within the university. When Dr. Catalona moved to another university, Washington University refused to let him transfer the biobank. Dr. Catalona obtained declarations from about 6,000 donors requesting the relocation of their samples to Northwestern, but Washington

[2] Y*earworth and Others v. North Bristol NHS Trust* [2009] EWCA Civ 37. However, the Court of Appeal in that case was concerned with the status of gametes from a living body, stored under licence and intended for use by the men whose bodies had produced them. Their Lordships focused on control and 'the right to use' as a gauge of proprietary interest, and the implications of the judgment beyond the particular facts of this case may be limited. Different materials stored for different purposes should be considered on a case-by-case basis.

[3] Originally established in the case of *Moore v. Regents of the University of California* (1990) in which the US Supreme Court rejected a claim for a share of profits generated from a valuable cell line derived from the claimant's tissue because it was held that the claimant did not remain owner of the tissue following its removal.

University refused to comply with the requests on the grounds that the biobank was an institutional asset because it contained samples from other university clinicians, and the university had paid Dr. Catalona's salary and provided funding for the biobank. The US Court of Appeals ruled that Washington University owned the tissues and that the donors could not force transfer of their samples; they could only withdraw their consent for the use of them in identifying research (whereupon the samples would be anonymised). This brings us to the next legal theme with regard to the rights of donors, namely consent.

1.2 Consent

Consent features as a leading factor in any review of the legal issues and challenges arising in relation to biobanking. This is partly due to variation, both in terms of the variation between contemporaneous requirements in different jurisdictions and in relation to different uses or material, and in terms of variation over time, as expectations and requirements for valid consent evolve. These factors are themselves significant because of the need for interoperability between banks and the sustainability of collections over time.

With regard to variation between jurisdictions, a recent European Commission study of 126 biobanks found acute contrasts in consenting practice [12]. Whilst the majority used at least one type of consent form, 13 banks (spread throughout Europe) took no written consent from donors at all. Forty-eight asked donors to consent to a specific study, 42 preferred to obtain consent to a research area, and 11 obtained 'blanket' consent. Such variety highlights the potential challenges of cross-border collaboration between such banks, though—notably—the authors found that more than half of the banks sampled had been involved in international collaborations and reported no major problems in sample sharing. Checking the consent provisions put in place by collaborators, however, remains a key consideration.

The difficulties created by variation in consenting standards over time are illustrated by the concerns surrounding the embryonic stem cell lines approved for federal research funding by the US National Institutes of Health (NIH). The NIH lines were derived from embryos donated before 2001 and when the bioethicist Robert Streiffer came to review the donors' consent forms in 2008 he found that they did not comply with more recent guidelines set by the US National Academy of Sciences, and some deviated egregiously [13]. As a consequence of this analysis, Stanford University decided that five of the approved lines should not be used as a result of concerns that the women who donated their embryos did not give properly informed consent for their use in research [14]. The head of the NIH Stem Cell Task Force responded to the criticism by emphasising that 'Streiffer's paper deals with application of 2008 standards to cell lines that were put on the registry in 2001' but the episode highlights the need to consider both the potential future use of banked material and the capacity of consents to withstand robust scrutiny going in a different, subsequent context.

A particular sensitivity arose in relation to the NIH lines because they were derived from human embryos. In many jurisdictions, embryo research is the subject of specific legislation, which commonly prescribes particular consent requirements. An example of this is the complex framework governing the creation, use, and banking of human embryonic stem cells in the UK created by the Human Fertilisation and Embryology Act 1990 and its related regulations. Amongst other things, the regulations require that, prior to giving consent for the use of material to derive embryonic stem cells and/or lines, donors must be informed that they will have no control over the future use of such cells or lines, that they may continue indefinitely and may be used in many different projects and/or therapy, and that they may be patented or used for commercial purposes but that the donors will not benefit financially from this. It is also a condition of every embryo research licence that samples of derived cells or lines are deposited in the UK Stem Cell Bank and may be used nationally or internationally. It follows that, although donors are asked to give broad consent to a potentially wide range of different uses of their material, they are well informed about some of the possible permutations of such research and application, hopefully reducing scope for future disagreement.

Of course, no matter how informed the donors may be, they may still decide at some future point to withdraw their consent. Where a biobank is to be used for research or prognostic purposes, the withdrawal of patient data during the course of a project is likely to have a detrimental impact on the integrity of the study. To this end, the UK Biobank [15] (a population study of 500,000 samples from the UK citizens) included detailed information about the donors' ability to withdraw their consent during the enrolment process, including an explanation of three different levels of withdrawal (no further contact, access, or use). The consent process made clear, however, that it would not be possible to remove donors' data from analyses that had already been done [16]. Similarly, the Human Fertilisation and Embryology Act 1990 referred to above provides that consent to the use can be varied or withdrawn at any time until the resulting embryo has been used for the purposes of a project [17]. Such 'bright line' guidance should help mitigate against misunderstanding and dispute, and should usually be comparatively straightforward to identify in relation to stem cells and lines.

The mere existence of valid and enduring consent, however, does not mean that the stem cell bank has no other duties to the donor. In the following we will consider duties arising with regard to donor privacy, a potential duty to contact the donor with certain findings, and a duty to take reasonable care of the donors' stem cells.

1.3 Privacy

Stem cell lines derive from a particular individual and can be considered as an aspect of that individual's privacy relating to health and genetic data. This notion that biological samples can in some respects and circumstances constitute personal data has been tacitly affirmed by some courts [18]. Through this route, data protection legislation emerges as a 'backdoor' mechanism for the regulation of biobanks [19].

In the United States, the Health Insurance Portability and Accountability Act (HIPAA) is a significant privacy law with relevance for research stem cell biobanks. HIPAA, however, does not apply to data that has been 'de-identified', i.e. information that does not identify an individual or provide 'a reasonable basis to believe that the information can be used to identify an individual'. One way of ensuring the anonymous character of data under the HIPAA regime is if a statistician or other person with appropriate knowledge and experience formally determines that the data is not individually identifiable [20].

This mechanism to authoritatively classify certain data as anonymous does not exist in Europe, where Directive 95/46/EC on the protection of individuals with regard to the processing of personal data and on the free movement of such data deals with 'personally identifiable' information. The Directive is currently under revision and will soon be recast as a regulation that has direct if not uniform effect in all European Member States, but it is unlikely that even the revised version will unequivocally clarify when tissue and cell lines can be considered personal data. Accordingly, the way that the data protection principles are implemented specifically for biobanks varies across Europe.[4]

In any event, a binary distinction between 'personally identifiable' and 'anonymous' information fails to appreciate that almost any seemingly anonymous item of information could potentially be personally identifiable given a particular set of circumstances [21]. In the UK, the Information Commissioner has suggested that organisations should evaluate whether individuals could be re-identified from the anonymised data by a 'motivated intruder' who is 'reasonably competent, has access to resources […] and would employ investigative techniques such as making enquiries of people who may have additional knowledge of the identity of the data subject' [22]. Courts have condensed this to an 'investigative journalist' test [23], and given the demonstrated capacity of investigative journalists to piece together information this makes for a high threshold in establishing anonymisation. Moreover, complete anonymisation that precludes the linking of existing data items with other health data from the same donor could greatly limit the value of some stem cell biobanks and not be practicable in others.

In this context, the concept has arisen that data can be 'coded', i.e. that identifiers can be (reversibly) obscured or removed. The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) has adopted concepts of 'single' and 'double coding', whereas it is currently expected that the revised European framework for data protection will pursue the concept of 'pseudonymisation'. 'Coded' or 'pseudonomous' data can be processed with fewer restrictions, but—crucially—it remains subject to the general framework of data protection regimes and its quality control stipulations. Thus, at least in Europe (and in a much expanded manner likely to come into existence under the new European privacy regulation), stem cell banks will need to appoint a data protection officer and conduct regular data protection impact assessments and audits, establish reporting and mitigation mechanisms for breaches, and not just establish a data protection policy but also integrate a 'data protection compliance' element into many of its standard operating procedures.

[4] Sample, data use, and protection in biobanking in Europe: legal issues.

1.4 Duty to Feedback

We have discussed some of the issues relating to donors' commercial interests in ownership and commercialised cells and lines, but different considerations apply to donors' interests in the data and findings that emerge from studies involving that material. Do biobanks owe a duty of care to donors?

As with the other areas discussed above, a range of different options have been adopted in relation to feedback. The UK Biobank is notable for the depth of its consideration of this issue, identifying that there are three stages at which the bank could in principle provide donors with feedback: at their initial assessment (for example, blood pressure measurements), in the initial stages before their cells are stored (for example, their white cell count), and later as the results of studies involving their cells emerge (for example, genomic data). However, UK Biobank decided that it would not provide any feedback to donors beyond basic measurements taken during their enrolment on the basis that the value of such information is questionable when communicated outside a clinical setting without being properly explained and supported with counselling. The UK Biobank does, however, provide general information about the results of studies based on the resource. This approach is not uncontroversial [24], though the bank's approach to feedback is clearly explained during the consenting process.

By contrast, the US Biobank established by NIH at the National Cancer Institute has adopted a slightly different approach whereby they will notify the institution at which a donor was registered in the event that researchers 'learn something important about [a donor's] health' [25].

Whilst most professional bodies acknowledge that the duty of care owed to a research participant is different to that owed to a patient in a clinical setting, it is likely that the full extent of that duty will only become clear when it is tested in the courts. The OECD has, however, published '*Guidelines on Human Biobanks and Genetic Research Databases*' which largely endorses a 'no feedback' approach but provides that the operators of biobanks 'should ensure that aggregate and general results of research conducted using its resources, regardless of outcome, are made publicly available either in the form of publications or through other means' [26], further emphasising the importance of explaining this approach during the consenting process.

2 Duties to Stem Cell Recipients

Where a clinical stem cell bank provides cells that are used either directly as a 'raw material' or as an 'ingredient' for an investigational or other therapy, the relevant legal requirements that relate to these types of cells will apply. It is beyond the scope of this chapter to rehearse the myriad legislative provisions relating to stem

cell-based therapies, but certain aspects that are of particular interest for clinical stem cell biobanks should be mentioned.

Firstly, the provenance of the biological material that was used to derive the stem cell lines will need to be considered. Here, regulators have suggested that the rules applying to cells and tissues for human treatment should be combined with the rules for good manufacturing practice [27]. In Europe, for example, Directive 2004/23/EC on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage, and distribution of human tissues and cells stipulates a number of tests that must be conducted on stem cell donors of tissues and cells for use in treatment. It can be argued that by simply adding the rules from another regulatory paradigm (where donor testing is important for technical and time reasons) into the quality requirements for clinical cell banking (where a comprehensive portfolio of tests can and will be conducted on the final cell therapy product), an unwarranted regulatory burden is created, and in some fields (such as in embryonic stem cell banking), practitioners have made the case for a cell banking regime that places a greater emphasis on the final 'product' than on donor testing [28]. Nonetheless, a clinical stem cell bank will often aim to keep comprehensive provenance testing records on file or may at least require cell line depositors to make representations regarding the existence, completeness, and safe keeping of these records.

The regulations also govern the further processing of cell lines in a biobank, including the generation of 'master cell banks'. In the United States, of particular interest for stem cell banks are perhaps the stipulations 'on cell lines used for manufacturing biological products' [29], which explicitly leaves space for the Directors of both the Center for Biologics Evaluation and Research or the Center for Drug Evaluation and Research to stipulate further tests that should be performed.

Even if they have yet to become (investigational) medicinal products, tissues and cells are 'products' in the legal sense and subject to rules for liability for defective products.[5] A stem cell bank will therefore need to consider what product-related representations and warranties are made when the initial cell or tissue material is procured, and whilst in transit to the bank. Unless specified otherwise in an agreement, the provider may be liable to the owner of the stem cell bank for defects. Conversely, where the bank sends out samples from the cell lines that it maintains, it will be liable (unless that liability has been expressly and lawfully allocated elsewhere) for the quality of the cells and any accompanying information that it provides.

[5] For the EU Directive 85/374 on liability for defective products, Art.2 [1985] (as amended by Directive 1999/34) defines 'product' as 'all moveables even if incorporated into another moveable or into an immoveable, and including electricity'—confirmed for organs: Case C-203/99 Veedfald v Århus Amtskommune [2001] E.C.R. I-3569.

3 Conclusion

In summary, the donor's rights and property interests in stem cell banks are perhaps not as prominent as might be expected. A stem cell bank can exist as a legal entity and own the stem cell samples it curates, though the requirements for consent and the protection of the donor's data embodied in the cell line place certain obligations on the stem cell bank. Recipients of stem cell therapies are protected via a strong set of legal safeguards focused on the manufacturing therapeutic products. A clinical stem cell bank will need to find its role in this process and comply with 'upstream' and 'downstream' regulatory obligations.

As we have seen above, for both research- and clinical-oriented stem cell banks, many of the applicable 'compliance' considerations are not actually laid out in law. Many of the apparently specific regulatory requirements have their origin in discussions between practitioners and regulators trying to establish testable parameters and appropriate risk factors in order to meet a much more general legal obligation, and many interpretations of what constitutes 'good practice' are not enshrined in law or regulation. It is therefore important that practitioners and regulators alike remain alert to the fact that compliance with requirements should not become a *pro forma* exercise and such requirements, whether legal or 'quasi-legal', should be regularly evaluated for gaps and shortcomings—but also for proportionality and appropriateness—to secure the greatest benefits for donors and recipients.

References

1. Laurie G. Reflexive governance in biobanking: on the value of policy led approaches and the need to recognise the limits of law. Hum Genet. 2011;130:347–56.
2. Isasi R, Knoppers BM. From banking to international governance: fostering innovation in stem cell research. Stem Cells Int. 2011;2011:498132.
3. European Court of Justice. CopyGene A/S v. Skatteministeriet. 2010. Case 262/08. I-5053. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:62008CJ0262:EN:NOT
4. European Parliament and the Council of the European Union. Directive 96/9/EC of 11 March, 1996 on the legal protection of databases. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31996L0009:EN:HTML
5. North American Free Trade Agreement. Copyright. Article 1705(1)(b). http://www.sice.oas.org/trade/nafta/chap-171.asp
6. Copyright Law of the United States of America. Definitions. Section 101. http://www.copyright.gov/title17/92chap1.html#101
7. World Trade Organization. Analytical index: agreement on trade-related aspects of intellectual property rights. Computer programs and compilations of data. Article 10(2). http://www.wto.org/english/res_e/booksp_e/analytic_index_e/trips_01_e.htm#article10
8. World Intellectual Property Organization. Copyright treaty. Compilation of data (databases). Article 5. http://www.wipo.int/treaties/en/ip/wct/trtdocs_wo033.html#P59_6206
9. The UK Human Tissue Act 2004. Prohibition of commercial dealings in human material for transplantation. Section 32(9)(c). http://www.legislation.gov.uk/ukpga/2004/30/section/32
10. European Court of Human Rights. Gillberg v. Sweden. Application no 41723/06. 2012 Apr 3. http://hudoc.echr.coe.int/sites/eng/pages/search.aspx?i=001-110144#{"itemid":["001-110144"]}

11. Washington University v. Catalona (2007) WL 1758268 (8th Cir. June 20, 2007).
12. Zika E, Paci D, Braun A, Rijkers-Defrasne S, Deschênes M, Fortier I, Laage-Hellman J, Scerri CA, Ibarreta D. A European survey on biobanks: trends and issues. Public Health Genomics. 2011;14:96–103.
13. Streiffer R. Informed consent and federal funding for stem cell research. Hastings Cent Rep. 2008;38:40–7.
14. Baker M. Consent issues restrict stem-cell use. Nature. 2008;454:556.
15. www.ukbiobank.ac.uk
16. UK Biobank Ethics and Governance Framework. Adoption, implementation and revision. 2007. Version 3.0, Section 6. http://www.ukbiobank.ac.uk/wp-content/uploads/2011/05/EGF20082.pdf
17. Human Fertilization and Embryology Act 1990. Consent to use gametes or embryos. Schedule 3. http://www.legislation.gov.uk/ukpga/1990/37/schedule/3
18. European Court of Human Rights. S. and Marper v. the United Kingdom. Applications nos. 30562/04 and 30566/04. Dec 04, 2008 [68] http://hudoc.echr.coe.int/sites/fra/pages/search.aspx?i=001-90051#{"itemid":["001-90051"]}
19. Bygrave LA. The body as data? Biobank regulation via the "back door" of data protection law. Law Innov Technol. 2010;2:1–25.
20. Code of Federal Regulations. Implementation specifications: requirements for de-identification of protected heath information. 2002. Title 45, Vol. 1, Section 164.514 (b) (2). http://www.gpo.gov/fdsys/pkg/CFR-2002-title45-vol1/pdf/CFR-2002-title45-vol1-sec164-514.pdf
21. Ohm P. Broken promises of privacy: responding to the surprising failure of anonymization. UCLA Law Rev. 2010;57:1701–76.
22. Information Commissioner's Office. Anonymisation: managing data protection risk code of practice. 2012. http://ico.org.uk/for_organisations/data_protection/topic_guides/~/media/documents/library/Data_Protection/Practical_application/anonymisation-codev2.pdf
23. The Information Commissioner v Magherafelt District Council [2012] UKUT 263 (AAC) http://www.osscsc.gov.uk/Aspx/view.aspx?id=3536
24. Johnston C, Kaye J. Does the UK Biobank have a legal obligation to feedback individual findings to participants? Med Law Rev. 2004;12:239–67.
25. Information for Donors. http://cahub.cancer.gov/you/?p=58
26. OECD guidelines on human biobanks and genetic research databases 2009;1.H. http://www.oecd.org/sti/biotech/44054609.pdf
27. European Commission. EudraLex, the rules governing medicinal products in the European Union, volume 4—EU guidelines for good manufacturing practice for medicinal products for human and veterinary use; annex 2—manufacture of biologically active substances and medicinal products for human use. http://ec.europa.eu/health/files/eudralex/vol-4/vol4-an2__2012-06_en.pdf
28. Murdoch A, Braude P, Courtney A, Brison D, Hunt C, Lawford-Davies J, Moore H, Stacey G, Sethe S; Procurement Working Group of National Clinical hESC Forum; National Clinical hESC Forum. The procurement of cells for the derivation of human embryonic stem cell lines for therapeutic use: recommendations for good practice. Stem Cell Rev. 2012;8:91–9.
29. Code of Federal Regulations. Cell lines used in manufacturing biological products. 2013. Title 21, Vol. 7. Section 610.18 (c). http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=610.18

Chapter 6
European Regulations and Ethical Issues on Cord Blood Banking

Carlo Petrini

1 Introduction

The first attempt to transplant hematopoietic stem cells was made at the beginning of the 1970s [1], although the first case of clinical success was in 1988, when Eliane Gluckman and her team transplanted cord blood stem cells to a 5-year-old child suffering from a serious form of Fanconi's anaemia [2].

Since then the transplantation of cord blood as a source of hematopoietic cells has been shown to be effective in treating a large number of hematological diseases (e.g. hemoglobinopathies of genetic origin, such as sickle-cell disease and thalassaemia, genetic diseases affecting the immune system, such as severe combined immunodeficiencies, some types of anaemia and marrow aplasia, some metabolic diseases). Cord blood is transplanted into children and adults with neoplastic malignancies, in particular acute leukaemia [3], who need an allogeneic transplant of hematopoietic stem cells and do not have a compatible donor among their relatives [4].

The potential advantages of cord blood stem cells are due in part to the low incidence of graft-versus-host disease (GVHD) associated with their use compared with that associated with stem cells from other sources, thus enabling the use of cord blood from Human Leukocyte Antigens (HLA)-discordant donors [5].

The discovery in cord blood of different types of stem cells (endothelial progenitor cells, mesenchymal stem cells, pluripotent simil-embryonic cells) has generated considerable interest and hope for a multitude of possible uses, especially in the field of regenerative medicine, although their possible applications in this field are only at the experimental stage.

In the case of transplantation of cord blood stem cells for functional neuroregeneration in children with cerebral palsy, recently published research findings are a source of considerable optimism [6].

C. Petrini (✉)
Italian National Institute of Health,
Via Giano della Bella 34, 00162 Rome, Italy
e-mail: carlo.petrini@iss.it

D. Ilic (ed.), *Stem Cell Banking*, Stem Cell Biology and Regenerative Medicine,
DOI 10.1007/978-1-4939-0585-0_6,

The use of cord blood to treat a wide range of diseases has led to the establishment of numerous biobanks specialized in its collection, storage, and distribution [7]. The first public cord blood bank was established in 1991 at the New York Blood Center [8].

There are essentially two main types of cord blood bank: public (for allogeneic, philanthropic use) and private (for autologous use) [9, 10].

1.1 Public Cord Blood Banks

Public accredited non-profit cord blood banks receive umbilical cord blood following informed parental consent. Cell count and volume are the key criteria that determine the eligibility of cord blood units for storage. Units of blood that meet the requisites for therapeutic use are screened in a series of tests, recorded in international registries and are available to national and foreign transplant centers [11, 12]. Once accepted, the cord blood becomes the property of the public bank for subsequent clinical use. Approximately 90 % of the cord blood collected to be stored for transplant purposes fails to meet the very strict criteria for possible use [13]. Samples unsuitable for storage and transplantation (e.g. because they contain too few cells) or that which subsequently become unsuitable (e.g. as a result of deterioration) can potentially be used for research [14]. In this way donated blood that is not suitable for storage is not wasted [15].

Most blood banks are linked through international registries that list publicly banked cord blood units in searchable databases such as Bone Marrow Donors Worldwide (BMDW), the NetCord Foundation, the National Marrow Donor Program (NMDP), and other national registries that are accessible to all patients in need. International accreditation bodies, such as the NetCord Foundation for the Accreditation of Cellular Therapy (FACT) and governmental regulatory requirements ensure that cord blood units available to the public satisfy strict quality standards. The BMDW registries show that in 2013 almost 600,000 cord blood units were stored worldwide in public biobanks linked through international networks [16].

1.2 Private Cord Blood Banks

Private cord blood banks collect and store cord blood for use exclusively by the person from whom it was taken or a family member. These banks promote the storage of cord blood for preventive purposes, i.e. in the event that the individual concerned or a relative should in the future develop some disease that can be cured by a stem cell transplant. In contrast to blood stored in public biobanks, units held in private banks for either autologous or allogeneic transplants (for the infant donor or a relative) remain the property of the child under the guardianship of the parents and are not available to the public. There is no scientific evidence for the clinical

usefulness as a means of prevention of autologous stem cells, and their storage in private biobanks for possible future autologous use is not envisaged in any European Union directives on the subject: in fact it is discouraged in all the most authoritative documents [17]. This type of collection and storage is not only useless (both scientific and clinical data show that the probability of cord blood stored in private biobanks being used for autologous use is around 1/75,000 or 0.0013 % [18, 19]), but actually harms the community at large, because it removes from the international network resources that could otherwise be of great therapeutic benefit to persons other than the donor [20]. Notwithstanding this, almost one million cord blood units are stored in over 130 private cord blood banks worldwide [21]. Private banks levy a charge (usually between $1,500 and $2,000) on acceptance of the units, plus an annual storage fee (usually between $90 and $200) [22].

1.3 Other Types of Cord Blood Banks

Other types of blood banks also exist [21], such as family-directed [23] and mixed public–private [24], but these are less numerous, although family-directed biobanks are potentially extremely promising and it is to be hoped that their numbers will increase [25].

In Europe both international and supranational institutions (European Union, Council of Europe) have published regulations governing the storage of cord blood, as have national institutions such as governments and parliaments. The following paragraphs describe the key regulations at all levels and offer a brief note on some of the opinions expressed by National Bioethics Committees, which in some cases influenced the stance of national regulatory measures.

2 International and Supranational Documents

Directive 2002/98 [26] states that: “Modern blood-transfusion practice has been founded on the principles of voluntary donor services, anonymity of both donor and recipient, benevolence of the donor, and absence of profit on the part of the establishments involved in blood transfusion services” (“Whereas” no. 20); “Member States shall take the necessary measures to encourage voluntary and unpaid blood donations with a view to ensuring that blood and blood components are in so far as possible provided from such donations” (Article 20).

Three EU Directives (2004/23/EC, 2006/17/EC, 2006/86/EC) establish the minimum requisites of quality and safety for the donation, procurement, testing, processing, preservation, storage, and distribution of human cells in the European Union.

Directive 2004/23/EC [27] requires member states to designate one or more “competent authorities” to be responsible for implementing the Directive, in particular not only with regard to authorizations, accreditations, and licenses but also

organization and oversight. The Directive specifies binding requisites to ensure the traceability of tissues and cells, for the control of import and export, the registration of businesses, and the notification of adverse reactions and events. The Directive entered into force on 7 April 2004, and the deadline for its transposition in the Member States was 7 April 2006.

Two later Directives were subsequently passed to implement Directive 2004/23.

The implementing Directive 2006/17/EC [28] establishes specific technical requirements for each step in the processing of human tissues and cells, in particular: requirements for the procurement of human tissues and cells, selection criteria for donors of tissues and cells, laboratory tests required for donors, procedures for the donation and procurement of tissues and/or cells and for their reception at the tissue bank, requirements for the direct distribution to the recipient of specific tissues and cells.

The implementing Directive 2006/86/EC [29] defines the technical requirements for: coding, processing, preservation, storage, and distribution of tissues and cells; authorization (quality system requirements), notification of serious adverse events and reactions, and traceability.

Recommendations drawn up by the Committee of Ministers of the Council of Europe are addressed to the Governments of the Council of Europe member States. The conclusions of the Recommendations Rec(2004)8 on autologous cord blood banks, adopted on 19th May 2004, effectively summarise the position on this issue of numerous competent institutions and the policies adopted in the majority of European countries:

1. If cord blood banks are established, they should be based on altruistic and voluntary cord blood donation and used for allogeneic transplantation and related research
2. The promotion of donation for autologous use and the establishment of cord blood banks for autologous use should not be supported by member states or their health services
3. Accurate information should be provided to the population about the advantages and disadvantages of cord blood banks
4. Where autologous cord blood banks are being established, the promotional material or information provided to families must be accurate, and fully informed consent to cord blood storage must be obtained
5. Autologous cord blood banks that are being established must meet the quality and safety standards set out in the Council of Europe's Guide to safety and quality assurance for organs, tissues, and cells [30].

3 National Regulations

Although the EU member states have transposed the same EU Directives, significant differences between the laws of member countries nonetheless remain.

3.1 In Some States Only Public Cord Blood Banks Are Authorized

3.1.1 Belgium

In Belgium, for example, the storage of cord blood is allowed only for philanthropic allogeneic use or for directed use. This is not stipulated by regulations applying to blood as such, but by the Law of 19 December 2008 on the collection and preservation of tissues and other human body parts for therapeutic and research purposes [31]. Article 8(1) of this Law prohibits:

1. The removal or any other procedure performed on human body materials under the present law that is not carried out for precise and scientifically based preventive, diagnostic or therapeutic purposes, or for precise and pertinent scientific research for a specified purpose
2. All uses of human body materials under the present law that are not performed for precise and scientifically based preventive, diagnostic or therapeutic purposes, or for pertinent and specified scientific research purposes for which approval has been granted by an Ethics Committee pursuant to the law of 7 May 2004 on experiments involving human beings
3. The removal of any body materials for purposes for which the expected consequences for the living donor are not in proportion to the goal pursued
4. The removal and storage of human body materials for future autologous or allogeneic use for a specific identified recipient, unless
   (a) At the time of both removal and/or procurement, the person for whom the human body materials are destined suffers from or presents an exceptionally high and scientifically recognized risk of suffering from a pathology for which the usefulness of the above operations is scientifically proven
   (b) The human body materials remain available for therapeutic use by third parties and are registered.

3.1.2 France

In France Article 7 of Law 2011-814 [32] acknowledges the therapeutic potential of cord blood and hematopoietic stem cells (treating them in the same statutory terms as tissues, cells, and products of the human body) and confirms the pre-existing ban [33, 34] on the preservation of cord blood for personal use except where a proven therapeutic need for the newborn or a family member is recognized at the time of birth. There is also a ban on the preservation for hypothetical future needs that are

not scientifically proven, as well as on exportation. Commercial blood banks are thus not authorized in France. Article 8 of the law lays down the requisites for facilities authorized to collect cord blood for therapeutic purposes.

3.1.3 Italy

In Italy, the Ministerial Decree of 18 November 2009, "Provisions concerning the storage of umbilical cord blood stem cells for autologous-directed use" [35], allows:

- The conservation of cord blood for allogeneic use—in other words for persons other than those from whom the cells were harvested—for altruistic purposes, in dedicated public health facilities
- The storage for the benefit of the newborn or of a consanguineous child suffering, at the time of the collection or previously, from a pathology that it is clinically appropriate to treat with cord blood stem cells
- The storage for autologous/directed use in cases of specific pathologies not yet evident (listed in the annex to the decree) for which there is proven scientific evidence for potential use, including for clinical experimentation

The same decree also allows for exportation of the sample of umbilical cord blood for autologous use, at the expense of the exporter and after authorisation and counselling have been given in accordance with the Agreement between the State and the Regions (i.e. Permanent Conference for relations between the State, the Regions, and the autonomous Provinces of Trento and Bolzano) [36].

Several other European countries have introduced similar legislation banning the private storage of cord blood, although it should be noted that in many countries in which the private storage of cord blood for autologous use is banned, agencies of foreign-based commercially-operated biobanks offer the possibility of exporting and storing cord blood units in other nations.

3.2 Commercially-Operated Private Cord Blood Banks Are Allowed in Some States

3.2.1 Germany

In Germany there are six non-profit banks for the allogeneic preservation of cord blood, which also offer directed preservation where indicated by the family, subject to a request from the physician. Transplant centers use the German National Registry of Blood Stem Cell Donors to locate cells [37].

Private banks are allowed and the same guidelines apply to both public banks for allogeneic preservation and private banks. Nonetheless, the German Federal Medical Association recognizes that "There are currently no known indications for autologous preservation" [38].

3.3 Mixed Public–Private Banks Exist in Some States

3.3.1 United Kingdom

The collection of cord blood in the United Kingdom is regulated by the Human Tissue Authority (HTA) and governed by the Human Tissue Act [39] in England, Wales, and Northern Ireland and in Scotland by the very similar Human Tissue (Scotland) Act of 2006 [40]. The current regulations applying to cord blood came into force on 5th July 2008 [41], and require that both public and private maternity units in which cord blood is collected for storage ensure the presence of properly trained personnel for harvesting, implement procedures to guarantee that this in no way prejudices assistance to mother and child, and ensure the traceability of samples.

There are currently three non-profit banks for the collection of cord blood for allogeneic use: the National Health Service Cord Blood Bank (NHS-CBB) (formerly the London CBB), the Newcastle University Hospital Bank, and the Northern Ireland Cord Blood Bank (NI-CBB). One bank is registered in Scotland but is not yet fully operational. Cord blood units are registered with the British Bone Marrow Registry (BBMR) [42] and the BMDW Registry [16] for international access. Several hospitals store cord blood for clinical purposes but use it only for their own needs or those of neighboring hospitals, and do not register it.

An innovative experiment in dual banking (mixed public–private storage) was set up in the UK by Richard Branson. Twenty percent of each cord blood sample harvested by Virgin Health Bank is stored for private use by the child from whom it was taken or by a family member, while 80 % is donated to the public arm of the bank, which is accessible to anyone in the world who needs it, at no cost. Virgin Health Bank's challenge is to combine the known potential of public-sector allogeneic storage with the possible, albeit at present remote, applications of autologous storage in specific fields of regenerative medicine [43].

Reviews of European regulations can be found in the literature [44–46].

4 Opinions of National Bioethics Committees

Several European national bioethics committees have expressed opinions regarding the storage of cord blood [47]. These committees usually draw up their opinions at the request of national governments or parliaments, for which their reports are useful points of reference, although it is worth noting that the bioethics committees have frequently published their opinions subsequent to the enactment of relevant legislation. In the latter case, the opinions expressed remain as significant reference points distinct from national laws on the subject.

In Europe the national bioethics committees of several nations have expressed opinions in the matter of cord blood: Austria [48], Belgium [49], Cyprus [50], France [51], Greece [52], Ireland [53], Italy [54], Portugal and Spain [55], and United Kingdom [56]. Most of these documents were issued after 16th March 2004, when the European Group on Ethics in Science and New Technologies of the European Commission (EGE) published its Opinion no. 19, on which they draw heavily [57, 58].

According to the EGE "The legitimacy of commercial cord blood banks for autologous use should be questioned as they sell a service, which has presently, no real use regarding therapeutic options. Thus they promise more than they can deliver. The activities of such banks raise serious ethical criticisms. While some members of the Group consider that this activity should be banned, the majority of the Group considers that the activities of these banks should be discouraged but that a strict ban would represent an undue restriction on the freedom of enterprise and the freedom of choice of individuals/couples. These banks should operate under strict conditions" [57].

Notwithstanding subtle differences in their approaches to cord blood banking, national bioethics committees are unanimous in recommending the promotion of cord blood storage by public banks for altruistic purposes and in discouraging private storage facilities [47, 59]. Their positions can be efficiently summarised by comparing the recommendations contained in the earliest and in the most recent documents on cord blood banking published by national bioethics committees in Europe. The two reports were issued respectively: in 2002, by the French committee [51], and in 2012, jointly by the Portuguese and Spanish committees [55]. The recommendations proposed in the two documents are shown in the Appendix.

Finally, it is worth recalling that numerous other institutions (scientific institutes and societies, organizations, scientific and professional associations among others) have issued different types of documents: guidelines, recommendations, opinions, reviews, etc. Although these documents are not binding, many of them are important as reference points on account of the high repute of their authors. Reviews that present and discuss these documents can be found in the specialized literature [60].

5 Appendix

Recommendations from the Comité Consultatif National d'Éthique pour les Sciences de la Vie et de la Santé (France) [51] and from the Conselho Nacional de Ética para as Ciências da Vida and the Comité de Bioética de España (Portugal and Spain) [55].

5.1 France

"Ethical difficulties arise because the concept of cord blood banks for exclusively autologous use carries with it a number of perils:

1. The gravest danger is for society in so far as setting up such banks is likely to contradict the principle of solidarity, without which no society can survive.
2. Such banks raise hopes of utopia and disguise a mercantile project using assistance to children as a screen.
3. They jeopardize justice and equity. If any reasonable indications existed, then the offer should be systematic, organized, managed, and supervised by public authorities; cost and broadness of scale then enter the picture. The disproportionate, and for the time being useless, cost of generalised autologous storage is in total contradiction with the obligation to provide public health based on solidarity and awareness of priorities.
4. Management by the private sector may be seen as discrimination based on wealth. However, this would hardly be exceptional in the healthcare sector, and those who use these programs cannot be blamed for their ingenuousness.
5. The futility of autologous banks and their cost would be provocation in the eyes of the very poor, in particular in the Southern hemisphere".

5.2 Portugal and Spain

"The CNECV and the CBE are of the opinion that one must:

1. Promote the free and altruistic donation of cord blood, the umbilical cord itself and placenta, for use in allogeneic transplants (…).
2. Disclose the importance of the solidary donation of such products at the time of delivery, for use in allogeneic transplants, and provide all the information necessary for the consent process, during the prenatal consultations, starting from the second trimester of pregnancy.
3. Establish a routine collection of blood and tissue from the umbilical cord and placenta in all pregnant women, for a public biobank, which always considers the possibility of refusal on the part of the woman, ensuring the ethical process of obtaining informed consent.
4. Request accreditation for the licensing process of all banks, public or private, and demand the same quality criteria for all samples used in the country.
5. Require that all public or private banks comply with identical technical and scientific internationally established quality standards, as well as with the ethical and legal requirements that assure respect for the dignity of those involved and for social justice in the community.
6. Strongly discourage the commercial appeals for cryopreservation of these fetal products exclusively for autologous use, as they compete with samples available for allogeneic transplant, consequently harming the common good.

7. Provide public biobanks with the necessary means to test, process and store the derived cells, maintain a quality system and their connection to European and international networks, and safeguard their continued sustainability.
8. Allow the conservation in public banks of samples suitable for use in close relatives, in case there is a proven clinical indication.
9. Verify that advertised claims of therapeutic applications have proven validity and clinical usefulness.
10. Provide the public obstetrics services and maternity hospitals with the means needed for that collection, and include it in their functional duties.
11. Recommend special attention from the regulatory authorities to the advertisement by commercial services in maternity hospitals, obstetric services, and health centers.
12. Prohibit any type of direct remuneration or compensation to health professionals from public entities who promote or make collections for private companies.
13. Regulate and supervise the activities of banks operating in each of both States and verify their compliance with international quality standards.
14. Ensure the representativeness of the samples preserved with respect to resident populations, with particular attention to population minorities and rare haplogroups.
15. Promote research into the methods of processing and preservation of cells derived from the umbilical cord and placenta, and new clinical applications.
16. Prevent the offer of other health-related genetic tests, without medical prescription, on the products collected at the time of delivery or on blood samples from newborns".

References

1. Ende M, Ende N. Hematopoietic transplantation by means of fetal (cord) blood. A new method. Va Med Mon. 1972;99:276–80.
2. Gluckman E, Broxmeyer HA, Auerbach AD, Friedman HS, Douglas GW, Devergie A, et al. Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical cord blood from an HLA-identical sibling. N Engl J Med. 1989;321:1174–8.
3. Moyer MW. Cell banks: life blood. Nature. 2013;498:S16.
4. Eapen M. Cord blood transplantation for leukemia in children. In: Broxmeyyer HE, editor. Cord blood: biology, transplantation, banking, and regulation. Bethesda: AABb Press; 2011. p. 467–75.
5. Ballen KK, Gluckman E, Broxmeyer HE. Umbilical cord blood transplantation: the first 25 years and beyond. Blood. 2013;122:491–8.
6. Jensen A, Hamelman E. First autologous cell therapy of cerebral palsy caused by hypoxic-ischemic brain damage in a child after cardiac arrest—individual treatment with cord blood. Case Rep Transplant. 2013;2013:951827.
7. Navarrete C, Contreras M. Cord blood banking: a historical perspective. Br J Haematol. 2009; 147:236–45.
8. Rubinstein P, Adamson JW, Stevens C. The placental/umbilical cord blood program of the New York blood center. A progress report. Ann NY Acad Sci. 1999;872:328–35.
9. Sullivan MJ. Banking on cord blood stem cells. Nat Rev Cancer. 2008;8:555–63.
10. Webb S. Banking on cord blood stem cells. Nat Biotechnol. 2013;31:585–8.

11. Brand A, Rebulla P, Engelfriet CP, Reesink HW, Beguin Y, Baudoux E, et al. Cord blood banking. Vox Sang. 2008;95:335–48.
12. Querol S, Rubinstein P, Marsh SGE, Goldman J, Madrigal JA. Cord blood banking: 'providing cord blood banking for a nation'. Br J Haematol. 2009;147:227–35.
13. Sun J, Allison J, McLaughlin C, Sledge L, Waters-Pick B, Wease S, et al. Differences in quality between privately and publicly banked umbilical cord blood units: a pilot study of autologous cord blood infusion in children with acquired neurologic disorders. Transfusion. 2010;50:1980–7.
14. Bordet S, Minh Nguyen T, Knoppers BM, Isasi R. Use of umbilical cord blood for stem cell research. J Obstet Gynaecol Can. 2010;32:58–61.
15. Faden R, Dawson L, Bateman-House A, Mueller AD, Bok H, Chakravarti A, et al. Public stem cell research. Considerations of justice in stem cell research and therapy. Hastings Cent Rep. 2003;33:13–27.
16. Bone Marrow Donors Worldwide. 2013. www.bmdw.org
17. Comité Italo-Français pour le bon usage du sang du cordon ombilical. Audition au Parlement Européen sur le don de la solidarité, collection privée et la préservation du sang de cordon ombilical. 15 Mars 2011. www.adoces.it/donazione-sangue-cordone/category/lazione-europea/
18. Nietfeld JJ, Pasquini MC, Logan BR, Verter F, Horowitz MM. Lifetime probabilities of hematopoietic stem cell transplantation in the U.S. Biol Blood Marrow Transplant. 2008; 14:316–22.
19. Associazione Donatori Cellule Staminali (ADoCeS), Contu L. Il dibattito sul sangue del cordone ombelicale: a chi e a che cosa serve la conservazione privata del sangue cordonale? (Rapporto 2011), 25 May 2011. www.adoces.it/materiale/rapporto2011.pdf
20. Smith FO. Why do parents engage in private cord blood banking: fear, realistic hope or a sense of control? Pediatr Blood Cancer. 2011;56:1003–4.
21. Butler MG, Menitove JE. Umbilical cord blood banking: an update. J Assist Reprod Genet. 2011;28:669–76.
22. Ballen KK. Challenges in umbilical cord blood stem cell banking for stem cell reviews and reports. Stem Cell Rev. 2010;6:8–14.
23. Smythe J, Armitage S, McDonald D, Pamphilon D, Guttridge M, Brown J, et al. Directed sibling cord blood banking for transplantation: the 10-year experience in the national blood service in England. Stem Cells. 2007;25:2087–93.
24. Fisk NM, Atun R. Public-private partnership in cord blood banking. BMJ. 2008;336:642–4.
25. Gluckman E, Ruggeri A, Rocha V, Baudoux E, Boo M, Kurtzberg J, et al. Family-directed umbilical cord blood banking. Haematologica. 2011;96:1700–7.
26. European Parliament, Council of the European Union. Directive 2002/98/EC of the European Parliament and of the Council of 27 January 2003 setting standards of quality and safety for the collection, testing, processing, storage and distribution of human blood and blood components and amending Directive 2001/83/EC. Official J Eur Union. 2003;L33:30–40.
27. European Parliament, Council of the European Union. 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. Official J Eur Union. 2004;L102:48–58.
28. European Commission. 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. Official J Eur Union. 2006;L038:40–52.
29. European Commission. Commission Directive 2006/86/EC 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. Official J Eur Union. 2006;L294:32–50.
30. Council of Europe (Committee of Ministers). Recommendation Rec(2004)8 of the Committee of Ministers to Member States on Autologous Cord Blood Banks. Adopted by the Committee

of Ministers on 19 May 2004 at the 88th meeting of the Ministers' Deputies. 2004. https://wcd.coe.int/ViewDoc.jsp?Ref=Rec%282004%298&Language=lanEnglish&Site=COE&BackColorInternet=DBDCF2&BackColorIntranet=FDC864&BackColorLogged=FDC864
31. Agence Fédérale des Médicaments et des Produits de Santé. Loi relative à l'obtention et à l'utilisation de matériel corporel humain destiné à des applications médicales humaines ou à des fins de recherche scientifique. Moniteur Belge 30 Décembre 2008;68774–87.
32. Assemblée Nationale de la République Française, Sénat de la République Française. Loi n. 2011-814 du 7 juillet 2011 relative à la bioéthique. Journal Officiel de la République Française 8 Juillet 2011;157.
33. Ministère de la Santé et des Sports. Arrêté du 16 décembre 1998, article II-2 relatif au prélèvement de cellules souches hématopoïétiques issues de sang placentaire. Journal Officiel de la République Française 30 décembre 1998;302.
34. Ministère de la Santé et des Sports. Arrêté du 14 septembre 2009 fixant le contenu du dossier accompagnant la demande d'autorisation ou la demande de renouvellement d'autorisation d'effectuer des prélèvements de cellules à des fins thérapeutiques. Journal Officiel de la République Française 11 Octobre 2009;236.
35. Ministero del Lavoro, della Salute e delle Politiche Sociali. Decreto 18 novembre 2009. Disposizioni in materia di conservazione di cellule staminali da sangue del cordone ombelicale per uso autologo dedicato. *Gazzetta Ufficiale della Repubblica Italiana*—Serie generale 31 dicembre 2009;n. 303.
36. Conferenza permanente per i rapporti tra lo Stato, le Regioni e le Province autonome di Trento e di Bolzano. Accordo tra il Governo, le Regioni e le Province autonome di Trento e di Bolzano sull'esportazione di campioni di sangue da cordone ombelicale per uso autologo. Accordo ai sensi dell'articolo 4 del decreto legislativo 28 agosto 1997, n.281, previsto dall'articolo 3, comma 1, dell'Ordinanza del Ministro del lavoro, della salute e delle politiche sociali 26 febbraio 2009 recante: "Disposizioni in materia di conservazione di cellule staminali da sangue del cordone ombelicale". www.statoregioni.it/dettagliodoc.asp?idprov=8136&iddoc=26575&tipodoc=2&conf=csr
37. Zentrales Knochenmarkspender Register Deutschland. 2013. www.zkrd.de
38. Bundesärztekammer (Federal Medical Association). Richtlinien zur Transplantation von Stammzellen aus Nabelschnurblut ("Guidelines for the transplantation of cord blood"). Deutsches Ärzteblatt 1999;96:69–76. www.bundesaerztekammer.de/downloads/transnabel_pdf.pdf
39. Human Tissue Authority. Human Tissue Act. 15 November 2004. www.legislation.gov.uk/ukpga/2004/30/contents
40. Scottish Parliament. Human tissue (Scotland) Act 2006 (asp 4). 116 March 2006. www.legislation.gov.uk/asp/2006/4/pdfs/asp_20060004_en.pdf
41. Human Tissue Authority. New rules for cord blood collection. 30 April 2008. www.hta.gov.uk/media/mediareleases.cfm/418-New-rules-for-cord-blood-collection.html
42. British Bone Marrow Registry. 2013. www.nhsbt.nhs.uk/bonemarrow
43. Mayor S. World's first public-private cord blood bank launched in UK. BMJ. 2007;334:277.
44. Meijer I, Knight M, Mattson P, Mostrert B, Simmonds P, Vullings W (editors), Technopolis Group. Cord blood banking in the UK. An international comparison of policy and practice Amended version January 2009. www.technopolis-group.com/resources/download/reports/2f952_final_09011\4.pdf
45. Petrini C. Banques de sang cordonal en Europe: législation et implications éthiques. Rev Gén Droit Méd. 2011;40:171–95.
46. Petrini C. A comparative analysis of the opinions from European National and International Ethics Committees regarding the collection, storage and use of umbilical cord blood. Blood Transfus. 2012;10:279–89.
47. Petrini C. European regulations on cord blood banking: an overview. Transfusion. 2012;52:668–79.
48. Austrian Bioethics Commission at the Federal Chancellery. Cord blood banking. 2008. www.bka.gv.at/DocView.axd?CobId=31001

49. Comité Consultatif de Bioéthique de Belgique: Avis n. 42 du 16 avril 2007 relatif aux banques de sang de cordon ombilical. 2007. https://portal.health.fgov.be/pls/portal/docs/page/internet_pg/homepage_menu/gezondheidzorg1_menu/overlegstructuren1_menu/comitees1_menu/bioethischecommissie1_menu/avis25_menu/avis25_docs/opinion%2042%20web.pdf
50. Cyprus National Bioethics Committee. Opinion of the Cyprus National Bioethics Committee regarding bioethical dilemmas of umbilical cord blood banking by private profit making companies. 2004. www.bioethics.gov.cy/law/cnbc/cnbc.nsf/all/b5d91dc497a2eb49c2257308004b46b2?opendocument
51. Comité Consultatif National d'Éthique pour les Sciences de la Vie et de la Santé (National Ethics Advisory Committee for Life Sciences and Health). Avis 74—Les banques de sang de cordon ombilical en vue d'une utilisation autologue ou en recherche (Opinion 74—Umbilical cord blood banks for autologous use or for research). 2002. www.ccne-ethique.fr/docs/en/avis074.pdf
52. National Bioethics Commission—Greece. Opinion on umbilical cord blood banking. 2007. www.bioethics.gr/media/pdf/recommendations/opinion_uc_eng.pdf
53. Irish Council for Bioethics. Stem cell research: hope or hype? Exploration of ethical questions. 2008. http://www.bioethics.ie/uploads/docs/StemCellScience&Ethics.pdf
54. Comitato Nazionale per la Bioetica. Mozione del Comitato Nazionale per la Bioetica sulla raccolta e la conservazione di cellule staminali derivate da cordone ombelicale. Approvata nella seduta plenaria del 13 luglio 2007. 2007. http://www.governo.it/bio3tica/mozioni/Mozione_cordonali.pdf
55. Conselho Nacional de Ética para as Ciências da Vida e do Comité de Bioética de España. Parecer n.º 67 CNECV/CBE sobre os Bancos de Sangue do Cordão Umbilical (Parecer conjunto do Conselho Nacional de Ética para as Ciências da Vida e do Comité de Bioética de España). 2013. www.cnecv.pt/admin/files/data/docs/1356002356_Parecer%2067%202012%20CNECV%20CBE%20SgCordao-APROVADO.pdf
56. Nuffield Council on Bioethics. Human bodies: donation for medicine and research. 2011. www.nuffieldbioethics.org/sites/default/files/Donation_full_report.pdf
57. European Group on Ethics in Science and New Technologies. Opinion 19. Ethical aspect of umbilical cord blood banking. 2004. http://ec.europa.eu/bepa/european-group-ethics/docs/avis19_en.pdf
58. European Group on Ethics in Science and New Technologies. Opinion 19. Ethical aspects of umbilical cord blood banking. 2004. Accompanying document. http://ec.europa.eu/bepa/european-group-ethics/docs/publ_op19_en.pdf
59. Petrini C. Umbilical cord blood collection, storage and use: ethical issues. Blood Transfus. 2010;8:139–48.
60. Petrini C. Ethical issues in umbilical cord blood banking: a comparative analysis of documents from national and international institutions. Transfusion. 2013;53:902–10.

Part II
Stem Cell Banking Worldwide

Chapter 7
Donor Recruitment and Eligibility Criteria for HLA-Homozygous iPS Cell Bank in Japan

Megumu K. Saito, Ayumi Matsunaga, Naoko Takasu, and Shinya Yamanaka

1 Introduction

In 2006, Shinya Yamanaka and Kazutoshi Takahashi at Kyoto University succeeded in inducing pluripotent stem cells from somatic cells by forcefully expressing four defined factors within mouse fibroblasts using retroviral vectors, and named these cells iPS cells [1]. In 2007, iPS cells were also generated from human skin cells [2]. Although iPS cells are very similar to embryonic stem (ES) cells in terms of their morphology, capacity for self-renewal, multi-potency, and gene expression profile, they have a great advantage in that they can be generated from somatic cells of any donor if they are made in accordance with a certain procedure. Therefore, iPS cell-based cell/tissue transplantation has been regarded as one of the most attractive applications for these cells.

From the perspective of transplantation immunology, the most appropriate source of cell transplantation is autologous cells/tissues, in which iPS cell-derived target cells/tissues are transplanted into the same patient. However, there are several problems associated with autologous transplantation:

M.K. Saito (✉)
Department of Clinical Application, Center for iPS Cell Research and Application, Kyoto University, Kyoto 6068507, Japan
e-mail: msaito@cira.kyoto-u.ac.jp

A. Matsunaga • N. Takasu
Medical Applications Promoting Office, Center for iPS Cell Research and Application, Kyoto University, Kyoto 6068507, Japan

S. Yamanaka (✉)
Department of Reprogramming Science, Center for iPS Cell Research and Application, Kyoto University, Kyoto 6068507, Japan

Gladstone Institute of Cardiovascular Disease, San Francisco, CA 94158, USA
e-mail: yamanaka@cira.kyoto-u.ac.jp

D. Ilic (ed.), *Stem Cell Banking*, Stem Cell Biology and Regenerative Medicine,
DOI 10.1007/978-1-4939-0585-0_7,

Table 7.1 Frequent HLA haplotypes in Japan

Rank	A	B	DRB1	Frequency of haplotype (%)
1	*24:02	*52:01	*15:02	8.275
2	*33:03	*44:03	*13:02	4.248
3	*24:02	*07:02	*01:01	3.769
4	*24:02	*54:01	*04:05	2.695
5	*02:07	*46:01	*08:03	1.940
6	*11:01	*15:01	*04:06	1.391
7	*24:02	*59:01	*04:05	1.097
8	*11:01	*54:01	*04:05	0.995
9	*24:02	*40:06	*09:01	0.857
10	*26:01	*40:02	*09:01	0.797

17,325 Haplotypes obtained from 4,743 families were analyzed. Downloaded from the website of HLA laboratory [3]

1. Since it takes nearly a year to establish iPS cell clones and to differentiate them into target cells/tissues, in emergency settings when immediate cell transplantation is required due to an unexpected disease or injury, any attempt to start generating iPS cells after the onset of the disease or injury would be useless
2. iPS cells established from somatic cells of a patient with a hereditary disease carry the same genetic mutation, and therefore require genetic correction before being used
3. Considering the potential costs, it would be financially prohibitive to generate iPS cells for each individual for autologous transplantation

Therefore, it is necessary to consider allogeneic transplantation from HLA-matched donors.

Ideally, the HLA types should perfectly match between the donor and the recipient in order to successfully engraft iPS cell-derived differentiated cells/tissues after allogeneic transplantation and to make them active to exert their functions. To realize this ideal, it is necessary to keep cells from donors of random HLA haplotypes in bank, similar to the case of a bone marrow bank or a cord blood bank. Unlike these somatic cell banks, however, the generation of iPS cells and their quality control is so time consuming and costly that it makes this unfeasible. Therefore, in Japan, we have aimed to establish multiple clinical grade iPS cell lines from donors homozygous for three HLA loci: HLA-A, -B, and -DR, in order to establish an iPS cell bank for medical use that can be used for cell/tissue transplantation in the future. If iPS cells are generated from donors homozygous for HLA haplotypes that are found in the Japanese population at a high frequency (these are called high-frequency HLA homozygous donors), cells derived from these iPS cells will be able to be transplanted into recipients heterozygous for the same haplotypes with a reduced risk of rejection.

Since Japan has a relatively homogenous ethnic population, the required size of the Japanese HLA-homozygous iPS cell bank seems to be relatively small (Table 7.1) [3]. Nakatsuji et al. have estimated that 30 homozygous ESC lines would match

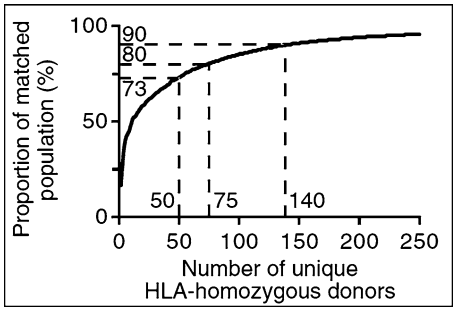


Fig. 7.1 Estimated cumulative coverage of the Japanese population by theoretical unique HLA homozygous donors at HLA-A, HLA-B, and HLA-DRB1 loci with four-digit specification [6]

82.2 % of the Japanese population, while 50 homozygous lines would match 90.7 % of the population [4, 5]. Okita et al. also estimated that 50 and 140 homozygous iPS cell lines would be sufficient to allow for HLA-matched transplantation for 73 % and 90 % of the Japanese population, respectively (Fig. 7.1) [6].

We are currently establishing the general design of the Japanese iPS cell bank for regenerative medicine. This project is mainly being conducted by the Center for iPS Cell Research and Application (CiRA), Kyoto University, in collaboration with Kyoto University Hospital. In this chapter, we focus on the donor eligibility criteria and contents of the informed consent for the Cell Bank of Kyoto (CiBK) project.

2 Regulatory Framework for iPS Cell-Based Cell Therapy in Japan

Before discussing the topic at hand, the authors wish to mention Japan's regulatory framework in regard to iPS cells. In Japan, there are two types of regulatory systems that must be adhered to when trialing new cell therapies [7]. These relate to clinical trials and clinical research, each of which is covered by different laws. Clinical trials are implemented based on the Pharmaceutical Affairs Act. Substances receiving pharmaceutical approval as part of this track may be sold as medicinal products and materials, and used in a treatment provided under Japanese public health insurance coverage. Clinical research is physician-led research, implemented based on "Guidelines on Clinical Research using Human Stem Cells" [8], under the provisions of the Medical Practitioners Act. The administration of cells derived from human stem cells to humans requires the pharmaceutical approval of the Pharmaceuticals and Medical Devices Agency (PMDA) and the Ministry of Health, Labor and Welfare on the clinical trial track, while as clinical research, it requires a review by the Institutional Review Board (IRB) of the relevant institution, as well as by the Ministry of Health, Labor and Welfare. Clinical research results are within the scope of combined (insured and uninsured) medical treatments as advanced medical technologies, but are not covered for regular use by insurance, and as such they cannot be retailed as pharmaceutical products and are restricted to limited

clinical applications. The important thing to remember is that an iPS cell bank is not in itself an end product, and as such is not administered directly to patients, with the result that applications cannot be made to either system for the bank itself. As a result, we have proposed research plans for this project and are in the process of discussing it with the various related ministries and agencies in order to establish informed consent/donor selection criteria, which would ensure that researchers and doctors using terminally differentiated cells are able to avail themselves of, and meet the requirements of, both systems.

3 Current Outline of the Japanese HLA Homozygous iPS Cell Bank

In the CiBK project, we envisage establishing iPS cells from homozygous HLA donors with roughly the top five to ten types of high-frequency HLA haplotypes over the next 5 years. During this time we will verify the usefulness and safety of the high-frequency HLA haplotype iPS cell bank, and providing we determine that the creation of the high-frequency HLA haplotype iPS cell bank is both appropriate and viable, we will go on to establish the iPS cell bank of up to approximately the top 100 haplotypes, with the objective of thereby providing coverage for around 90 % of the Japanese population. If this plan is executed simply by recruitment of healthy volunteers with unknown HLA haplotypes, it is estimated that around 100,000 people would need to be screened in order to establish the top ten types, which is unrealistic for both physical and financial reasons. Therefore, in the CiBK project, we intend to obtain information regarding people who have, for whatever reason, already had their HLA type examined, and ask those who are HLA homozygous to take part in the research project of their own free will.

At present the Ethics committee in Kyoto University has approved the establishment of an iPS cell bank from donor candidates undergoing HLA screening at Kyoto University Hospital and donor candidates undergoing HLA screening as platelet transfusion donors with the Japanese Red Cross, as well as from umbilical cord blood stored as part of the cord blood bank. The establishment of iPS cells is to be done at clinical grade, from peripheral blood or umbilical cord blood, in the Cell Processing Center that is part of CiRA; further details of this are currently under consideration.

As stated in the preceding section, there are currently two routes by which cell therapy products can be administered to patients, and we are in the process of creating donor eligibility criteria to ensure that the program is compatible with both systems. The guidelines, laws, and regulations to be adhered to are as follows: for clinical research, the “Guidelines on Clinical Research using Human Stem Cells” [8]; and for clinical trials, the “Japanese Standards for Biological Ingredients” [9] and the “Guidelines relating to ensuring the Quality and Safety of Pharmaceuticals, etc., Processed from Human (homogenous) iPS (type) Cells” [10]. Donor interview

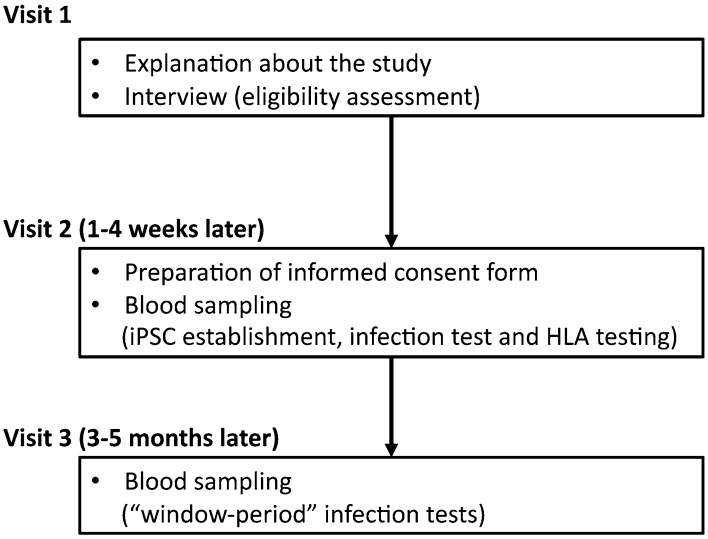


Fig. 7.2 Flow of study participation

sheets have been created based on the interview sheet used for blood donors by the Japanese Red Cross, which is based on the Japanese Standards for Biological Ingredients, but is already in use in regard to humans [11].

In order to avoid false positives being created as a result of a window period, some Japanese guidelines require a second infection screening several months after samples are harvested. This requirement has been adhered to in this project, in which secondary screening is to be carried out (Fig. 7.2).

The inclusion and exclusion criteria for CiBK donors are as follows:

3.1 Inclusion Criteria

Subjects should meet all the following criteria.

- Age ≥20 years old at the time of informed consent
- Capable of giving written consent on the basis of his/her free will after receiving a thorough explanation of the project and understanding the explanation (those who require a legally authorized representative for signing the consent form will not be included)
- Any race and sex
- The homozygocity of at least three loci of HLA, including HLA-A, -B, and -DR, should be confirmed by genetic methods
- Candidates who have type O in the ABO blood type are preferred

3.2 Exclusion Criteria

Subjects who meet any of the following criteria should be excluded from the project based on the "Guidelines to Ensure the Quality and Safety of Human (homologous) iPS (like) Cell-Derived Drugs," "Standards for Biological Substances" and the "Guidelines for Clinical Research Using Human Stem Cells" (issued by the Ministry of Health, Labor and Welfare, Japan).

Regarding the following infections, interviews and blood tests will be performed, and those with positive results will be excluded from the project.

1. Hepatitis B (HBV) (HBs-Ag positive)
2. Hepatitis C (HCV) (HCV-Ab positive)
3. Human immunodeficiency virus (HIV) infection (HIV-Ab positive)
4. Human T-cell leukemia virus 1 (HTLV-1) infection (HTLV-1 Ab positive)
5. Parvovirus B19 infection (Human parvovirus B19 DNA, PCR-positive)
6. *Treponema pallidum* infection (Treponema pallidum Hemaglutination Assay/ TPHA/-positive)

Subjects who have a present or past history of any of the following diseases confirmed through interviews will be excluded:

1. Bacterial or protozoal infections, such as chlamydia infection, gonorrhea, tuberculosis, malaria, leishmaniasis, Chagas' disease, African trypanosomiasis and babesiosis
2. Confirmed or suspected sepsis
3. Malignancies
4. Confirmed or suspected transmissible spongiform encephalopathy or other cognitive disorders
5. Apparent hereditary diseases, which may affect the application of iPS cell bank

Other

1. Female subjects who are pregnant, breastfeeding, or may be pregnant
2. Subjects assessed by the principal investigator as not being suitable as a donor

4 Details of the Informed Consent

The explanation of the study to the subjects, obtainment of informed consent, and sampling of tissues will be performed at Kyoto University Hospital. The informed consent document and the informed consent form prepared by the CiRA will be used in the project. The informed consent document and the informed consent form prepared will be used after being reviewed by the Ethics Committee of Kyoto University.

In order to avoid any possibility of coercing subjects to consent to participate in the project and to ensure the anonymity of the personal information of subjects and

Table 7.2 Items of the informed consent document/form for CiBK

1. Purpose of the study
2. Review of the study by the Medical Ethics Committee
3. Study participants
4. Study period
5. iPS cell bank for medical use (use for research, use for medical care)
6. What you are requested to do
7. Risks and burden [risks associated with sampling of your blood, risk of leakage of personal information, other burdens on participants: frequency of visits]
8. Costs to you
9. Genetic analysis
10. Voluntary participation and withdrawal from the study
11. Handling of test results
12. Expected benefits and risks of participating in the study
13. Handling of samples after completion of the study
14. Publication of study progress and results
15. Handling of intellectual property
16. Study organization and financing
17. Contact information

the voluntary nature of their participation, a research coordinator who has no interest in the project will be in charge of providing the explanation, helping at the time of obtaining informed consent and handling personal information under the supervision of the personal information manager. Considering the subjects' need to think about whether they wish to participate in the study, they will be sent home after the explanation has been provided (Fig. 7.2). After a certain period of time to allow the subjects to make their final decision, the subjects who intend to participate in the study will be asked to return to the hospital and give their informed consent. Personal information will be strictly managed with linkable anonymization by the research coordinators under the supervision of the personal information manager. The items included in the informed consent document are shown in Table 7.2.

4.1 Withdrawal of Consent

Subjects can withdraw their consent in writing. Subjects are able to withdraw their consent even after iPS cell bank has been generated and their use for transplantation therapy has started. However, once the use of the iPS cells for a cell therapy for a specific recipient has been initiated, the iPS cells for the recipient will not be retractable in consideration of the potential impact on the therapy for the recipient.

4.2 Prohibited Procedures

The CiBK project will not involve the following procedures in compliance with the prohibition rules stipulated under Article 6 of the current "Guidelines on the Utilization of Human Embryonic Stem Cells" in Japan [12]:

- Create an individual through the transplantation of embryos produced by utilizing human iPS cells into a human or animal uterus or through any other method.
- Introduce human iPS cells into a human embryo.
- Introduce human iPS cells into a human fetus.

Additionally, in the CiBK project, germ cells are excluded as target cells.

4.3 Intellectual Property Rights Generated from This Study

If any intellectual property rights are generated from the results of this study, the rights will be managed by Kyoto University and do not belong to the participants who participated in this study. The ownership of any established iPS cells and cells that differentiate from these cells will be held by Kyoto University.

4.4 Application of iPS Cell Bank for Cell Therapy

As mentioned above, the cells from the iPS cell bank[s] themselves are not the final products transplanted into recipients, and therefore, are not subject to Japanese guidelines and regulations such as the "Guidelines on clinical research using human stem cells" [13] and "Japanese Standards for Biological Ingredients" [9]. If cells from iPS cell bank[s] generated by this study are to be used as the source of cell transplantation therapy for humans, a study plan submitted by each research institution, medical institution, or pharmaceutical company in compliance with relevant laws and guidelines stipulated by the government must be submitted and approved before use in each transplantation therapy. Moreover, in such cases, consent from the participants will not be obtained again for each transplantation or study plan.

Even after the death of subjects, iPS cells from the bank will continue to be used when transplantation therapy is planned using the cells, unless the subjects have expressed their objection to the posthumous use of their cells.

5 Conclusions

The creation of bank systems for use in the application of human iPS cells to cell transplantation therapy is also underway in the United States and Europe. At present, however, the interview categories and infection screening categories currently required by the US Food and Drug Administration and European Medicines Agency in regard to human specimens for cell therapy differ to those required in Japan. In order for cells to be exchanged between banks in different countries and regions it will therefore be necessary to define a unified set of criteria not only for these categories but also for quality control in regard to completed iPS cells.

Since the quality varies for each iPS cell line under the current technology, it is costly and time consuming to control/assure the quality of a number of iPS cell lines. Since iPS cells have almost infinite capacity for self-renewal, it is more practical to generate cell lines that are as versatile as possible and to establish a system that enables the cell lines to be supplied under strict quality control. Also from this viewpoint, it is preferable to generate high-frequency HLA donor-derived allogeneic iPS cells for medical use and to conduct strict quality control than to custom-make autologous iPS cells and to control their quality.

The goal of this project is to establish a significant basis for the use of iPS cells for cell transplantation therapy in the near future. Regarding the methods used to induce the differentiation of iPS cells into various functional cells (e.g. neurons, retinal cells, cardiac muscle, pancreatic β cells, and hematopoietic cells) and to transplant them, a number of studies have been performed over recent years, and the technology has been developing at an amazing speed. These methods are likely to be established as feasible technologies applicable to many cell types in the near future. If iPS cell banks are used effectively, they may contribute to the treatment of patients suffering from a refractory disease or injury for which conventional medicine has found no cure.

Acknowledgments We thank Tomoko Kosaka for donor coordination, Kentaro Azuma, Shinji Asonuma, Naoki Nagata, Rie Kato and Toshihiko Hoshino for the administrative assistance; Shinji Uemoto and Toshio Heike for their work at Kyoto University Hospital; Keisuke Okita, Kazutoshi Takahashi, Masato Nakagawa, Shin Kaneko, and Shin Kawamata for the management of cell processing units and scientific discussions; Shimon Tashiro, Ayako Kamisato, and Kaori Muto for discussions and comments regarding potential ethical and regulatory issues.

Funding for the CiBK is provided by the Ministry of Education, Culture, Sports, Science and Technology (MEXT) and the Funding Program for World-Leading Innovative Research and Development on Science and Technology (FIRST Program) of the Japan Society for the Promotion of Science (JSPS).

Shinya Yamanaka is a member without salary of the scientific advisory boards of iPierian, iPS Academia Japan, Megakaryon Corporation, and HEALIOS K. K. Japan.

References

1. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006;126:663–76.
2. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007;131: 861–72.
3. HLA Laboratory K Japan. Haplotype frequency. http://www.hla.or.jp/haplo/haplodl.php?type=&lang=en. Accessed 6 Sept 2013.
4. Nakatsuji N, Nakajima F, Tokunaga K. HLA-haplotype banking and iPS cells. Nat Biotechnol. 2008;26:739–40.
5. Nakajima F, Tokunaga K, Nakatsuji N. Human leukocyte antigen matching estimations in a hypothetical bank of human embryonic stem cell lines in the Japanese population for use in cell transplantation therapy. Stem Cells. 2007;25:983–5.
6. Okita K, Matsumura Y, Sato Y, Okada A, Morizane A, Okamoto S, et al. A more efficient method to generate integration-free human iPS cells. Nat Methods. 2011;8:409–12.
7. Yanagi K, Fukuda E, Jotatsu Y, Shikano M, Miyake S. Regulatory frameworks for cell therapy products in Japan. J Artif Organs. 2012;15:325–30.
8. Ministry of Health L.a.W. Guidelines on clinical research using human stem cells. http://www.mhlw.go.jp/english/policy/health-medical/medical-care/dl/guidelines.pdf. Accessed 6 Sept 2013.
9. Agency P.a.M.D. Japanese standards for biological ingredients (In Japanese). http://www.pmda.go.jp/operations/shonin/info/saisei-iryou/pdf/H170331_001.pdf. Accessed 6 Sept 2013.
10. Ministry of Health L.a.W. Guidelines relating to ensuring the quality and safety of pharmaceuticals, etc., processed from human (homogenous) iPS (type) cells (In Japanese). http://www.pmda.go.jp/kijunsakusei/file/guideline/biologics/120907-5.pdf. Accessed 6 Sept 2013.
11. Society T.J.R.C. Interview form for blood donation (In Japanese). http://www.jrc.or.jp/vcms_lf/ketsueki_monshinhyo_2013ver.pdf. Accessed 6 Sept 2013.
12. Ministry of Education C. Sports, Science and Technology. Guidelines on the utilization of human embryonic stem cells. http://www.lifescience.mext.go.jp/files/pdf/n743_01.pdf. Accessed 6 Sept 2013.
13. Ministry of Health L.a.W. Guidelines on clinical research using human stem cells. 2006. http://www.mhlw.go.jp/english/policy/health-medical/medical-care/dl/guidelines.pdf. Accessed 6 Sept 2013.

Chapter 8
Banking of Pluripotent Stem Cells: Issues and Opportunities from the NIH Perspective

Anastasia G. Efthymiou, Mahendra Rao, and Justin Lowenthal

1 Introduction

The first NIH funding for research involving hES cells was authorized more than a decade ago by President George W. Bush. This funding decision effectively limited the use of hES cell lines to lines generated before the President's decision date of August 9, 2001 [1]. The rationale was based primarily on moral concerns related to destroying embryos for research purposes. Though over 200 lines had been generated by this time point, ultimately only 21 cell lines were of adequate quality and number to be available for distribution [2], and many of those 21 lines were subsequently shown to have significant scientific flaws and questionable consent documentation. As time progressed, it became clear that this arbitrary time stamp was inhibiting the field significantly. Still, despite such constraints, the NIH strongly supported the regenerative medicine field, and, since fiscal year (FY) 2008, has allocated approximately $1.5 billion to human stem cell-related research, with about $396 million designated for hES cell research and $1.1 billion to human adult stem cell research [3].

Several fundamental advances have been made using ES cells in research, and the NIH helped spur the effort by creating the National Stem Cell Bank at the University of Wisconsin in 2005, which distributed the 20 or so lines that were approved under early government guidelines [4]. As private funding supplemented NIH funding and once regulations were relaxed under President Barack Obama (allowing embryos originally collected for clinical in vitro fertilization purposes to

A.G. Efthymiou (✉) • M. Rao
Center for Regenerative Medicine, National Institutes of Health,
Bethesda, MD, USA
e-mail: anastasia.efthymiou@gmail.com; efthymioua@mail.nih.gov

J. Lowenthal
Department of Bioethics, Clinical Center, National Institutes of Health,
Bethesda, MD, USA

D. Ilic (ed.), *Stem Cell Banking*, Stem Cell Biology and Regenerative Medicine,
DOI 10.1007/978-1-4939-0585-0_8,

be donated for research under strict consent provisions), many more lines have subsequently been generated worldwide and submitted for NIH approval. Currently, more than 200 hES cell lines have been generated worldwide that are approved for research using federal funding [5].

In addition to hES cell lines available for basic research, several lines have been generated using clinically-compliant materials and processes ("clinical-grade" or "current Good Manufacturing Practice/cGMP/-compliant") that would allow hES cell-derived products to be used clinically [6]. Cell lines have been generated by academic centers and private entities, and several trials are underway using hES cell-derived cells. Similar clinically-compliant lines have been developed in other countries as well, but no comparisons between these lines and no common distribution of such lines has been implemented or is envisioned, partially because of legal and policy barriers impeding distribution of hES cell lines across international jurisdictions [7].

The field of PS cell research shifted seismically with the discovery of the ability to reprogram any adult cell using defined factors pioneered by Yamanaka and colleagues [8, 9]. Due to their efforts and the efforts of others, it is now possible to consider obtaining cells from a patient with an obscure disease, transforming those somatic cells into induced pluripotent stem (iPS) cells, and growing them in sufficient numbers to make this rare phenotype widely available to individual investigators to allow them to assess the phenotype in a multitude of differentiated cell types and to develop potential drug and cell transplantation therapies. This ability of researchers to use a stage-specific differentiation process to obtain particular differentiated cell phenotypes allows investigators to examine the etiopathology of the human disease in vitro or in vivo, without the confounding influences of immortalization, genotypic background, and allelic variability (among others) that often plague in vitro cell culture.

An additional promise of iPS cells is the possibility that "personalized medicine" may be feasible. Advances in iPS cell generation have reduced the probability of insertional mutagenesis as a result of reprogramming, thus decreasing the chance deleterious effects of integration, persistent expression, or reactivation of the inducing genes. Current techniques range from using excisable all-in-one constructs (e.g.: CRE_LOX flanked, piggyback, or sleeping beauty transposon-based vectors), episomal vectors (plasmids, minicircles, and non-integrating episomal viruses), and mRNA, protein or small molecules that activate the specific pathways [10]. Now cells from an individual's own tissue can be harvested and reprogrammed with integration free methods to produce a stock of clinically-compliant pluripotent cells for personalized drug development, toxicity screening, and even autologous transplantation. To ensure this is commercially viable, groups have been working to reduce the cost of development of iPS cells and develop clinical grade and scalable processes to generate iPS cell lines. Other groups have approached the problem by suggesting that Human Leukocyte Antigen (HLA)-matched (rather than fully personalized) banks of lines can be developed. This is the approach that is being operationalized in Japan by Yamanaka and colleagues.

The rapid development of the field—the exponential increase in the number of types of pluripotent cell lines being generated and the multiple different uses of the

cell types proposed—has underscored the need to develop a mechanism to identify, collate, and distribute PS cell lines and their derivatives.

2 NIH Support for Generation and Banking of Lines

2.1 hES Cells

The practice of "biobanking" has been construed broadly to involve storing health and genetic information and/or various types of biological materials in banks, repositories, or collections. These biobanks are administered under many different infrastructures with many different sizes and purposes, both broad and specific. In recent years, biobanks have been particularly prevalent and important as resources for genetic research, coupling storage of tissue and DNA samples with genome sequencing data for genetics research and genome-wide association studies (GWAS). The practice of banking somatic cells, as well as primary cell lines and immortalized cancer lines, has been a vital research resource for many years, particularly in the form of anonymized lines from commercial vendors such as American Type Culture Collection (ATCC) and Sigma. This banking is currently supported by and governed under existing U.S. Food and Drug Administration regulation.

Banking of stem cells is a logical extension of these precedents—banks of well-characterized, normal, and disease-specific pluripotent cell lines are already a vital resource for basic research, disease modeling, and drug discovery, and the current regulatory and policy precedents for somatic cells and DNA biobanking could be extended to the banking of stem cells. Banking of stem cells may involve some important special considerations, as noted below (particularly in the areas of informed consent, therapeutic development, and commercial product development/intellectual property); however, as noted by Lomax et al. [11], current policy should be sufficient to govern much of PS cell banking.

The concurrent advancement of stem cell technology and information technology, along with the evolution of federal regulations involving stem cells, has established biobanking as a crucial measure in the progression of stem cell research. The obvious advantage of biobanking lies in a standardized and regulated way to preserve stem cells for use in future scientific research or for use in future therapy. As a leader of scientific research, the NIH has made a concerted effort to promote the establishment of public biobanks to house stem cell samples that can later be distributed for research purposes. Although the NIH is not currently developing therapeutic stem cell banks, it is actively engaged in promoting other technologies that will complement the research and development of therapeutic strategies involving stem cells. In this section, we highlight NIH involvement in stem cell banking, including early efforts of hES cell banking, the establishment of the NIH National Stem Cell Bank, and the establishment of the hES Cell Registry.

On August 9, 2001, President Bush announced a limit on federal funding for hES cell research, such that funding would only be approved for lines developed before August 9, 2001, and only for lines from which the embryo had been created for reproductive purposes, was no longer needed for those purposes, and was donated with proper informed consent and without financial inducements [1]. After these initial restrictions imposed on stem cell research in 2001, it became clear that further research would be dependent upon the small number of eligible hES cell lines.

In response to these regulatory measures governing the use of stem cells, the NIH articulated policies and procedures allowing for the formation of the hES Cell Registry as a means to allow researchers to easily identify hES cell lines approved for NIH funded research. All hES cell lines that met the eligibility criteria were added to the Registry, along with a corresponding NIH code and the provider's information. The NIH also participated with hES cell providers to draft Memoranda of Understanding (MOU) and Material Transfer Agreements (MTA) to further facilitate the use of these lines. Intramurally, the NIH developed the NIH Stem Cell Unit (SCU), in order to characterize the 21 lines approved under the Bush administration's policy under a standard paradigm and within one laboratory. The SCU has also created a database of all information regarding tested cell lines, and has established standards for culture, quality control, and monitoring [12].

As hES cell lines were developed and characterized, they were stored and collected within organizations and institutions to develop stem cell banks. Early banks included biorepositories at public and private institutions, national institutes, and individual laboratories. However, the wide variability of these institutions hindered rapid progression of the field, and researchers requested that the NIH consider options for more affordable and timely access to well-characterized and quality-assured lines. In response, the NIH awarded a grant of $16.1 million over 4 years to the WiCell Research Institute in 2005 to fund a National Stem Cell Bank [4]. Using this award, the National Stem Cell bank consolidated the federally funded hES cell lines in one location and provided a means for quality control and distribution of these cell lines. Under this grant, the National Stem Cell Bank was required to fully characterize all hES cell lines on the NIH registry, perform quality control testing, and distribute cell lines at a reduced cost with minimum intellectual property barriers. The hES cell collection at the National Stem Cell Bank included 21 cell lines approved for NIH-funded research in 2001, and distributed these lines from 2005 to 2010. By consolidating these cells in one location, the NIH hoped to optimize and standardize the techniques necessary for proper characterization of these cells for best use in both basic and translational research. The National Stem Cell Bank also had several non-research centered imperatives, including public availability of data and information, training other researchers in stem cell techniques, and developing MTAs that would serve as a template for all future MTAs involving stem cells.

In March 2009, President Obama issued the Executive Order 13505 *Removing Barriers to Responsible Scientific Research Involving Human Stem Cells*, which altered the criteria necessary for NIH to conduct and support research on hES cells [13]. In response, the NIH published the "National Institutes of Health Guidelines for Human Stem Cell Research" (Guidelines) on July 7, 2009, which established

policy procedures under which NIH funds human stem cell research and ensure that NIH-funded research in this area is ethically responsible, scientifically worthy, and conducted in accordance with applicable law [14]. Under these Guidelines, all hES cells subjected to internal review by the NIH and deemed eligible for federal-funding are posted on the new NIH Registry, along with an NIH Registration number for use in NIH Applications. Currently, there are upwards of 200 cell lines available on the hES Cell Registry, and a full list can be accessed at http://grants.nih.gov/stem_cells/registry/current.htm.

2.2 iPS Cells

iPS cells possess a unique advantage in that cells can be obtained from healthy or disease patients, and can be obtained from a variety of original tissue sources. Advances in cell biology have shown that iPS cells can be created from fibroblasts, blood, and urine samples [15, 16]. Reprogramming methods also vary, and researchers can choose from a variety of integrative and non-integrative methods of reprogramming, each with varying rates of effectiveness [10]. As iPS cells are developed from somatic cells, they do not involve the destruction of an embryo, and therefore are not subject to the same regulatory constraints as hES cells. The lack of regulatory constraints and freedom of choice granted by these cells has made them a very attractive cell source for stem cell research. Despite these advantages, there are some patent restrictions on the generation and use of iPS cells [17]. Currently, patents are held on the choice of factors for reprogramming, the methods used to deliver reprogramming factors, starting cell type, culture conditions, and products comprising iPS cells [18].

As research efforts regarding iPS cells have expanded, NIH has been exploring initiatives aimed at tapping large existing collections of biological material—healthy and diseased—for use as iPS cell source material. Many investigators have been adding collection of fresh punch biopsies to existing protocols, seeking a collection of dermal fibroblasts. Especially with the advent of iPS cells from peripheral blood and with the use of increasingly small amounts of material, however, the options have greatly increased. Of particular interest are several collections of existing tissue and blood samples whose donors, disease profiles, and even genomes are already well characterized. In particular, the NIH has been exploring blood and cord blood banks as potential sources of material for both research-grade and clinical-grade iPS cell lines. In parallel to the increasing clinical use of umbilical cord blood (UCB) worldwide, an entire industry to collect and store UCB has developed. Two competing models have evolved: a public cord blood bank model supported by public funds akin to the blood bank and bone marrow programs and a competing private cord banking business where commercial "private" entities offer to store UCB for use by a particular child or family.

The availability of well-characterized, HLA-typed cells collected with appropriate consent coupled with recent breakthroughs in iPS cell generation suggest that cord

blood cells may represent a good source of cells for such an effort. Two important points are worth emphasizing. Not only is the efficiency of generating iPS cells from cord blood-derived stem cells higher, it is also faster, as the absolute number of cells that can be obtained from a small fraction of the cord blood aliquot far exceeds what is required for generation of an iPS cell line. In addition, good patient histories are available, and if consent forms are well written (as is common with blood bank registries) it is also possible to collect additional cells or additional data for further follow-up through re-contact with tracked, permissive donors.

On a practical level, it is likely that the residual blood that is present in the tubing of a sample is sufficient, meaning that the entire sample need not be thawed for the purposes of iPS cell derivation. This suggests even more flexibility, as the sample itself could be preserved in nearly its entirety for a future clinical use of its own. Of course, a small aliquot of UCB could also easily be frozen separately at the time of collection or iPS cell generation could be planned at the time of use of a cord blood unit. These options mean that iPS cell generation can be included readily in the workflow without any major changes to current processes.

iPS cell generation also may allow private blood banks to be able to share samples without compromising on established contracts with donors who pay to have their samples stored for individual use. It may also allow private blood banks to consider additional sources of revenue and increase the use of their stored samples, which in turn is likely to increase the number of potential donors willing to store samples.

One can imagine a workflow process where UCB is shipped to a facility, a small aliquot is removed and iPS cell lines are generated at the same site using a zero-footprint process such as plasmids, or minicircles or Sendai virus (each of which can be manufactured using a process that can be made cGMP-compliant) and stored in a regulated environment, thawed, and then used when required. Alternatively, a small sample is separated from an existing stored unit when there is need for such a specific HLA typed sample and this is then processed to generate an iPS cell line for potential therapeutic use or when a cord blood unit is shipped for use, a small sample is retained and iPS cell lines are made to provide a replenishment for the used unit.

An alternative source of prospectively collected specimens is blood or marrow donors. This is particularly important in considering clinical use and autologous therapies for obtaining HLA matched samples. Blood collection is relatively straightforward, and detailed processes for collection, storage, and distribution have been codified for decades, along with the development of sophisticated databases. Blood banking is an international effort and shipping of blood derivatives is routine. Investigators have developed techniques to use different types of blood cells for iPS cell generation and have developed clinically compliant methods of selection of the best starting material. Bone marrow donor databases likewise offer similar advantages and since donors are prospectively HLA matched, it is possible to consider developing specialized banks from carefully screened and tested donors with well-documented clinical histories.

In response to the discovery of iPS cells and related technological and scientific advances in stem cell research, the NIH responded by creating the NIH Center for Regenerative Medicine (NIH CRM) to provide intramural and extramural support for stem cell researchers and to collaborate with international entities in order to facilitate the use of stem cell technologies. The NIH CRM has been closely involved in developing appropriate MTAs for researchers seeking iPS cell lines, and establishing biorepositories for the housing and distribution of PS cell lines. Under the initiatives put forth by the NIH CRM, researchers can generate or obtain iPS cell lines that can be then distributed to academic collaborators, banked in a cell repository, or sent to a commercial entity. Additionally, cell banked within these repositories are able to share lines with academic collaborators and license lines with commercial entities (Fig. 8.1).

2.3 Biorepositories

The NIH has provided three institutions with grants to support the housing and distribution of hES cells and iPS cells. There are currently three NIH-funded biorepositories at RUCDR Infinite Biologics (Rutgers), the Coriell Institute for Medical Research, and Wisconsin Stem Cell Bank (WISC) (Table 8.1). Each of these repositories holds a variety of specimens, and some contain both human and mouse embryonic and iPS cells. These banks are responsible for maintaining all deposited lines, maintaining an up-to-date database, performing necessary quality control tests, and tracking licenses and FTO. All banked lines are properly identified and characterized, and are subject to quality control measures including karyotyping, parent line identity matching, sterility testing, and viability after thawing. Additional quality control measures may include identification of pluripotency markers, and teratoma or embryoid body analysis to demonstrate functional pluripotency. Each of these repositories is then responsible for distribution of these lines to institutions in academia, to service providers, and to pharmaceutical companies. Human cells are available for distribution solely for research purposes under a proper MTA and cannot be used on human subjects, and every distributed line contains a Certificate of Analysis.

3 Differences Between Banking hES Cells and iPS Cells: NIH Engagement with Policy and Ethics

Banking of iPS cells differs from banking hES cells for a variety of reasons. Perhaps the most important is that tissue used to generate iPS cells can be readily obtained from a variety of easily accessible sources and subsequently banked. This differs from hES cells in a variety of ways: the ease and ubiquity of derivation; the

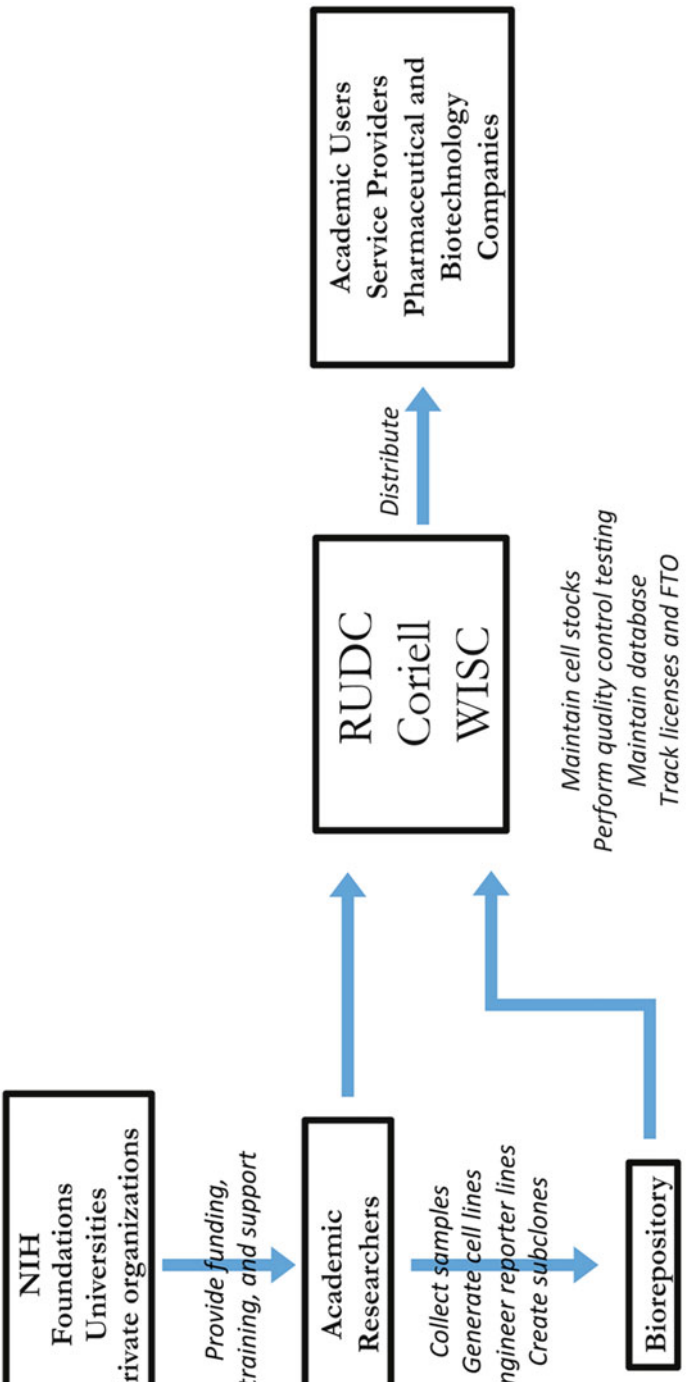


Fig. 8.1 Flowchart of stem cell banking with biorepositories established by NIHCRM. Funding, training, and support are given to academic researchers through public and private entities. Researchers will be able to collect samples, generate cell lines and subclones of those lines, and deposit the lines within individual biorepositories or NIH-funded repositories. These repositories will be responsible for maintenance of cell stocks, quality control (QC) testing, database maintenance, and tracking licenses and FTO. Cells may be distributed from these repositories to academic users, service providers, and private companies

Table 8.1 List of biorepositories containing agreements with NIHCRM for banking of stem cell lines

Repository	Location	Website	Contact
Rutgers	Piscataway, NJ	www.rucdr.org	Michael Sheldon, sheldon@biology.rutgers.edu
Coriell	Camden, NJ	www.ccr.coriell.orf	Dorit Berlin, dberlin@corriel.org
WISC	Madison, WI	www.wicell.org	Robert Drape, rdrape@wicell.org
WiCell			Andy DeTienne, adetienne@warf.org

connection between the donated tissue and a donor who has confidentiality concerns and moral values (not the case in an hES line derived from a blastocyst, although the blastocyst and germ cell donors have indirect claims); the ownership rights of the donors of the blastocyst; restrictions on the timing of donation; and the lack of HLA data on blastocysts.

Because of these fundamental differences, consent requirements that pertain to hES cells being eligible for the NIH registry are not analogous to the ethical and policy issues of informed consent for somatic cell donation. At the NIH, it became clear that, as investigators began to use iPS cells in their research, the status of iPS cell lines vis-à-vis previous consent for obtaining the source tissue caused concern and confusion among investigators, Institutional Review Board (IRB) members, and policy makers. Some investigators were interested simply in deriving iPS cell lines simply as flexible disease models for studying basic biology—in effect, simply as a means to an end, a tool for study. Others were interested in developing panels of iPS cell lines as a drug screening assay, or using iPS cell lines for new disease types, or for experimenting with cell transplantation techniques. Further complications arose with efforts to share and distribute iPS cell lines, to sequence iPS cell genomes, and (especially) to bank these cells for future use—for multiple difference applications, by multiple different investigators. As ideas for iPS cell-based therapies began to develop, there also was a desire to track and re-contact donors (i.e. not have lines be "anonymized"—or de-identified—as was regularly required) to get new consent, new samples, and updated health information (the latter to facilitate US FDA approval).

Several IRB officials (though by no means a universal sentiment) have indicated that making iPS cells is ethically akin to making an immortalized line and as such, as long as the donor consent permits use in commercial and academic settings, there should be no requirement for obtaining specific consent to making iPS cells. Other IRBs, however, took a different approach, approving iPS cell protocols very narrowly and being hesitant to allow the possibility that donor iPS cells could potentially be banked and have future clinical use as transplants.

In order to ensure that appropriate consent standards could be developed and instituted as early as possible, the NIH CRM reached out to the NIH Department

of Bioethics within the Clinical Center. In its dual roles in clinical and research ethics consultation and as an academic bioethics research department, the Bioethics department had already been approached about the emerging issues related to iPS cells from several perspectives: investigators looking for guidance, and colleagues interested in academic analysis of the multitude of new "ethical twists" posed by deriving PS cells from somatic sources. NIH CRM and NIH Bioethics thus undertook a full ethical and policy analysis of iPS cells and informed consent: comparing iPS cells to analogous past technologies, surveying current literature and policy frameworks, and proposing a standard template under a novel bioethics consent framework that could be used to harmonize the field [19].

From an ethics perspective, several aspects of iPS cells make them particularly complicated for obtaining consent under current models. The potential to derive any of a large variety of cell types from an iPS cell line means that the potential applications that need to be covered by a consent form are extensive (Fig. 8.2). Practically any tissue type and disease phenotype is possible, and the research applications range from very basic to very translational, including such potentially-sensitive issues as whole genome sequencing (and genetic privacy/discrimination), human reproduction (particularly as it became clear that functional gametes and embryos can be developed from iPS cells), commercial development, and allogeneic transplantation. The main question then became whether a "broad" consent could adequately cover all of the issues and twists posed by iPS cells, and whether such a broad consent would mean a donor would be adequately "informed" of the research to which they are contributing (particularly important given the revelation of the Henrietta Lacks case related to HeLa cells, among other problematic cases).

The need to have an avenue to identify and communicate with the original donors thus became important for a variety of reasons: in order to discuss new applications as they arose, in order to obtain more updated health information to satisfy the US FDA, and (for donors) in order to allow some limited form of donor control so that donors would feel respected and involved in the process. The latter is particularly important when considering the issue of participant withdrawal from research, and in particular where to draw the philosophical and policy line at which point a donor-participant is no longer allowed to withdraw their own biological material/derived iPS cell lines [20]. The standards for prospective consent developed by the team from CRM and Bioethics attempted to resolve all of these issues in one template aimed at prospective collection of fresh biological material for broadly-used iPSCs, with a particular emphasis on banking and sharing of lines (to facilitate NIH efforts to do thus) and re-contact of donors to foster a norm of "sustained interaction."

Further efforts by these teams have involved addressing the issue of previously collected specimens and determining what standards to hold previous consent processes to when determining whether a sample is eligible for repurposing of iPS cell derivation and downstream applications. The NIH partnered with policymakers

Sensitive Research and Use Categories:	• Large-scale genome sequencing • Genetic modification • Reproductive research, particularly gametes and embryos • Transplantation into animals, particularly neural applications		• Human-animal chimeras (regulated by NIH stem cell policy) • Commerical product development and profits • Sharing to other researchers, particularly internationally • Transplantation into humans for clinical trials and therapy	
Types of patients & consent situations	Types of biospecimens	Ethical issues for physicians and investigators	Ethical issues involving future research, banking and distribution, and related uses	Ethical issues involving commercial development, clinical banking, translation, and therapy
• Prospective vs. repurposing (previously collected) • Adults vs. pediatric • Alive vs. dead • Research donor vs. patient (clinical specimen) • Healthy donor vs. disease • One-time donation or long-term relationship • International (other jurisdictions) • Common vs. rare disease • Ethnic and racial minorities • Volunteer blood donor vs. research donor	• Biorepositories and tissue registries • EBV-derived lines • Cord blood banks (public and private) • Blood banks – hospitals, ARC, etc. • Bone Marrow registries • Private Tissue Banks • Tumor registries • Medical/surgical waste • Commercial entities • Plasmapheresis • Investigator-based research collections • Disease-specific tissue banks • Special collections (Rare diseases, genetic diseases)	• Obtaining informed consent • Confidentiality • HIPAA compliance • Compliance with OHRP guidelines and IRB approvals	• Traceability (of original donor)–de-identification vs. coding; sustained interaction • Governance/MTA structures • Tracking–of donor identity, of consent provisions • Limitations and gatekeepers for accessing samples • Ethics oversight by biobanks and secondary institutions • Varying legal/regulatory statues in international jurisdictions	• Re-contact of donors to satisfy FDA requirements • Patent and IP concerns • Benefits owed to donor • Adequate preclinical evidence • Ethical trial design

Fig. 8.2 A sampling of ethical, policy, and regulatory issues to consider along the spectrum from initial collection of tissue for iPS cell derivation through research to the development and testing of novel cell therapies

at CIRM, the International Stem Cell Forum, and McGill University to come up with a set of preliminary "points to consider"—the DISCUSS project—to address the variety of possible concerns related to previously-collected and banked specimens [21]. This project is meant to begin a conversation among stakeholders—investigators, biorepositories and banks, policymakers, ethicists, etc.—to determine a set of ethical and logistically-practicable requirements for using valuable banks of existing biospecimens in iPS cell research applications.

It should be noted that moving forward, NIH is attempting to proactively identify emerging ethical and policy issues related to iPS cells. For example, NIH CRM and Bioethics are working with partners at the NIH Stem Cell Task Force to survey the landscape of possible ethical concerns, from conceptual to legal to policy-oriented, and set an agenda for future analysis and guidance. Emerging issues include fresh analysis of the issue of human-animal chimeras in the iPS cell context, exploration of the issues raised by genome editing of iPS cell-derived gametes, neuroethical issues associated with transplanting iPS cell derivatives into the brains of animals and other humans, cost and access issues as iPS cells become viable as cell transplantation therapy, and further discussion of iPS cell banking. For more information, please contact Sara Hull at the NIH Department of Bioethics.

4 Issues to Consider with Banking

As can be seen from the discussion above, banking of PS cells is by no means confined to a simple process of building a repository and warehousing samples. Careful evaluation of the types of pluripotent cells being banked, ensuring compliance with rules of working with human subjects, and heeding the ethics of the donation process are all critical to a successful collection of tissue samples. Once a sample is collected, several additional determinations need to be made. The primary consideration is whether the sample is destined for clinical use or for research and screening use (or a mixture). The storage, database, and sharing requirements change dramatically. Additional important practical decisions include whether to store the collected tissue now and make PS cells later or make PS cells immediately upon collection, and whether source tissue samples should be stored as well as the PS cells where possible in case techniques evolve.

Another practical issue to consider is cost and the scale of the effort. Even though costs of making PS cells have progressively fallen, they are still not insignificant, ranging from $10 to $20,000 per line. Equally important is the fact that the time to obtain a PS cell line that is well tested and characterized is quite long—perhaps as long as 8 months. This will have important implications on whether personalized PS cells can be used for the treatment of acute disorders and whether making and storing matched HLA lines is an alternative strategy to consider for these cases. Likewise, given the time constraints associated with differentiation from PS cells to

the cell type required for use, it is important to consider whether key differentiated cell types should be produced in advance.

There are regulatory issues to consider in planning cell-based products as possible therapies. It is as yet unknown whether regulatory agencies will consider a differentiated cell from each PS cell line as a separate product, with its own requirements for testing and safety studies, or whether there be specific special regulations for certain kinds of autologous cell-based therapy that are more akin to, for example, organ transplants or surgical procedures. These are still important unanswered questions that will need to be addressed as one proceeds to applications of these important technical breakthroughs.

5 The Role and Vision of NIH: Moving Forward

The NIH has responded to these challenges in many different ways [22–24]. The NIH negotiated the first agreements to enable hES cells to be widely distributed and set up a characterization unit to evaluate the quality and stability of the cells. As mentioned, the NIH has strongly supported the regenerative medicine field and has allocated approximately a billion dollars a year to stem cell related research with about a third being allocated to the PS cell work.

The NIH has recognized that reducing the cost of banking iPS cells will require developing new models of storage and alternate models of funding. More recently the NIH CRM has introduced a crowd-sourcing model for deposit and distribution of PS cell lines using the non-profit repositories as a model [25]. This model utilizes the paid-for existing infrastructure and the expertise of existing repositories to store, expand, and distribute tissue and PS cell lines that are characterized by using a consensus panel of tests. The repositories coordinate with each other to ensure that panels of lines can be assembled, and all stakeholders work to ensure that derivatives, subclones and new variants that may be developed from the initial lines will be redeposited to a repository using the same distribution strategy used for the original line(s). This redeposit and redistribution mechanism is critical to a self-sustaining model because it not only allows for panel expansion, but also allows for continuous repository updates. To jumpstart the program, the NIH has funded the development of several lines and deposited them at these repositories to ensure comparability between repositories and act as well-characterized control or reference lines that can be freely exchanged between repositories. The NIH has also worked to ensure that common MTAs and use agreements are in place, and navigated among patents and Limited Use Label Licenses (LULLs) to negotiate agreements. These steps ensure that both academic and non-academic users can access the lines and that customized panels of lines can be assembled from cells residing in different repositories. By enabling companies (service providers) to access these lines, the NIH has also made it possible for a laboratory that does not have the skill sets in the entire generation,

characterization, and differentiation to a particular phenotype to still be able to work with such cells to analyze them with their own expertise in other screening and characterization technologies.

In a similar fashion, the NIH has developed and is currently developing clinically compliant protocols for generating, storing, and distributing PS cells, with a goal of making Standard Operation Procedures (SOPs) and reagents as widely accessible as possible. Additionally, the actual clinical-grade cells made by these processes will also be available for testing and evaluation in individual clinical protocols. This will give individual investigators the ability to test an off-the-shelf product at a relatively modest cost and reduce the risk of unexpected problems later when the final cell line is made for a particular cell transplantation or other therapeutic use. The NIH believes that this is particularly important for autologous products where data is required outlining the process and procedure for therapy before one moves forward. One can imagine having a small bank of 10–12 lines manufactured under a cGMP-compliant process that are widely available for preclinical testing and for developing modified differentiation protocols. Once such a protocol has been tested in three to four such lines, there can be reasonable comfort that a new PS cell line that has the right therapeutic profile can be used to generate the final product required using a cGMP process. The regulatory authorities also have some comparability data that can be used to determine regulatory compliance and time of development and by extension the cost has been significantly reduced. NIH CRM has been working on coordinating the development of clinical-grade protocols and is planning both internal and external (via contracts to outside vendors) processes, workflows, and infrastructure to allow investigators to develop potential cell therapies from banks of blood and tissue that are collected, reprogrammed, and stored in a cGMP-compliant manner.

6 Barriers That Remain

The NIH is currently evaluating how these processes will work, but is optimistic that this may be a viable model. Despite this optimism, we acknowledge that much further work needs to be done in several areas.

Perhaps the most important aspect is to harmonize international efforts [26]. Currently, cells made in one country may not be used in another because of conflicting regulations involving rules governing shipping of human material exposure to serum, lack of access to donors, (particularly in a field where ensuring ethnic and genetic diversity of samples used will be vital for both research and planning for cell therapy), or requirements for preclinical testing. Each of these issues, while solvable, requires time and effort to resolve.

A second important aspect is the absence of a searchable database of available PS cell lines akin to what is available for other large tissue or biological specimens. It is hard at this stage to avoid duplication, assemble panels, or develop unique

complimentary datasets simply because one does not know what samples are actually available worldwide.

A third important aspect that we perceive is the lack of forward planning to take into account the rapid technological advances that are being made in the stem cell field. For example, the development of iPS cells less than a decade ago has already spurred strategies for making these cells using alternative source materials, utilizing multiple reprogramming methods, and using these cells in a number of alternative models. As moving a single therapy forward takes more than a decade (on average) and immense and coordinated effort, we are faced with a very real possibility that we may be building banks and distributing cells that no one will use. Planning for obsolescence is a critical component of planning in most rapidly moving fields, but historically some fields such as drug development have not, and funding agencies are only now beginning to consider this issue.

Thus we feel that while the NIH has made an important contribution to funding the critical research and enabling translation through its support of biorepositories and developing clinically compliant cell lines, much remains to be done. Moving forward, the NIH will need to coordinate activity, leverage existing resources, and develop self-sustaining models that can evolve with changing needs. These tasks will require international coordination, careful planning, and budgetary commitments to develop a consensus solution.

7 Summary

Despite early regulatory hurdles, the use of PS cells in the field of regenerative medicine has continued to expand. Many different models of delivery of cell-based therapy have been successful and creative pioneers have shown what can (and equally importantly what does not) work. Many of the hurdles we have described are already becoming less of a challenge. Government policies have been more stable, and federal funding for regenerative medicine, in the USA and internationally, has increased in recent years. Coupled with state funding, these increases have been able to support the field. Technical advances, including the optimization of integration-free iPS cell reprogramming, and making iPS cells with small amounts of tissue sample at significantly reduced costs and these technological advances will help resolve some existing roadblocks to translational work. Although current manufacturing costs remain high, it is likely that as companies develop efficient methods of large scale manufacturing that costs will fall. The NIH looks forward to supporting the banking of PS cells and developing efficient methods of their differentiation.

Acknowledgements This work was supported by the NIH Center of Regenerative Medicine, an NIH Common Fund Initiative and the Intramural Research Program of the NIH. The opinions expressed in this article represent the views of the authors and in no way can be construed to reflect the opinion or policy of the NIH, DHHS, or US Federal Government.

References

1. Bush GW. The white house, fact sheet on embryonic stem cell research. (http://georgewbush-whitehouse.archives.gov/news/releases/2001/08/20010809-1.html). 9 Aug 2001.
2. NIH Human Embryonic Stem Cell Registry Under Former President Bush (9 Aug 2001–9 Mar 2009). Stem cell information [World Wide Web site]. Bethesda, MD: National Institutes of Health, U.S. Department of Health and Human Services; 2012. http://stemcells.nih.gov/research/registry/Pages/eligibilitycriteria.aspx. Accessed 05 Nov 2013.
3. NIH Stem Cell Research Funding, FY 2002–2012. Stem cell information [World Wide Web site]. Bethesda, MD: National Institutes of Health, U.S. Department of Health and Human Services; 2013. http://stemcells.nih.gov/research/funding/pages/Funding.aspx. Accessed 5 Nov 2013.
4. National Institutes of Health. NIH awards a national stem cell bank and new centers of excellence in translational human stem cell research. Press release from the NIH Office of the Director (http://www.nih.gov/news/pr/oct2005/od-03.htm) 3 Oct 2005.
5. NIH Human Embryonic Stem Cell Registry. Stem cell information [World Wide Web site]. Bethesda, MS: National Institutes of Health, U.S. Department of Health and Human Services; 2013. http://grants.nih.gov/stem_cells/registry/current.htm. Accessed 5 Nov 2013.
6. Crook JM, Peura TT, Kravets L, Bosman AG, Buzzard JJ, Home R, et al. The generation of six clinical-grade human embryonic stem cell lines. Cell Stem Cell. 2007;1:490–4.
7. Stem Cells and Diseases. Stem cell information [World Wide Web site]. Bethesda, MD: National Institutes of Health, U.S. Department of Health and Human Services; 2012. http://stemcells.nih.gov/info/pages/health.aspx. Accessed 5 Nov 2013.
8. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006;126:663–76.
9. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007;131:861–72.
10. Rao MS, Malik N. Assessing iPSC reprogramming methods for their suitability in translational medicine. J Cell Biochem. 2013;113:3061–8.
11. Lomax GP, Peckman SR. Stem cell policy exceptionalism: proceed with caution. Stem Cell Rev. 2012;8:299–304.
12. NIH Stem Cell Unit. Stem cell information [World Wide Web site]. Bethesda, MD: National Institutes of Health, U.S. Department of Health and Human Services; 2012. http://stemcells.nih.gov/research/nihresearch/scunit/Pages/Default.aspx. Accessed 5 Nov 2013.
13. Executive Order 13505, 3 C.F.R. 10667; 2009.
14. 2009 Guidelines on Human Stem Cell Research. Stem cell information [World Wide Web site]. Bethesda, MD: National Institutes of Health, U.S. Department of Health and Human Services; 2011. http://stemcells.nih.gov/policy/pages/2009guidelines.aspx. Accessed 5 Nov 2013.
15. Okita K, Yamakawa T, Matsumura Y, Sato Y, Amano N, Watanabe A, et al. An efficient nonviral method to generate integration-free human-induced pluripotent stem cells from cord blood and peripheral blood cells. Stem Cells. 2013;31:458–66.
16. Zhou T, Benda C, Dunzinger S, Huang Y, Ho JC, Yang J, et al. Generation of human induced pluripotent stem cells from urine samples. Nat Protoc. 2012;7:2080–9.
17. Eisenstein M. Up for grabs. Nat Biotechnol. 2010;28:544–6.
18. Georgieva BP, Love JM. Human induced pluripotent stem cells: a review of the US patent landscape. Regen Med. 2010;5:581–91.
19. Lowenthal J, Lipnick S, Rao M, Hull SC. Specimen collection for induced pluripotent stem cell research: harmonizing the approach to informed consent. Stem Cells Transl Med. 2012;1:409–21.
20. Lowenthal J, Hull S. Framing the "Right to Withdraw" in the use of biospecimens for iPSC research. Ethics Biol Eng Med. 2013;4(1):1–14.

21. Lomax GP, Hull SC, Lowenthal J, Rao M, Isasi R. The DISCUSS project: induced pluripotent stem cell lines from previously collected research biospecimens and informed consent: points to consider. Stem Cells Transl Med. 2013;2:727–30.
22. Rao MS, Collins FS. Steering a new course for stem cell research: NIH's Intramural Center for Regenerative Medicine. Stem Cells Transl Med. 2012;1:15–7.
23. Rao M. Public private partnerships: a marriage of necessity. Cell Stem Cell. 2013;12:149–51.
24. Rao MS. LULL(ed) into complacency: a perspective on licenses and stem cell translational science. Stem Cell Res Ther. 2013;4:98.
25. Rao M. iPSC Crowdsourcing: a model for obtaining large panels of stem cell lines for screening. Cell Stem Cell. 2013;13:389–91.
26. Turner M, Leslie S, Martin NG, Peschanski M, Rao M, Taylor CJ, et al. Toward the development of a global induced pluripotent stem cell library. Cell Stem Cell. 2013;13:382–4.

Chapter 9
Sibling Donor Cord Blood Program: Children's Hospital Research Center Oakland and Beyond

Nahal Rose Lalefar and Bertram Lubin

1 Introduction

Hematopoietic stem cell transplantation (HSCT) has become an important treatment option not only for patients with malignancies but those with other diseases such as hemoglobinopathies, inborn errors of metabolism, immunodeficiencies, and bone marrow failure syndromes. Umbilical cord blood (UCB) has proven to be an important stem cell source, particularly in non-Caucasian patients, who have a lower chance of identifying a matched unrelated adult donor in the National Bone Marrow Donor Bank because of the genetic heterogeneity within human leukocyte antigen (HLA) among non-Caucasian donors and the limited number of potential non-Caucasian donors enrolled in the national program. Despite the fact that there is only a one-fourth chance of an HLA match, sibling donor UCB serves is as an important resource for parents who currently have a child that might be considered for HSCT and who are having another child.

The first cord blood transplant was performed in France for a child with Fanconi anemia and involved a sibling donation [1]. Since then, it has become a suitable alternative to bone marrow transplantation (BMT). If the UCB contains sufficient numbers of hematopoietic stem cells for engraftment and is at least 4/6 HLA matched, the chance that it will engraft in a young recipient is high, given the cell dose/kg of recipient. Compared to bone marrow or peripheral blood harvest of hematopoietic stem cells, when a decision to transplant is made, the readily available banked UCB is easier to obtain with no risks to the donor and it carries less risk of transmission of blood-borne infections [2]. Furthermore, the chance of developing significant graft versus host disease (GVHD) is less when UCB is used compared to other sources of stem cells derived from older donors or adults due in part to the immunologic naiveté of UCB graft [3].

N.R. Lalefar (✉) • B. Lubin
Department of Hematology/Oncology, Children's Hospital and Research Center Oakland, 747 52nd Street, Oakland, CA 94609, USA
e-mail: nlalefar@mail.cho.org

D. Ilic (ed.), *Stem Cell Banking*, Stem Cell Biology and Regenerative Medicine,
DOI 10.1007/978-1-4939-0585-0_9,

However, graft failure and delayed engraftment may occur when the cell dose is limited. Under these circumstances, augmentation of the UCB with bone marrow derived cells from the sibling may be required.

Having a fully HLA matched sibling, an option that is more likely to exist with a sibling UCB collection or a sibling donor than with an unrelated donor, is of great importance in situations where a transplant is considered for a patient with sickle cell anemia or thalassemia. Due to concerns over engraftment and GVHD, transplants for patients with these two hemoglobinopathies have been restricted to situations where a complete HLA compatible donor exists. Unfortunately, this is often not the case and methods to overcome this limitation are under intense study. In sickle cell anemia, transplantation has traditionally been performed in patients with advanced organ damage whereas in patients with thalassemia, transplantation is performed before evidence of iron related tissue damage. If an available test to predict clinical severity in sickle cell disease (SCD) existed, young children with SCD could be considered for transplant. In such cases, related UCB units would be a valuable resource.

2 Sibling Donor Cord Blood Program

Over the past two decades, a number of cord blood banks have been developed to help with UCB availability and delivery. With support from the National Institutes of Health (NIH), a "Sibling Donor Cord Blood (SDCB) Program" was initiated at Children's Hospital Oakland Research Institute (CHORI) in 1998 as a resource to collect, characterize, and release CBUs from parents who currently had a child affected by a nonmalignant disorder that might be considered for transplantation [4]. The program started as a multi-center collaborative, open-label phase I–II study assessing the utility of stem cells from sibling donor UCB with the goal of enrolling 30 patients over 5 years. This program was the first related cord program in the world and while it was initiated to assist families who had children with sickle cell anemia or thalassemia, it rapidly became used by families who currently had a child with a malignant or non-malignant disease that could potentially be treated with an HLA matched sibling donor.

The primary outcome measurement was engraftment of donor cells. Secondary outcome variables included evaluations of the effect of donor engraftment on clinical and laboratory manifestations of β-thalassemia and sickle cell anemia. The specific aims were initially to establish UCB banking as a resource to families and hematologists and to implement, validate, and monitor CBU collection and processing procedures where collections were offered at community and university birthing centers across the United States [5]. Informed consent procedures, collection, and shipping procedures that secured the stem cells within UCB, processing procedures within 48 h of collection to optimize stem cell collection, storage and release procedures, infusion procedures and post-transplant monitoring systems were all developed and validated. Costs associated with the operations of the program were covered by the NIH grant awarded to Children's Hospital Oakland.

Families in the United States were recruited by a variety of procedures including telephone calls after referrals by community and academic physicians and presentations at national hematology, sickle cell, and thalassemia meetings. In 2007, when NIH funding no longer was available as research questions regarding collection and banking were answered, Children's Hospital and Research Center Oakland developed a medical collaboration with ViaCord, called the Sibling Connection, to sustain the free sibling UCB collection services. This program now offers an UCB collection and banking program to expectant parents who have a child that may be in need of a stem cell transplant.

Given the value of cord blood, particularly its ability to be used without a complete HLA match and lower risk of severe GVHD, programs now exist across the world to collect and cryopreserve UCB. Public banks promote allogeneic (related or unrelated) donation under no charge for cord collection. Private banks, who charge fees for cord blood banking, were initially developed to store stem cells from UCB for autologous use by a child if the child were to develop disease later in life. No accurate estimates exist of the likelihood of children to need their own stored UCB stem cells in the future. One estimate is approximately 1 in 2,700 individuals, however, there have been other estimates with much lower rates [6]. Those cord blood units stored publically are made available through procedures similar to that in the National Marrow Donor Program (NMDP) to patients in need. The limited amount of stem cells in UCB makes it difficult to use a single unit of UCB for an adult and multiple units have been used successfully. Procedures are also being developed to expand the number of stem cells in UCB so that they can be used for transplantation in an adult. Although UCB is currently considered discarded human material, it should only be collected for banking with an Institutional Review Board (IRB)-approved protocol. Furthermore, informed consent must be obtained prior to delivery. Targeted efforts are being made to recruit underserved minorities (black, Hispanic, American Indian/Alaska Native individuals) in public UCB banking programs.

3 Informed Consent

Informed consent, which is obtained during the second or third trimester of the mother's pregnancy, must address the risk of collection, the type of testing done on the unit, medical and genetic history of the mother and father, and the ownership of the unit. In the case of the Sibling Connection, the legal guardian/ViaCord Client owns the UCB and/or cord tissue sample. For related UCB collections, the consent should indicate that collection of the unit does not imply that there has been a decision on transplantation [7]. Parents would be informed that autologous UCB would not be used as a stem cell source if the donor developed leukemia later in life as the genetics of leukemia are likely to be present in the sibling donor. Parents should also be advised that there is no scientific data to support the claim that autologous UCB is a tissue source proven to be of value for regenerative medical purposes or to serve as "biologic insurance". If UCB was collected from a newborn who subsequently

developed a genetic, immunologic, or malignant neoplastic disorder, parents should notify the UCB bank so that the unit is not used for transplantation. Low volume and low cell count may limit the plan to store the cord blood and this must also be discussed with the family.

4 Procedure

The following are the procedures for UCB collection, processing, laboratory testing, and cryopreservation used by the Sibling Connection.

4.1 Collection

At approximately 28–30 weeks gestation, the UCB collection kit is delivered to the participating family. The family is required to complete and submit the enrollment forms before the kit can be shipped. However, the collection kit can be delivered expeditiously if the forms are received after 30 weeks gestation. The kit consists of a standard thermally insulated platelet shipper that contains a 250-mL blood bag with 35-mL citrate-phosphate-dextrose anticoagulant as well as standardized materials to prepare the umbilical cord for sterile venipuncture. UCB is collected during the third stage of labor when the placenta remains in the uterus or after placental delivery. A 16-gauge needle is introduced into the umbilical vein and the blood drains via gravity into the tubing and collection bag connected to the needle. The bag is rotated intermittently to prevent clotting. It is encouraged to collect as much UCB as possible to enhance the utility of the final product and increase the potential cell dose, ideally greater than 100 mL. The UCB stem cell-collection program should not alter routine practice for the timing of umbilical cord clamping and cord blood collection is not performed on complicated deliveries.

4.2 Processing and Cryopreservation

The unit is shipped in the platelet shipper at 20 °C directly to the stem cell laboratory for processing, testing, and cryopreservation. Samples are processed within 48 h of collection. Standardized freezing and storage conditions follow standards per the Federation for the Accreditation of Cellular Therapy (FACT). The CBU is both volume reduced and red blood cell depleted for preservation. Units are stored in the vapor phase of liquid nitrogen.

Prior to cryopreservation, segments should be attached to the cord blood for testing and confirmation of identity. Aliquots are used to quantitate the total cell number, hematopoietic stem cell number, determine HLA typing, and cell viability. The

retained samples would rarely be used for genetic testing. Since these are directed units for related family members it is more likely that the donor sibling would be tested and the retained samples would be used for other required testing such as HLA-typing and viability. A minimum of 1.0×10^8 total nucleated cells (TNC) is required for banking. However, since the CBUs belong to the families, the UCB banking program will provide the TNC to the family along with assessment of possible utility if the CBU is below this number. The family decides if they want to continue storage, have the unit discarded or donate it to research.

For the UCB transplantation itself, an expected infused cell dose of greater than 5.0×10^7 TNC per kilogram of the recipient's body weight is recommended for hemoglobinopathy transplantations based on Eurocord and the CIBMTR registry data [8]. In a study of stem cell transplantation for children with both malignant and non-malignant diseases who received *unrelated* donor cord blood with no more than two HLA mismatches, a higher probability of survival was shown in CB grafts containing at least 1.7×10^5 CD34+ cells per kilogram of the recipient's body weight [9].

4.3 Laboratory Testing

Testing is performed on a sample of UCB retained at the time that the unit is processed (regulations require that this sample be obtained within 7 days of collection of the UCB product).

Initial typing to determine if the CBU is a match for the recipient, includes HLA-A, -B, -DRB1 intermediate resolution. If the product is a match, confirmatory HLA typing is performed on an additional CBU sample. Any other HLA typing of the mother, other family members, the donor, or the recipient are performed by the transplant facility where the recipient will be treated. CBUs are tested for sickle cell anemia and thalassemia.

Infectious testing is done on the mother at approximately 36 weeks gestation. Infectious testing on the cord blood includes hepatitis B surface antigen (HBsAg) nucleic acid-cased test (NAT), antibody to hepatitis C virus (HCV), antibody to human immunodeficiency virus (HIV), HIV antigen NAT, HCV antigen NAT, antibody to cytomegalovirus, syphilis (STS), and antibody to human T-cell lymphotrophic virus I/II. CBUs with a confirmed positive test for Anti-HIV, HbsAg, or HCV are not used.

5 Results

As of March 2006, there were 1,797 sibling cord collections across the United States, the majority occurring in California, Texas, New York, and New Jersey [10]. Forty-nine percent of the collections were for siblings with malignant diseases, 28 % were for sickle cell anemia, 6 % were for thalassemia, 17 % were for other and

rare diseases. Of these collected units, 65 were released (18 % were for thalassemia). With a median follow-up time of 16 months, overall survival was 65 % in patients who had underlying malignant disorders and 93 % for those with non-malignant disorders.

As of January 2010, 3,060 sibling CBUs had been collected across the United States. The categories of participation included malignant disorders ($n=1,685$ or 55 %), SCD ($n=828$ or 27 %), thalassemia ($n=142$ or 5 %), and other rare hematological conditions ($n=405$ or 13 %) [11]. Of those cord blood units collected, 4 % ($n=123$) were released for transplantation. However, once again utilization was greatest for families of patients with thalassemia (18 % of collected cord blood units). Of the transplants performed, 41 had a malignancy, 26 had thalassemia, 31 had sickle cell anemia, and 25 had a rare hematological disorder. All but nine donor/recipient pairs were HLA-identical and 30 % combined marrow with cord blood from the same sibling donor. The overall 1-year survival rate after CBT was 87 % (98 of 113 patients treated by March 2009) and the 8-year probabilities of survival and event-free survival were 84 % and 78 %, respectively. The incidence of graft rejection was 7 %. The incidence of Grade II–IV acute GVHD was 15 %, and the incidence of chronic GVHD was 9 %.

Since late 2007, US transplant centers have been required to register all allogeneic transplants with the CIBMTR, which collects outcome data for allogeneic transplants. Non-US centers can voluntarily submit transplant data but it is not a requirement. Between 2000 and 2011, 315 stem cell transplants were registered with CIBMTR using HLA-identical sibling cord blood for patients under the age of 21 years at the time of transplant. These transplants were performed at 117 centers internationally, 38 % of which were for inherited abnormalities of erythrocyte differentiation or function, including thalassemia major and sickle cell anemia. Refer to Table 9.1. A total of 61 pediatric (age <21 years) recipients of HLA-identical sibling donor cord blood transplants were registered with the CIBMTR by US centers between 2008 and 2011.

Given that the sibling cord donor program was initiated for patients with hemoglobinopathies and the greatest ratio of collected versus released CBUs was utilized by patients with hemoglobinopathy disorders, it was important to examine outcome differences of bone marrow and cord blood transplantation (CBT) in these patients. In 2013, the outcomes of 485 patients with thalassemia major or SCD receiving HLA-identical sibling CBT ($n=96$) or BMT ($n=389$) between January 1994 and December 2005 were reported [12]. It was noted that the patients who received sibling cord transplants were significantly younger (median ages 6 versus 8 years) and were treated more recently than those who underwent bone marrow transplants (median year 2001 versus 1999). Patients who received UCB transplants had slower neutrophil recovery but less acute GVHD and no chronic GVHD. The outcome for both groups was excellent, and in multivariate analysis, the disease-free survival did not differ between the cord blood or bone marrow transplant groups for either thalassemia major or SCD.

Table 9.1 Indications for transplant in pediatric patients (age <21 years) registered with the CIBMTR that received a HLA-identical sibling-donor cord blood transplant between 2000 and 2011

Characteristic	*N* (%)
Total *N*	315
Number of centers	117
Disease	
Acute myelogenous leukemia	30 (10)
Acute lymphoblastic leukemia	79 (25)
Chronic myelogenous leukemia	5 (2)
Myelodysplastic/myeloproliferative disorders	12 (4)
Other acute leukemia (biphenotypic, bilineage or hybrid leukemia)	1 (<1)
Non-Hodgkin lymphoma	5 (2)
Severe aplastic anemia	31 (10)
Inherited abnormalities erythrocyte differentiation or function[a]	119 (38)
SCID and other immune system disorders[b]	21 (7)
Inherited disorders of metabolism[c]	9 (3)
Histiocytic disorders[d]	3 (<1)

Courtesy of CIMBTR, 2013, unpublished

[a] Inherited abnormalities of erythrocyte differentiation or function include: thalassemia, $n=54$; sickle cell anemia, $n=29$; Fanconi anemia, $n=21$; Diamond-Blackfan anemia, $n=6$; beta thalassemia, $n=4$; E-beta thalassemia, $n=1$; Schwachmann-Diamond, $n=1$; sickle thalassemia major, $n=1$; dyskeratosis congenita, $n=1$; congenitial dyserythropoietic anemia, $n=1$

[b] SCID and other immune system disorders include: Wiskott Aldrich syndrome, $n=5$; chronic granulomatous disease, $n=5$; Kostmann agranulocytosis, $n=3$; CD40 ligand deficiency, $n=2$; cartilage hair hypoplasia, $n=1$; SCID ADA deficiency, $n=1$; Bare lymphocyte syndrome, $n=1$; X-linked lymphoproliferative syndrome, $n=1$; combined immunodeficiency disease, $n=1$; X-linked ectodermal dysplasia, $n=1$

[c] Inherited disorders of metabolism include: Hurler syndrome, $n=3$; adrenoleukodystrophy, $n=2$; Krabbe disease, $n=1$; metachromatic leukodystrophy, $n=1$; osteopetrosis, $n=1$; Wolman disease, $n=1$

[d] Histiocytic disorders include: FELH, $n=1$; hemophagocytosis, $n=1$; histiocytic disorder not otherwise specified, $n=1$

6 Conclusion

The CHORI-ViaCord experience with sibling cord blood donation and transplant has shown that sibling cord blood will continue to be utilized, particularly for patients with thalassemia, SCD, and other hereditary and malignant disorders. Despite many inherent challenges in coordinating and collecting the cord blood, uniform standard procedures have allowed for the generation of cord blood products that can be a suitable alternative to bone marrow.

References

1. Gluckman E, Broxmeyer HA, Auerbach AD, Friedman HS, Douglas GW, Devergie A, et al. Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical-cord blood from an HLA-identical sibling. N Eng J Med. 1989;321:1174–8.
2. American Academy of Pediatrics Section on Hematology/Oncology, American Academy of Pediatrics Section on Allergy/Immunology, Lubin BH, Shearer WT. Cord blood banking for potential future transplantation. Pediatrics. 2007;19:165–70.
3. Reed W, Walters M, Trachtenberg E, Smith R, Lubin BH. Sibling donor cord blood banking for children with sickle cell disease. Pediatr Pathol Mol Med. 2001;20:167–74.
4. Walters MC, Quirolo L, Trachtenberg ET, Edwards S, Hale L, Lee J, et al. Sibling donor cord blood transplantation for thalassemia major: experience of the Sibling Donor Cord Blood Program. Ann N Y Acad Sci. 2005;1054:206–13.
5. Reed W, Smith R, Dekovic F, Lee JY, Saba JD, et al. Comprehensive banking of sibling donor cord blood for children with malignant and nonmalignant disease. Blood. 2003;101:351–7.
6. Committee on Obstetric Practice and Committee on Genetics. ACOG committee opinion number 399, February 2008: umbilical cord blood banking. Obstet Gynecol. 2008;111:475–7.
7. Lubin BH, Eraklis M, Apicelli G. Umbilical cord blood banking. Adv Pediatr. 1999;46: 383–408.
8. Ruggeri A, Eapen M, Scaravadou A, Cairo MS, Bhatia M, Kurtzberg J, et al. Umbilical cord blood transplantation for children with thalassemia and sickle cell disease. Biol Blood Marrow Transplant. 2011;17:1375–82.
9. Wagner JE, Barker JN, DeFor TE, Baker KS, Blazar BR, Eide C, et al. Transplantation of unrelated donor umbilical cord blood in 102 patients with malignant and nonmalignant diseases: influence of CD34 cell dose and HLA disparity on treatment-related mortality and survival. Blood. 2002;100:1611–8.
10. Walters M, Quirolo L, Edwards S, Lee J, Robertson S, Falcon K, Briddell R, Lubin B. Abstract presentation, directed donor cord blood transplantation for hematological disorders. American Society for Blood and Marrow Transplantation: Boston, MA; 2007.
11. Walters M, Edwards S, Robertson S, Falcon K, Briddell R, Lubin B. Abstract poster, sibling donor cord blood transplantation for hemoglobinopathies. In: 8th Annual cord blood symposium, San Francisco, CA; 2010.
12. Locatelli F, Kabbara N, Ruggeri A, Ghavamzadeh A, Roberts I, et al. Outcome of patients with hemoglobinopathies given either cord blood or bone marrow transplantation from an HLA-identical sibling. Blood. 2013;122:1072–8.

Chapter 10
Anthony Nolan Experience: Issues and Strategy for Cord Blood Banking from Ethnic Minority Groups

Fatma Hatimy, Monica Del Olmo, Linda Moss, Velibor Milovanovic, Sergio Querol, Alejandro J. Madrigal, and Susana G. Gomez

1 Introduction

Developing and expanding the cord blood programme in the UK has several advantages. Most significantly, the cost to the NHS of stem cell transplants is reduced if stem cells are sourced locally. A well-stocked cord blood bank (CBB) will also provide rapidly available stem cells, reducing the interval between intention to treat and transplant time [1]. The CBB also runs as a human biomaterials bank, and thus enables research for potential therapies.

Anthony Nolan's Cord Blood Program commenced in 2008, with the initial site at Kings College Hospital in London, supported by the 'state of the art' Cell Therapy Centre in Nottingham where the cord blood is processed. The expansion of the program has been rapid, and now boasts five collection sites in England, with continuing planned expansion following the government award of the Regional Growth Fund in 2013. Producing high quality stem cell units for transplants is a particular focus of the program.

F. Hatimy • M. Del Olmo • L. Moss • V. Milovanovic • A.J. Madrigal • S.G. Gomez (✉)
Anthony Nolan Cord Blood Program, Nottingham, UK
e-mail: susana.gomez@anthonynolan.org.uk

S. Querol
Barcelona Cord Blood Bank, Programa Concordia, Banc Sang i Teixits,
Passeig Taulat 116, Barcelona 08005, Spain

Anthony Nolan Cord Blood Program, Nottingham, UK

Cord Blood Working Group, World Marrow Donor Association, Leiden, The Netherlands

D. Ilic (ed.), *Stem Cell Banking*, Stem Cell Biology and Regenerative Medicine,
DOI 10.1007/978-1-4939-0585-0_10,

2 Issues

The UK offers a woman-centered model of maternity care. This differs from some other countries that offer a public cord blood service. Most recently, delayed cord clamping has become a feature of care offered to women or care that woman choose to have. Only one of the hospitals within Anthony Nolan's cord blood program offers this as a routine feature of care. It may be argued that this affects the quality of the unit collected; however, local examination of the cord blood collecting practice may indicate that it is the collector that affects the quality of the unit collected. Studies in America have found differences between collection sites and have hypothesized that these differences may be due to race/ethnic differences and/or environmental factors [2]. Certainly further study is required to establish what affects the quality of the cord blood unit.

Overall, there is great enthusiasm and support for the cord blood program at all five collection sites within the program. There is goodwill from the women and their families. This is evident from the interest generated from the information and pre-consent leaflets available at the hospitals. There are strict screening criteria for participation in the program following The Human Tissue Authority (HTA; www.hta.gov.uk) and NetCord-Foundation for the Accreditation of Cellular Therapy (FACT; www.factwebsite.org/Standards/) standards.

2.1 Selection of the Hospitals as Collection Sites

The selection of the hospitals as collection sites is based on birth rate and ethnic diversity for the hospital catchment area. However, these hospitals within the UK, inevitably, by these criteria, are the regional referral centers, and will have a population with higher risk factors, excluding women from the cord blood program. The ethnic minority groups of people will have a proportionally higher risk than their Caucasian counterparts [3]. This may stem from the overall socio-economic status of minority groups within the UK. Exclusion of women based on risk factors will reduce the specific target groups that the program wishes to collect from.

2.2 Language Barriers

Language barriers are another issue limiting the proportion of ethnic minority people participating in the program. These differences will vary regionally as various ethnic groups selectively locate themselves. Leicester and Birmingham collection sites are situated in the regions' hospitals with the highest birth rates. Leicester has a large group of Asians from the Indian sub-continent, many of whom may have emigrated from East Africa. According to the 2011 census, this group represents 28 % of Leicester's community [4]. Conversely, Birmingham's largest ethnic group are those from the Pakistani community, representing 13.5 % of the local community [5]. London has a

greater diversity from Sub-Saharan Africa. All of these groups have particular language barriers, as English is not their mother-tongue. There are also various languages and dialects within these communities, which makes interpreting an arduous task. An example of this would be when considering translation needs for the Pakistani community, whilst the national language for Pakistan is Urdu, there are regional dialects and languages such as Mirpuri, Pushtu, and Punjabi. Whilst Leicester's community may have a high proportion of second generation Asians and will have spoken English having emigrated from Africa, a high proportion of Birmingham's Asian women are very often newly immigrant from Pakistan and have limited English, if any. Second generation Pakistani women may have newly immigrant husbands. This becomes an issue for informed consent. Consent forms are only provided in English, and the verbal information given by the collectors is in English. Interpreters are currently not used within Anthony Nolan's Cord Blood Program as information governance would be difficult. This will be discussed further. Multi-lingual collectors are ideal for combatting this problem with communication. Within the NHS, the use of family members as interpreters is discouraged, as informed consent cannot be guaranteed, and the patients' wishes are at risk of being superseded by family tradition and culture. Anthony Nolan would concur with this view, and seeks to impart the necessary information directly to the woman by trained collectors, thus complying with the law in the UK.

2.3 Cultural and Religious Background

Reluctance and refusal to donate is sometimes attributed to religious restrictions, particularly within the Pakistani community (anecdotal evidence). As Islam is the major religion in the Pakistani community, investigations were conducted to examine the teachings of Islam in relation to stem cell donations and transplants [6]. The Muslim Council in London is clear that the donation of stem cells is not prohibited in Islam. Any act that prolongs life is encouraged within Islam [6, 7]. This would therefore appear to be a cultural restriction, which may be harder to change, and one could argue ethically, whether it is the remit of Anthony Nolan to do. Certainly, the advantage would be for the ethnic group, as more stem cell units would be available for transplant. Stem cell donation within the Indian community and other Muslim communities is more accepted.

2.4 Medical Risk Factors

Peculiar to the African community, the reluctance to participate is due to the amount and type of information taken during the consenting process (anecdotal evidence). This is an issue for investigation. Assurances of confidentiality do not allay fears of divulgence or misuse. Other fears anecdotally, expressed are the use of the cells in research. Human Immunodeficiency Virus (HIV) is reported to be endemic in Sub-Saharan Africa, and refusal to donate may be a self-excluding act, as testing will be carried out if donating. As the scope for treating blood borne diseases with stem cell

transplants grows, sourcing Africans as donors will be of particular importance and one Anthony Nolan will need to examine. Another feature that reduces this cohort of people is the fact that many have been exposed to malaria. Donation criteria exclude all women who have had malaria from donating cord blood. With the prolific nature of the malarial infection in Africa, most immigrants from Africa will have had malaria, again reducing the number of those eligible to donate.

2.5 Other Aspects

Internationally, ambivalence and attrition for stem cell donation has been noted in the Black and minority ethnic groups. It has been difficult to isolate the founding causes for this ambivalence and attrition. Cultural attitudes towards disease may harbor the answers. Certain cultures may view disease as stigmatizing and/or weakening. Donating blood and/or stem cells may also be seen as weakening an individual. Ownership of individual cell DNA may be important, if there is mistrust of the scientific community.

Medical risk factors, language, religion/culture mistrust of information governance are issues that reduce the cohort of those within the ethnic minorities that donate stem cells. Anthony Nolan has been keen to stress the fact that the stem from cord blood would otherwise be treated as clinical waste, and for some people this is the deciding factor, encouraging them to donate.

3 Strategies for Improvement

The strategy for Anthony Nolan has been to locate collection sites in areas of high ethnic diversity in order to capture the people least likely to be represented on the adult donor register. As discussed, this cohort of people will be reduced further by the particular nuances unique to their communities. As the public CBB industry develops, this decision has been a global feature in the loci of collection sites. Umbilical cord blood stem cells offer a more equitable service to those with rare tissue types and reduces search times for this group of patients This is made possible by increasing the availability of hematopoietic stem cells to those that may otherwise not find a match within a more opportune time due to disease progression.

Anthony Nolan worked closely with the UK's Blood transfusion service, NHSBT, to establish the ideal size of the cord blood inventory that would cater to the needs of the UK patients in need of stem cell transplants. It was decided that 50,000 would be the target size of the cord blood inventory [1]. The advantages to the country would be the reduction in cost to the NHS, a rapid availability reducing intention-to-treat transplant interval, and increasing match potential for those rare HLA tissue types. An earlier study in Australia determined the ideal size for the Australian cord blood inventory. They recognized the challenges in finding matches for particular groups of people, and resolved to focus on ethnicities whose home

country did not have an established cord blood service. Similar studies determined the American cord blood inventory. The Indian service took a slightly different perspective, and recognized the needs of emigrants and their descendants from India living abroad, as well as the national needs [8]. This will eventually reduce the disparity in treatment and improve overall survival rates and reduce treatment related mortality amongst ethnicities from the Indian subcontinent.

There is substantial Anthony Nolan investment in marketing cord blood services. Anthony Nolan provides a wealth of educational materials directed at women and their families attending the hospitals designated as the collection sites. This is in the form of leaflets delivered to women at various stages of their pregnancy. All material is printed in the English language, and extolls the benefits of the cord blood donation, featuring success stories of lives saved. This is mainly passive marketing and excludes non-English speaking families. Social media is also used as a tool to market the cord blood program. Again the target audience may exclude the very communities that the cord blood program would want to include. Recent changes to the literature to include photos featuring people from ethnic minorities have been made to be inclusive of minority groups. Using specific celebratory events within the ethnic groups has also been used to educate and disseminate the message more widely. This has been particularly successful in Leicester. Government funding was successfully achieved to target Asian groups with the '6 % campaign'. Whilst this was not specifically targeted at the cord blood program, the message can be used to promote donation within the program. Future considerations are being given to invite high profile community representatives to disseminate the message of the benefits of cord blood donation from ethnic minority communities.

Data suggests that a total number of nucleated cells in cord blood samples from non-Caucasian communities are lower [2], and the overall strategy in Anthony Nolan recognizes this. However, fewer non-Caucasian sourced units qualify for banking as the conversion target is uniform and set too high for these target units to qualify. Treatment-related mortality has been noted to be high in African–American patients [9]. An American study found that the number of cord blood units banked, which was sourced from African–Americans, was much lower than the number who had consented [9]. This is also reflected within one of Anthony Nolan's collection sites, where the majority of units banked are from Northwestern European descents. Future technologies may need to be employed to enhance units collected from minority groups to make more available for ethnic minority groups.

Within Anthony Nolan's Cord Blood Program it would be preferable to have multi-lingual collectors, as evaluating the quality and accuracy of the information given would be more cost effective and efficient, and this is considered during recruitment of collectors. Strict guidelines from the HTA and international accreditation body, NetCord FACT, require that informed consent is gained from the mother, and an understanding of the written material must be demonstrated. A large number of women are excluded from the program as they do not speak or read English. The program is in its infancy, and this may change in the future as developments are made in information technology.

The issues faced and strategies used by Anthony Nolan's Cord Blood Program are not unique but are similar to those experienced globally. The field of cord blood banking is an evolving one, and the pace of change is fast as collaboration enhances the knowledge base for all in the field. It must be acknowledged that not all ethnic groups will be catered for by Anthony Nolan's Cord Blood Program, and it is imperative that worldwide collaboration continues to exist for equity of hematopoietic cell transplant treatment for all ethnicities. Good practice examples are those of the public cord blood banking program in India that recognize the needs of the emigrant nationals and their descendants, and those of the Australian public CBB in recognizing that some ethnic groups may already be catered for by established public CBBs in countries of origin.

4 Conclusion

Anthony Nolan has established excellent worldwide links between registries and has paved the way for ethnic minorities in the UK to receive a greater chance of hematopoietic cell transplant, and continues to seek improvement in the number of hematopoietic cell transplants enabled. The limitations faced by Anthony Nolan in recruiting donors from ethnic minorities can be overcome, as more sites come on board and experience is gained by the collection site teams. As the collection sites become established and familiarity with the specific educational/knowledge needs of the ethnic communities in those areas becomes more evident, we can expect to gain more donors from the target groups.

Acknowledgments The authors would like to thank Professor N. Russell (Nottingham City Hospital) and Professor G. Mufti (Kings College Hospital) and Midwife Terie Duffy for their help and support of Anthony Nolan Cord Blood Program.

References

1. Querol S, Mufti GJ, Marsh SG, Pagliuca A, Little AM, Shaw BE, et al. Cord blood stem cells for haematopoietic stem cell transplantation in the UK: how big should the bank be? Haematologica. 2009;94:536–41.
2. Ballen KK, Kurtzberg J, Lane TA, Lindgren BR, Miller JP, Nagan D, et al. Racial diversity with high nucleated cell counts and CD34 counts achieved in a national network of cord blood banks. Biol Blood Marrow Transplant. 2004;10:269–75.
3. Gardosi J, Beamish N, Francis A, Wiliams M, Sahota M, Tonks A, et al. Stillbirth and infant mortality West Midlands 1997–2005: trends factors and inequalities. West Midlands Perinatal Institute report. http://www.pi.nhs.uk/pnm/WM_SB&IMR_2007report.pdf. Accessed 26 Nov 2013.
4. Leicester City Council. Census 2011. http://www.leicester.gov.uk/your-council-services/council-and-democracy/city-statistics/census2011/. Accessed 26 Nov 2013.
5. Birmingham City Council. Census 2011. http://www.birmingham.gov.uk/census. Accessed 26 Nov 2013.

6. Athar S. Enhancement technologies and the person: an Islamic view. J Law Med Ethics. 2008; 36:59–64.
7. Fadel HE. Developments in stem cell research and therapeutic cloning: Islamic ethical positions, a review. Bioethics. 2012;26:128–35.
8. Viswanathan C, Roy A, Damodaran D, Manira AK, Kabra P. Optimization of the inventory size of the public cord blood program—the Indian context. J Assoc Physicians India. 2010; 58:608–11.
9. Baker KS, Davies SM, Majhail NS, Hassebroek A, Klein JP, Ballen KK, et al. Race and socio-economic status influence outcomes of unrelated donor hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2009;15:1543–54.

Chapter 11
Establishment of Mesenchymal Stem Cell Banks in India

Chandra Viswanathan and Prathibha Shetty

1 Introduction

Stem cell-based therapies hold tremendous promise for clinical use. While there are a few cell-based products, which are commercially available, there are many more potential therapies in clinical development. Stem cell-based products can be of allogeneic or of autologous origin. In the allogeneic cell therapy model, cell banking is employed for manufacturing and storage of a large-scale inventory of a uniform, off-the-shelf product. In the autologous cell therapy model, cell banking is employed for manufacturing and storage of individual aliquots, to be used for the preparation of future repeat doses for self. As cell-based therapies begin to progress through Phase III clinical trials, an increasing need to have cells of the right type and numbers and of certain specificity can be anticipated [1]. This has kindled interest among investors and pharma companies alike. Thus, issues for the industry will be to tackle some of these challenges they face due to limited and obscure regulations, getting an idea of the research capabilities, and certainly achieving some return on investments. With increasing knowledge and understanding of several adult and hematopoietic stem cells in various stages of translation, banking of cells is becoming popular. We refer to mesenchymal stem cell (MSC) banking as an example of adult stem cell banking, as that is the most frequently researched cell type. We are not discussing umbilical cord blood banking in this chapter, as that is something already commercialized and discussed elsewhere in this book.

The banking of MSC must meet the following main objectives, at a minimum:

1. Ensure availability of high quality, reliable, and well-characterized human MSCs for clinical applications.

C. Viswanathan (✉) • P. Shetty
Regenerative Medicine Group, Reliance Life Sciences Pvt. Ltd.,
R-282, TTC Area of MIDC, Thane-Belapur Road, Rabale, Navi Mumbai 400 701, India
e-mail: chandra.viswanathan@ril.com

D. Ilic (ed.), *Stem Cell Banking*, Stem Cell Biology and Regenerative Medicine,
DOI 10.1007/978-1-4939-0585-0_11,

2. Optimization of cell numbers at the master and working cell bank (WCB) levels.
3. Establishment of stability studies at different time points during the process development.
4. Establishment of functional capability of cells stored for varying periods in liquid nitrogen.
5. Put in place documentation formats for raw material procurement, manufacturing process, quality control (QC), quality assurance (QA), and deviation if any.
6. Establish a comprehensive quality management system to support the current Good Manufacturing Practice (cGMP) facility requirements throughout the process of cell banking.
7. Define the minimum release criteria as per available international standards.

MSC banking involves three important steps: collection, processing, and cryopreservation of derived stem cells until use [2].

2 Logistics of MSC Banking

2.1 Raw Material Collection

The collection of the biological samples (autologous/allogeneic) for derivation of MSCs is done in vacutainers or tubes with anticoagulant or media containing antibiotics. Depending on the type of sample (e.g., bone marrow or umbilical cord tissue or adipose tissue), the proportion could vary. Collection of biological material should always be done from accredited, licensed, or authorized medical establishments by a qualified medical specialist.

2.2 Informed Consenting

The significance of the consenting process in stem cell banking cannot be underestimated. An informed consenting procedure should be administered to donors by trained staff or the clinicians themselves. The donors should be advised about the banking process and the potential use of the banked cells as an alternative for a possible future clinical application. The response of the donor should be documented in the donor evaluation form, which should also be designed to ascertain the risk of transfusion transmissible diseases. The donors should provide written consent for the collection of cord tissue postpartum and should also consent to provide blood samples for all the mandatory tests. The protocol of the informed consent should be approved by the respective Institutional Stem Cell Committee. The donor will need to cooperate with the facility with regard to medical follow up for extended periods of time, which could affect the cells in the long term.

2.3 Infectious Disease Testing

It is very important to screen the samples for infectious disease before processing the sample. For this the maternal blood or the donor peripheral blood is screened for infectious disease markers like HIV I & II and HCV antibodies, Hepatitis B Surface Antigen (HbsAg), Syphilis, and CMV IgG and IgM as per existing regulatory guidelines. If the donor's blood is reactive for any of the infectious diseases, the sample is generally not taken up for processing and banking. Should such samples be used, quality policies have to be in place to ensure prevention of mixing up samples, wrong labeling, etc. The policy may also include handling and processing of the samples in quarantine as per the respective organization's policy. If there are regulations pertaining to this, adherence to these is paramount. Information about the donor with respect to age, weight, volume of the sample, collection center, etc. is captured in the donor information sheet.

2.4 Processing Facility

All the samples for cell derivation should be processed in the GMP-compliant facility comprising of self-contained Grade B cell-processing suites. Each processing suite should have a Class II microbiological safety cabinet providing the Grade A air environment required to fit open processing of cell materials. The suites should be self-contained and independent of each other in order to minimize the risk of cross-contamination. When using processing methods that may result in contamination or cross-contamination of cellular therapy products, critical environmental conditions should be controlled where appropriate for temperature, humidity, ventilation, air quality, and surface contaminates. The processing facility should provide environmental monitoring for microorganisms.

2.5 Generation of Master and Working Cell Banks

Appropriate batch numbers should be assigned to the cell banks. In order to ensure supply of adequate cells for future requirements, a master cell bank (MCB) and a WCB concept should be adopted. This two-tiered cell banking system is to optimize the space in the liquid nitrogen storage containers. Thus in all cases, a MCB concept must be adopted from which a WCB should be made for future use.

2.6 QA and QC

Protocols should be developed, implemented, and documented for the validation or qualification of significant, processes, equipment, reagents labels, containers, packaging materials, and computer systems.

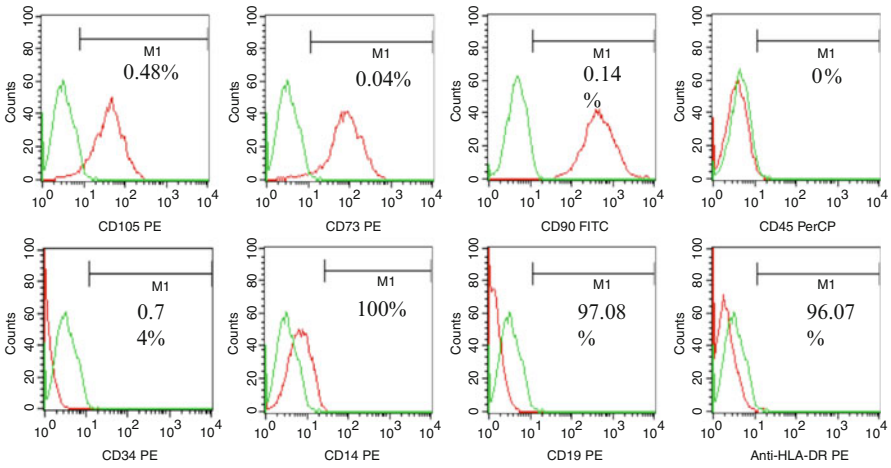


Fig. 11.1 Phenotypic characterization of MSCs by FACS analysis. The expanded MSCs should express MSC markers and should be negative for hematopoietic markers as per the ISCT criteria

The cell products for banking should undergo the following minimum QC tests based on available international guidelines:

1. Cell enumeration.
2. Phenotypic characterization.
3. Viability determination.
4. Microbiological tests for sterility, mycoplasma, and endotoxin levels on the cell supernatants.

2.6.1 Cell Enumeration

Post harvest, the cells are enumerated by automated cell counters. For generation of a MSB, cells should be frozen at passage 1. From the MSB, a WSB is generally created so that it can be used for cell derivation as and when required.

2.6.2 Phenotypic Characterization

Cells from each passage should be subjected to immunophenotypic analysis. Expanded cells should be characterized for the presence and absence of markers by using antibodies such as CD45, CD73, CD105, SSEA-4, HLA-DR, HLA-ABC, CD31, CD14, CD44, CD29, and vWF as per the criteria of the International Society for Cell Therapy (ISCT) (Fig. 11.1) to identify MSCs. Viability is determined using a specific viability dye to stain the dead cells. Both the marker expression and the viability can be assessed using the automated systems to give a quantitative output of expression [3].

2.6.3 Viability Determination

For viability determination, the cells are stained with 7-Amino Actinomycin D (7-AAD), (BD Pharmingen, USA), and acquired on the flow cytometer. Dead cells take up the fluorescent stain, while the live cells exclude this stain. Viability and phenotypic characterization studies are done at every passage.

2.6.4 Microbiological Tests

During the banking process, the untested samples are segregated from the tested one by storing them at different storage systems. We use two separate liquid nitrogen storage containers with corresponding labels. To distinguish the untested from fully tested samples they are labeled as "Under Quarantine" samples. Quarantined samples should be transferred to the final storage only after infectious disease markers clearance is received. Once the cells are requested for use, the cells from the MSB are passaged to receive adequate cell numbers. Then the mandatory tests are performed and cells are issued with a certificate of analysis.

2.7 Documentation

Documentation is an integral part of any process. It is very important to develop proper processing steps with validation of crucial steps. Using the international guidelines that are available, all relevant information and tests on raw materials should be performed at appropriate stages, and only approved materials should be taken up for processing. The details are documented in the respective Batch Manufacturing Records (BMR). QA team approves each BMR. All documents relating to cell bank manufacturing, such as the BMR, cell harvest details, freezing records, phenotypic characterization, viability data, and the details of the MCB and WCB and their location are reviewed regularly by the QA team and archived for future reference.

2.8 Labeling

Each biological sample is labeled with a unique barcode number as shown below. The unique barcode assigned to each of the samples should capture maximum information related to the cells banked. For example, the label shown below is a 15-digit barcode used for cord blood storage at the Reliance Life Sciences (RLS) facility, with a unique identity as narrated below (Fig. 11.2). This is also in line with the recommendations made by ISBT 128 for labeling.

Fig. 11.2 The 15-digit unique barcode denotes the following: *M* denotes the collection center, *0142* denotes the unique number of the collection center/medical establishment, *13* denotes the year of collection, *501234* denotes the 6-digit sample unique ID number of the donor, *01* or *02* denotes the case of cord blood banking, the mode of delivery, namely vaginal or cesarean, respectively

2.9 Cryopreservation Considerations

The science and the art of cryopreservation has been successfully utilized for the long-term storage of several different cell types like bone marrow cells, ovaries, sperm, etc. for many years, and is also considered the most effective method for other cellular preservation. Given the fact that the current process is not yet optimized for all cell-based products, there is a significant need to further improve cryopreservation techniques. An ideal cryoprotection solution should be nontoxic for cells and patients, nonantigenic, chemically inert, provide a high survival rate after thawing, and allow immediate implantation into the patient even without washing. However, cryopreservation protocols still often rely on 10 % dimethyl sulfoxide (DMSO) as a cryoprotectant, due to its marked ability to penetrate cell membranes and prevent cell rupture. It is well known that DMSO is potentially cytotoxic, and transplantation of DMSO-preserved human BM cells has been shown to cause severe adverse reactions. As a result, efforts are being made to reduce the DMSO content of cryoprotection media as much as possible, in some cases by replacing a portion of it with alternatives such as hydroxyethyl starch, and, in some cases, by replacing it completely. Furthermore, serum-free cryomedia are increasingly being developed. In addition to the above areas, efforts to optimize the cryopreservation process have focused on the use of more environmentally friendly cryopreservation agents, lowering the overall cost of reagents, manual handling and storage, and using slow-rate cooling methods. These efforts have resulted in good, reproducible yields, with maintenance of similar phenotypes and cell surface markers, cells remaining untransformed, practically no microbiological contaminations, and comparable growth rates to non-cryogenically treated cells [1]. Individual banks need to come up with the most optimum cryopreservation steps for their processes by running validation experiments during process development.

2.9.1 Validation Studies

Validation refers to the establishment of documented evidence that provides a high degree of assurance that a specific process will consistently yield a product that

meets its predetermined specifications and quality attributes. A process is validated to evaluate the performance of a system with regard to its effectiveness based on intended use.

2.9.2 Stability Studies

Stability studies should be designed and conducted to determine the optimum period for which the banked cells can be cryopreserved without having any effect on viability. For this, the expanded MSCs cryopreserved are thawed at specified time points (1 month, 6 months, 1 year, 2 years, and 3 years) and characterized along with their viability. The data obtained post-thaw is compared with the pre-freezing characteristics. Genotypic characterization such as the karyotyping analysis should be performed to check for any genetic variation in the cells post-thawing after varying periods in storage.

2.10 Cell Characterization and Release Criteria

Once the cell-based product has been thawed, the final characterization prior to expansion (when applicable) and delivery to the patient must be performed. Post-thawing release criteria, like, for example, post-culturing release criteria, include parameters such as viability, recovery, doubling time, phenotyping, and differentiation capacity. In addition, analytical methods must be used to rule out the presence of residual growth media supplements. The time and costs involved in this step, as well as in the subsequent expansion into the end product, could add significantly to the cost of the product. It is better if it is performed within an on-site cell therapy processing unit of a medical center as opposed to outsourcing it to an external testing facility, provided there are specially trained and qualified personnel proficient in carrying out these tests [4].

2.11 Packing and Shipping Considerations

Each of these aspects has unique benefits and disadvantages. One must consider ease of maintaining the temperature during transit to the hospital, ease of preparing the cells for the patient at the hospital, and storage of the cell-based product at the hospital till use. A biohazard label should be applied to each product prior to release from the collection facility if any test shows evidence of infection due to communicable disease agents. A biohazard label should be applied to each product if testing was not performed or final results are not available.

The transportation of the donor samples for processing should be validated to fix the most optimum transit temperature. Accordingly, temperature-controlled containers

usually ranging from 2 to 15 °C are designed to transfer materials. The samples should be received at the manufacturing plant within the stipulated time; delays in transportation will compromise the viability and yield of cells to be isolated. Every deviation from the laid down conditions needs to be analyzed and adequately addressed.

Today, many non-cell-based products, such as blood components, are shipped at −20 °C and cellular products are transported at −60 to −80 °C in dry ice. Viability is maintained till the temperature is maintained which requires replenishment of dry ice periodically. MSCs can be easily transported in cryovials at this temperature and can be transferred to liquid Nitrogen at −196 °C until use. The number of vials will vary as per their need. However, autologous MSCs or allogenic cells have been shipped predominantly by this method, and the quality of the final products has proven to be satisfactory. Cell therapy products in non-frozen states are more likely to be adopted in the short term until on-site cell therapy processing units become the norm in major medical centers. The actual logistics of moving the cells from one place to another can be challenging when attempting to ensure that the right patient gets the right cells at the right time. Companies should draw experience from entities that are experts in shipping blood/tissue/materials such as blood banks, specialized logistics companies, or academia in delivering stem cells for research through collaboration.

2.12 Storage and Delivery of Cells to Patients

The MSCs or MSC-based cellular products could be stored on site, frozen or fresh depending on the product, whether it is purely MSCs or a combination or on a scaffold, in the existing blood/tissue bank of the hospital for a few days until the patient is ready for the treatment. These hospital departments generally have liquid nitrogen supplies, dry ice, suitable canisters, and some basic validation in place. But allowing the medical team to receive the final ready-to-use end product without having to depend on the hospital cell-processing unit to perform thawing, characterization, expansion, and possible differentiation procedures on site would be most ideal. In the perfect world, a preloaded syringe shipped to the medical center, with the desired number of MSCs, would be ready for the physician to administer to the patient. Companies in this domain could learn from the biopharma companies marketing monoclonal antibody-based products with regard to product delivery methods. While these are still early days for cell therapy products, logistical and cost-related benefits will determine commercial success and be a differentiating factor.

3 Future Frontiers

Based on the research findings, it is evident that provisioning of an autologous or allogenic cell-based product like MSCs, for one's own future use through a dependable and validated banking system is definitely desirable. Controversies around the scientific basis will continue to be the subject matter for future conferences and

scientific forums. Assuming that an autologous and an allogenic biological cell-based product is banked through appropriate methods, other questions that need to be addressed are the uniformity of quality across the banks, uniformity in the cell types, the exchange and transfer of cells across geographies, the clinical application, and efficacy-related questions. Attempts should be made to harmonize these issues, failing which interchangeability and use of cells across borders will remain a challenge and defeat the very purpose of the banking [5]. Long-term storage in precise storage media can be expensive, and years later, the multiplication of cells from the MCB could become problematic. Despite several scientific, regulatory, and logistics' challenges, the medical world is looking forward to utilizing stem cells and cell-based products for treatment of several unmet lifestyle and degenerative medical needs in the future. The medical community should accept the medicine of this new era with its attendant problems, and continue to learn from research progress [6]. In the future, one can expect to see cells and stem cell-based products as lyophilized powders, noncellular conditioned media with equal functional capabilities, to help and ensure better bedside handling by medical and paramedical staff. Time alone will tell, but scientific research and innovations in this domain are worth watching [7].

4 Existing Regulations and Accreditations

Therapies based on stem cells and cell-based products involve extended development of stem cell banking, characterization studies, production processes, and pilot scale manufacture.

Regulatory structure for cord blood banking activities has been finalized by the US FDA under the category of "Human Cells, Tissues, Cellular and Tissue-Based Products." Both public and private cord blood banks are regulated under the CFR Title 21 Section 1271. All countries will eventually have their own regulations in the cell banking area depending upon the degree of activity in their vicinity. In countries like India, China, Japan, the general structure of regulations are just becoming framed [8, 9]. The American Association of Blood Banks (AABB) and the Foundation for Accreditation of Cellular Therapy (FACT) are two "not for profit" accrediting agencies that have taken a lead in laying down the standards for cord blood and cell banking [10]. Companies and academia can choose to participate and upgrade their quality by complying with the standards of these agencies. As this work relates to biological tissue, there is a constant inflow of new research findings that will impact the quality of the stored material for future intended use. From 20th October 2011, all cord blood units used for transplant in US patients have been subject to licensure requirements including the sites from where the material was sourced and collected. Accordingly, member cord blood banks have had to comply with changes and processes to qualify their units for use within and outside the country [11, 12]. There are relatively very few banks that store other cell types like MSCs for prolonged periods. MSC banking, being relatively new, will go through intense discussion and

deliberation among cell biologists and clinicians for its usefulness before it becomes a standard of care. In this context, the role played by AABB and the FACT will go a long way in establishing the regulatory framework for MSC banking [13–15].

5 The Indian Scenario

In the Indian context, RLS had taken a lead in setting up the first public cord blood bank in 2001 (www.rellife.com) when there were no regulations in force. Only recently, a draft gazette on the establishment of cord blood banks has been made available by the Drug Controller's office, until which time, a blood bank licensing model was being adopted. The MSC banking services for autologous and allogenic use have also been available on request for some time now, although specific regulations for this are still under development. As there are several companies offering this service, patients and families would most benefit from selecting a service provider based on their capabilities and credibility. The laboratories of RLS that support stem cell research and translation have voluntarily opted for accreditation by the AABB, College of American Pathologists (CAP), and the National Accreditation of Biological Laboratories (NABL) for continuous quality improvement.

The Indian Council of Medical Research (ICMR) has in its guidelines outlined the role of Institutional Ethics Committees for Stem Cell Research and Therapy (IC-SCRT) and the National Apex Committee for Stem Cell Research and Therapy (NAC-SCRT). The Drug Controller has the responsibility to develop and enforce adherence to legal requirements [16]. While many issues are yet to be resolved with respect to regulatory policies in various stem cell-based therapy developments, the knowledge and experience gained thus far in MSC research and banking from end to end, with inputs from translational research, will only help better establishment of the various components. It appears that there is a huge scope for change as this is an evolving science and the only certainty is progress, which is expected to be continuous, and for the better.

6 Conclusions

1. Private banking of cells and stem cells for one's own use in the future is a subject of intense debate; however, scientific curiosity should not stop and research will continue. Timely availability of good quality cells in adequate numbers for research and clinical development is the force that drives establishment of cell banking.
2. Cord blood banking guidelines and the knowledge gained over the last three decades form the basis for banking of other cell types for research and potential future clinical use.
3. Research and development on use of MSCs and other cell types for degenerative and lifestyle diseases are on the increase.

4. A process development for banking consists of extensive validation of production process, repeatability of process, quantity and quality of cellular yields, raw material definition, phenotypic markers, and fitness for use.
5. One must define the production environment, suppliers qualification, training of staff, training on Standard Operation Procedures (SOPs) and policies, document control, label control, data integrity, safety, software integrity, proficiency testing, etc. which directly contribute to the success of the program.
6. Defining and specifying storage conditions, real-time stability, cryopreservatives, formulations, maintenance and handling of such cells including transport are very crucial to banking.
7. Regenerative medicine is the medicine for a new era; the regulations are still under development, but harmonization is being attempted between various accrediting agencies to minimize loss of time in translation, minimizing confusion.
8. Families and patients opting for banking the cells must choose a facility that has credibility, stability, and long-term vision.

Acknowledgment The authors acknowledge Reliance Life Sciences Pvt. Ltd. (www.rellife.com), for providing the infrastructure and financial support to work on this project.

Conflict of Interest: This is to inform that I am a current employee of Reliance Life Sciences Pvt. Ltd. and neither I nor the company have a financial gain from this article.

References

1. Harel A. Cryopreservation and cell banking for autologous mesenchymal stem cell-based therapies. Cell Tissue Transplant Ther. 2013;5:1–7. http://www.la-press.com/cryopreservation-and-cell-banking-for-autologous-mesenchymal-stem-cell-article-a3541. Accessed 26 Nov 2013.
2. Cooper K, Viswanathan C. Establishment of a mesenchymal stem cell bank. Stem Cells Int. 2011;2011:905621.
3. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006;8:315–7.
4. Thirumala S, Goebel WS, Woods EJ. Clinical grade adult stem cell banking. Organogenesis. 2009;5(3):143–54.
5. Coopman K. Large scale compatible methods for the preservation of human embryonic stem cells: current perspective. Biotechnol Prog. 2011;27:1511–21.
6. Viswanathan C, Kabra P. Umbilical cord blood—progress, process and promise. Frozen life—manual of cryobiology for assisted reproduction and stem cells. Jaypee Publication; 2009. p. 400–16.
7. Viswanathan C. Cord blood banking: public and private. Hematol Today. 2012;589–98.
8. United Kingdom Stem Cell Initiative (UKSCI). UK Stem Cell Initiative (UKSCI)—report and recommendations. 271735/UK. London: DH Publications; 2005.
9. U.S. Food and Drug Administration (FDA). Current good tissue practice (cGTP) and additional requirements for manufacturers of human cells, tissues, and cellular and tissue-based products (HCT/Ps). Guidance for industry. Center for Biologics Evaluation and Research. FDA/OCOD. HFM-40. Rockville, MD; 2009.
10. American Association of Blood Banks. FACT, ISCT guidelines.

11. U.S. Food and Drug Administration (FDA). Regulation of human cells, tissues, and cellular and tissue-based products (HCT/Ps). Guidance for industry. Small entity compliance guide. FDA/HFM-40. Center for Biologics Evaluation and Research. Rockville, MD; 2007.
12. U.S. Food and Drug Administration (FDA). Current good tissue practice for human cell, tissue, and cellular and tissue-based product establishments. Part IV. 21 CFR Parts 16, 1270, 1271. Washington, DC: Federal Register; 2004.
13. Food and Drug Administration. Draft document concerning the regulation of placental/umbilical cord blood stem cell products intended for transplantation or further manufacture into injectable products. Fed Regist. 1996;61:7087–8.
14. Harvath L. Food and Drug Administration and a proposed approach to regulation of hematopoietic stem/progenitor cell products for therapeutic use. Transfus Med Rev. 1999;14(2): 104–11.
15. NETCORD, FACT, international standards for cord blood processing, testing, banking, selection and release. 3rd ed. 2006.
16. Indian Council of Medical Research—Department of Biotechnology (ICMR-DBT). Guidelines for stem cell research and therapy; 2007.

Chapter 12
Stem Cell Banking in Iran

Newsha Haghparast, Mohammad Pakzad, Parvaneh Farzaneh, Marzieh Ebrahimi, Vahid Hajihosseini, Mahdi Tondar, and Hossein Baharvand

1 Introduction

The incredible potential of stem cells for self-renewal and differentiation into other cell lineages makes them great candidates for cell therapy and regenerative medicine. Due to the promise in this area, many countries have directed their main research programs towards this field and Iran has been one of them. It is worth mentioning that the number of published stem cell-related papers from 15 papers in 2004 has reached 145 in 2011 and is still continuing to grow [1].

In 1998, the whole world turned its attention to the newly derived human embryonic stem cells (hESCs) and the effects they could potentially have on cellular

Newsha Haghparast and Mohammad Pakzad contributed equally in this work.

N. Haghparast • M. Pakzad • V. Hajihosseini • M. Tondar
Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, Academic Center for Education, Culture & Research, Tehran, Iran

P. Farzaneh
Human and Animal Cell Bank, Iranian Biological Resource Center, Academic Center for Education, Culture & Research, Tehran, Iran

M. Ebrahimi
Department of Regenerative Medicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran

H. Baharvand (✉)
Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, Department of Developmental Biology, University of Science and Culture, Academic Center for Education, Culture & Research, Tehran, Iran
e-mail: Baharvand@RoyanInstitute.org

D. Ilic (ed.), *Stem Cell Banking*, Stem Cell Biology and Regenerative Medicine,
DOI 10.1007/978-1-4939-0585-0_12,

research and therapy, drug screening, and developmental studies [2]. Iran followed the progress of this discovery closely.

By introducing the concepts of cell and stem cell research and therapy, the existence of a cell bank became essential, as there was a need for an entity designed to provide access to qualified stem cell lines from different origins and grades [3]. A stem cell bank, which has been committed to depositing cells and has established and maintained long-term quality standards would enable researchers to economize better as well as assure them regarding the quality of stem cell lines they intend to work with. It is well accepted that long-term passaging and maintenance in vitro can affect the genetic integrity of the cell line. By establishing a cell bank and depositing cell line backups with low passage numbers, this problem can lose much of its significance [4]. In this chapter, we will introduce Iranian stem cell banks, including Royan Stem Cell Bank (RSCB), and their role in biomedical science in the country.

2 Cell Banking in Iran

Figure 12.1 showed chronologically arranged events of the cell banking in Iran. The Pasteur Institute of Iran (http://ncbi.pasteur.ac.ir/) was the founder of the first nonprofit Iranian cell bank in 1993, created with the purpose of centralized collection and storage of human and animal cell lines. The bank is divided into four collections, from which the cell lines are made available to applicants:

- General cell collection
- Hybridoma collection
- Human leucocyte antigen (HLA)-defined collection
- Human genetic disorders collection

In 2008, the Iranian Biological Resource Center (IBRC) was also established under the authority of the Academic Center for Education, Culture and Research

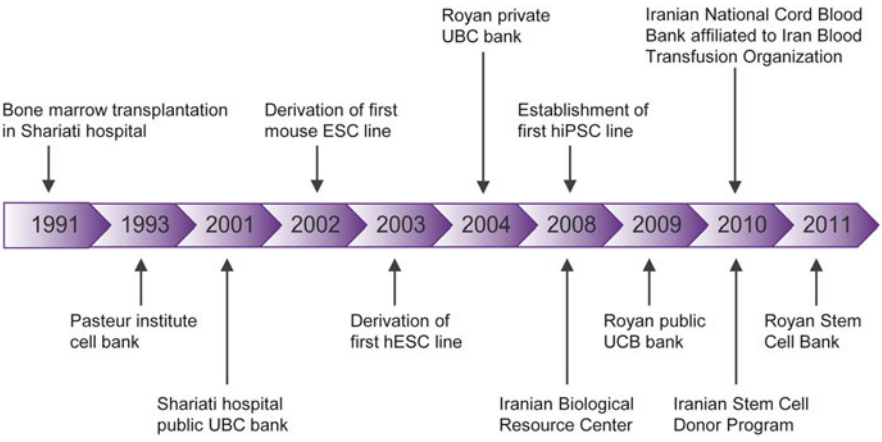


Fig. 12.1 Chronologically arranged events of the cell banking in Iran

(ACECR). This center acts as an infrastructure for research and biotechnology by providing different types of biological materials. The activities of the human and animal cells department of the IBRC include production, collection, characterization, quality control, expansion, cryopreservation, storage, distribution, and national patent deposition of human and animal cell lines. In addition, immortalized stem cells and primary cultures of human and animal cells are characterized and qualified in different steps of banking. The collections of this department are provided for the national demand for cells for research in different areas of biomedical science and technology, endangered native animals, human population genetics, and genetic diseases. This department also provides cell-related services and training in different areas of cell biology (http://www.ibrc.ir/Default.aspx?tabid=792).

The subject of bone marrow stem cell exploitation and application in transplantation gained much attention in 1991 when bone marrow transplantation was applied for the purpose of curing cancers and blood illnesses [1]. This step, which was taken in the Hematology-Oncology and Stem Cell Transplantation Research Center (HORCSCT) in Shariati hospital which is affiliated to Tehran University of Medical Science was the last one before reaching the exciting field of stem cell research and therapy [1].

But the subject of embryonic stem cells (ESC) remained undisclosed until Royan Institute successfully generated Iran's first mouse ESC (mESC) line in 2002. After this achievement, more focus was allocated to hESC line derivation. This goal was achieved in 2003 and the first Iranian hESC line was named Royan H1 [5] (in Persian, *Royan* means *embryo*). Since the ESC line derivation path was successfully paved, more research and therapeutic centers were interested in applying stem cells. In 2004, Royan Stem Cell Technology Company as a private company was established to bank private cord bloods (www.rsct.ir). Finally, in 2008 the first Iranian human-induced pluripotent cell (hiPSC) line was derived in Royan Institute, which showed great promise for future clinical programs [6].

3 Research Stem Cell Banks

In 2011, RSCB started to work primarily as a pluripotent stem cell depository. However, it also stored other stem cell lines such as adult stem cells including mesenchymal stem cells (MSCs) and neural stem cells (NSCs). The cells are maintained in vapor phase of liquid nitrogen to reduce risk of contamination (Fig. 12.2). Other institutes are following the same trend in establishing stem cell banks, but until now they have not officially started working.

3.1 Pluripotent Stem Cell Lines in RSCB

After the generation of the first mESC in 2002, around 165 mESC lines have been derived and deposited in RSCB from different mice strains such as *NMRI*, *C57BL/6*, *BALB/c*, *DBA/2*, *FVB/N*, and *SW* (Table 12.1). Also, 150 mESC lines were derived

Fig. 12.2 Vapor phase nitrogen tank and its supply tank in RSCB

Table 12.1 Mouse embryonic cell lines in RSCB

Strain	No. of cell lines
C57BL/6	16
BALB/c	40
♂ NMRI × ♀ BALB/c	2
DBA/2	35
FVB/N	8
NMRI	58
SW	6

Table 12.2 Human-induced pluripotent stem cell lines in RSCB

Phenotype/disease name	Abbreviation	No. of cell lines
Normal	–	14
Bombay blood group	BOM	9
Familial hypertrophic cardiomyopathy	FHC	9
Glycogen storage disease	GSD	6
Tyrosinemia (Type I)	TYR	7
Hereditary cholestasis	HER	6
Retinitis pigmentosa	RP	15
Leber's congenital amaurosis	LCA	4
Usher syndrome (Usher's syndrome)	USH	8
Age-related macular degenerative disease	ARMD	7
Leber hereditary optic neuropathy	LHON	4
Crigler–Najjar syndrome	CNS	17

from mouse single blastomere. Most of these cell lines were generated by dual inhibition of mitogen-activated protein kinase (MAPK) (also known as MEK) and transforming growth factor β (TGFβ) type I receptors (also known as activin receptor-like kinase [ALK]-4, -5, and -7) by PD0325901 and SB431542, respectively [7, 8].

Moreover, after derivation of the first hESC line, Royan H1 [5], this process continued and more hESC lines were generated using human spare embryos, abnormal embryos diagnosed in preimplantation genetic diagnostics (PGD) program, and low quality embryos. Until 2013, 62 hESC lines were derived, 55 of which were produced from single blastomeres [9–11].

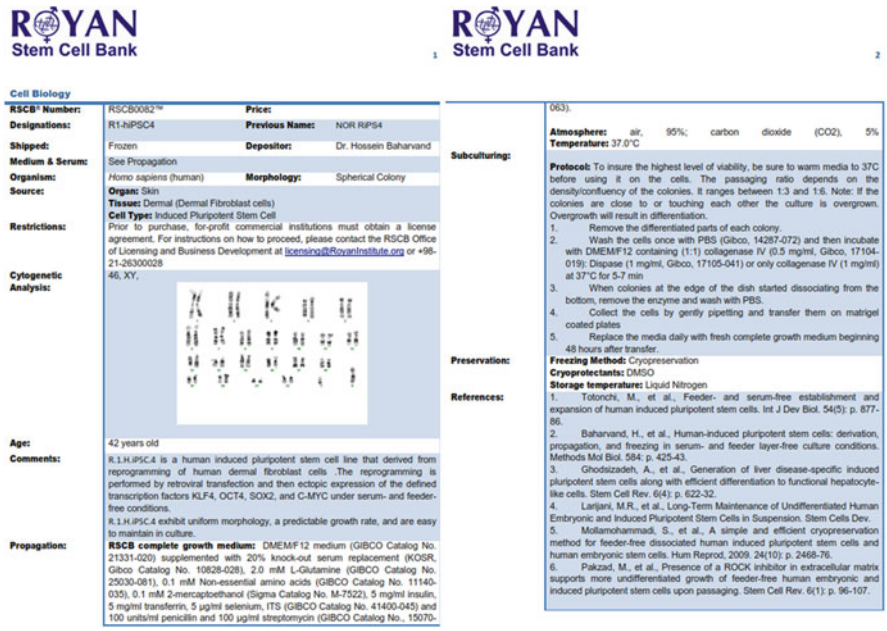
After the introduction of iPSCs to the area of stem cell research [12, 13] and considering the enormity of their potential, Royan Institute adopted this technology to produce iPSC lines. RSCB produced five mouse iPSCs and following on from this, 106 human iPSCs were derived from donors with normal and deficient phenotypes (Table 12.2) [14–16].

The most popular RSCB cell lines, which are purchased routinely by Iranian researchers, have catalogs which contain crucial information about their characteristics (Fig. 12.3). Other cell lines catalogs are being processed.

3.2 Quality Control in RSCB

RSCB cells are routinely tested for sterility—the presence of bacterial and fungal contaminants as well as Mycoplasma. After ensuring the sterility of the lines, they are deposited in liquid nitrogen tanks or are delivered to applicants. Karyotyping by G-banding and Giemsa staining is done to ensure normal karyotype of cell lines.

In RSCB, OCT4, SSEA3, SSEA4, TRA-1-81, TRA-1-60, and alkaline phosphatase activity are used as pluripotency markers for human pluripotent stem cells (hPSCs), while OCT4, Nanog, SSEA1, and alkaline phosphatase activity are used



ROYAN Stem Cell Bank 1

Cell Biology

RSCB® Number:	RSCB0082™	**Price:**	
Designations:	R1-hiPSC4	**Previous Name:**	NOR RiPS4
Shipped:	Frozen	**Depositor:**	Dr. Hossein Baharvand
Medium & Serum:	See Propagation		
Organism:	*Homo sapiens* (human)	**Morphology:**	Spherical Colony

Source:
Organ: Skin
Tissue: Dermal (Dermal Fibroblast cells)
Cell Type: Induced Pluripotent Stem Cell

Restrictions: Prior to purchase, for-profit commercial institutions must obtain a license agreement. For instructions on how to proceed, please contact the RSCB Office of Licensing and Business Development at licensing@RoyanInstitute.org or +98-21-26300028

Cytogenetic Analysis: 46, XY,

Age: 42 years old

Comments: R.1.H.iPSC.4 is a human induced pluripotent stem cell line that derived from reprogramming of human dermal fibroblast cells .The reprogramming is performed by retroviral transfection and then ectopic expression of the defined transcription factors KLF4, OCT4, SOX2, and C-MYC under serum- and feeder-free conditions.
R.1.H.iPSC.4 exhibit uniform morphology, a predictable growth rate, and are easy to maintain in culture.

Propagation: **RSCB complete growth medium:** DMEM/F12 medium (GIBCO Catalog No. 21331-020) supplemented with 20% knock-out serum replacement (KOSR, Gibco Catalog No. 10828-028), 2.0 mM L-Glutamine (GIBCO Catalog No. 25030-081), 0.1 mM Non-essential amino acids (GIBCO Catalog No. 11140-035), 0.1 mM 2-mercaptoethanol (Sigma Catalog No. M-7522), 5 mg/ml insulin, 5 mg/ml transferrin, 5 µg/ml selenium, ITS (GIBCO Catalog No. 41400-045) and 100 units/ml penicillin and 100 µg/ml streptomycin (GIBCO Catalog No., 15070-063).

Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C

Subculturing:
Protocol: To insure the highest level of viability, be sure to warm media to 37C before using it on the cells. The passaging ratio depends on the density/confluency of the colonies. It ranges between 1:3 and 1:6. Note: If the colonies are close to or touching each other the culture is overgrown. Overgrowth will result in differentiation.
1. Remove the differentiated parts of each colony.
2. Wash the cells once with PBS (Gibco, 14287-072) and then incubate with DMEM/F12 containing (1:1) collagenase IV (0.5 mg/ml, Gibco, 17104-019): Dispase (1 mg/ml, Gibco, 17105-041) or only collagenase IV (1 mg/ml) at 37°C for 5-7 min
3. When colonies at the edge of the dish started dissociating from the bottom, remove the enzyme and wash with PBS.
4. Collect the cells by gently pipetting and transfer them on matrigel coated plates
5. Replace the media daily with fresh complete growth medium beginning 48 hours after transfer.

Preservation:
Freezing Method: Cryopreservation
Cryoprotectants: DMSO
Storage temperature: Liquid Nitrogen

References:
1. Totonchi, M., et al., Feeder- and serum-free establishment and expansion of human induced pluripotent stem cells. Int J Dev Biol. 54(5): p. 877-86.
2. Baharvand, H., et al., Human-induced pluripotent stem cells: derivation, propagation, and freezing in serum- and feeder layer-free culture conditions. Methods Mol Biol. 584: p. 425-43.
3. Ghodsizadeh, A., et al., Generation of liver disease-specific induced pluripotent stem cells along with efficient differentiation to functional hepatocyte-like cells. Stem Cell Rev. 6(4): p. 622-32.
4. Larijani, M.R., et al., Long-Term Maintenance of Undifferentiated Human Embryonic and Induced Pluripotent Stem Cells in Suspension. Stem Cells Dev.
5. Mollamohammadi, S., et al., A simple and efficient cryopreservation method for feeder-free dissociated human induced pluripotent stem cells and human embryonic stem cells. Hum Reprod. 2009. 24(10): p. 2468-76.
6. Pakzad, M., et al., Presence of a ROCK inhibitor in extracellular matrix supports more undifferentiated growth of feeder-free human embryonic and induced pluripotent stem cells upon passaging. Stem Cell Rev. 6(1): p. 96-107.

Fig. 12.3 Sample catalog of a hESC line

for mouse pluripotent stem cells. Also, in vitro differentiation and in vivo teratoma formation of these cells are part of standard testing for pluripotency in RSCB.

The information about the state of RSCB stem cell lines, the number of them, and their location in nitrogen tanks are all inscribed in RSCB software (Appendix 1), which we generated and is an excellent resource for avoiding possible mistakes.

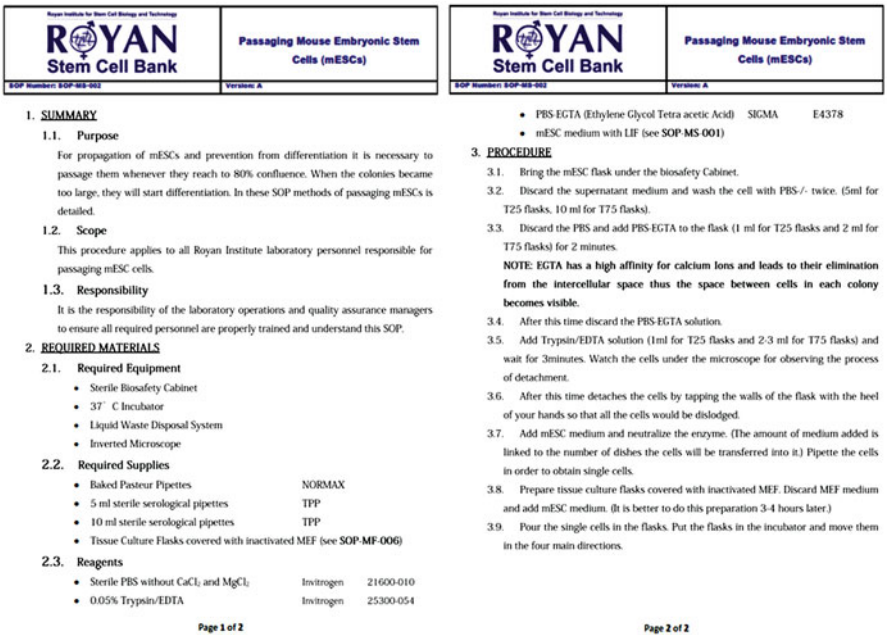
3.3 Documentation in RSCB

A proper documentation is of an utmost importance for both traceability and reproducibility. The technicians are writing down all the methods in the form of Standard Operating Procedures (SOP). After SOPs are verified, they are available for public use. For instance, RSCB, made available about 20 SOPs to those researchers who aim to work on different stem cell lines obtained from the bank (Table 12.3, Fig. 12.4).

Product characteristics such as company, catalogue number, and lot number are important for cell banks because cell lines can respond differently to the same products from different companies. RSCB uses validated material and equipment in order to prevent this type of variation and always keep products' batch labels in the RSCB filing system. RSCB validates samples of all crucial material on established cell lines before ordering any new product.

Table 12.3 The list of Royan Stem Cell Bank's SOPs for those researchers who work on PSC lines

Main subject		Related procedure	Related SOP
Mouse embryonic fibroblasts (MEFs)		Preparation of MEF medium	SOP-MF-001
		Derivation of MEFs	SOP-MF-002
		Passaging MEFs	SOP-MF-003
		Cryopreservation of MEFs	SOP-MF-004
		Thawing MEFs	SOP-MF-005
		Inactivation of cells with mitomycin C treatment	SOP-MF-006
Mouse embryonic stem cells (mESCs)		Preparation of mESC medium	SOP-MS-001
		Passaging of mESCs	SOP-MS-002
		Cryopreservation of mESCs	SOP-MS-003
		Thawing of mESCs	SOP-MS-004
		Separation of mESCs from MEFs	SOP-MS-005
Human pluripotent stem cells (hPSCs)	Feeder-free culture	Preparation of hPSC medium	SOP-HS-005
		Passaging of hPSCs	SOP-HS-006
		Cryopreservation of hPSCs	SOP-HS-007
		Thawing of hPSCs on Matrigel™	SOP-HS-008
		Aliquoting of Matrigel™ and preparation of Matrigel™-coated dishes	SOP-HS-009
	Feeder-dependent culture	Preparation of hPSC medium	SOP-HS-001
		Passaging of hPSCs	SOP-HS-002
		Cryopreservation of hPSC	SOP-HS-003
		Thawing of hPSCs	SOP-HS-004



ROYAN Stem Cell Bank | Passaging Mouse Embryonic Stem Cells (mESCs)

SOP Number: SOP-MS-002 Version: A

1. SUMMARY

1.1. Purpose

For propagation of mESCs and prevention from differentiation it is necessary to passage them whenever they reach to 80% confluence. When the colonies became too large, they will start differentiation. In these SOP methods of passaging mESCs is detailed.

1.2. Scope

This procedure applies to all Royan Institute laboratory personnel responsible for passaging mESC cells.

1.3. Responsibility

It is the responsibility of the laboratory operations and quality assurance managers to ensure all required personnel are properly trained and understand this SOP.

2. REQUIRED MATERIALS

2.1. Required Equipment

- Sterile Biosafety Cabinet
- 37° C Incubator
- Liquid Waste Disposal System
- Inverted Microscope

2.2. Required Supplies

- Baked Pasteur Pipettes NORMAX
- 5 ml sterile serological pipettes TPP
- 10 ml sterile serological pipettes TPP
- Tissue Culture Flasks covered with inactivated MEF (see **SOP-MF-006**)

2.3. Reagents

- Sterile PBS without $CaCl_2$ and $MgCl_2$ Invitrogen 21600-010
- 0.05% Trypsin/EDTA Invitrogen 25300-054

Page 1 of 2

ROYAN Stem Cell Bank | Passaging Mouse Embryonic Stem Cells (mESCs)

SOP Number: SOP-MS-002 Version: A

- PBS-EGTA (Ethylene Glycol Tetra acetic Acid) SIGMA E4378
- mESC medium with LIF (see **SOP-MS-001**)

3. PROCEDURE

3.1. Bring the mESC flask under the biosafety Cabinet.

3.2. Discard the supernatant medium and wash the cell with PBS-/- twice. (5ml for T25 flasks, 10 ml for T75 flasks).

3.3. Discard the PBS and add PBS-EGTA to the flask (1 ml for T25 flasks and 2 ml for T75 flasks) for 2 minutes.

NOTE: EGTA has a high affinity for calcium Ions and leads to their elimination from the intercellular space thus the space between cells in each colony becomes visible.

3.4. After this time discard the PBS-EGTA solution.

3.5. Add Trypsin/EDTA solution (1ml for T25 flasks and 2-3 ml for T75 flasks) and wait for 3minutes. Watch the cells under the microscope for observing the process of detachment.

3.6. After this time detaches the cells by tapping the walls of the flask with the heel of your hands so that all the cells would be dislodged.

3.7. Add mESC medium and neutralize the enzyme. (The amount of medium added is linked to the number of dishes the cells will be transferred into it.) Pipette the cells in order to obtain single cells.

3.8. Prepare tissue culture flasks covered with inactivated MEF. Discard MEF medium and add mESC medium. (It is better to do this preparation 3-4 hours later.)

3.9. Pour the single cells in the flasks. Put the flasks in the incubator and move them in the four main directions.

Page 2 of 2

Fig. 12.4 An SOP sample in RSCB

Daily observations of cell lines' morphology and detailed information on cell culture events such as passage, cryopreservation, and thawing are written in laboratory notebooks, which are part of official RSCB documentation. Apart from writing daily reports, all the information about the cryopreserved cells such as cell line name and origin, passage number, cryopreservation date, and their place in nitrogen tanks will be registered in RSCB software which enables users to gain information about cell lines' current state (Appendix 1). RSCB is also trying to get a feedback from researchers, who used the cell lines, and pass this information to future users.

4 Clinical Stem Cell Banks in Iran

Clinical stem cell banks are mainly designed for patients in need of cell transplantation. In 1991, 3 years after the first umbilical cord blood (UCB) transplantation [17–19], HORCSCT was inaugurated in Iran as the first national hematology-oncology center (http://horcsct.tums.ac.ir/). Now, this center is a prominent stem cell transplantation center in the world with more than 300 successful transplantations performed per year. Moreover, this center deposits UCB from 2001. By identifying donor's HLA types up to now, this bank has stored more than 2,500 samples. This bank processes UCB units manually in a semi-closed system and cryopreserves all the samples which have passed the quality tests successfully. UCB bank of HORCSCT is a member of International NetCord Foundation—Foundation for the Accreditation of Cellular Therapy (FACT). Up to now, more than 60 patients were treated with these cells. The disease of the patients were blood cancers, non-malignant hematologic disorders, primary immunodeficiencies, and inborn errors of metabolisms.

In 2004, the Royan private UCB bank (www.rsct.ir), the first private Iranian UCB bank, started its activities. At the moment there are more than 30,000 UCB units have been stored in this bank, 18,000 of which have identified HLAs. The processing of cord blood samples in this bank is performed by Sepax cell separation system (Biosafe SA, Switzerland), which are fully automated and mobile closed systems for efficient and consistent processing of UCB, bone marrow, peripheral blood, and other cell-based products. Families who want to store an UCB sample in this bank must pay an annual fee. More than half of the UCB owners, signed an informed consent to put the HLA information of their UCB in HLA bank to donate their UCB if needed for a patient.

Three years later in 2009, the public section of the Royan public cord blood bank was inaugurated. Currently, of the 5,000 samples deposited in this bank, 2,100 have identified HLAs. The donors have been selected from all ethnic groups in Iran to increase chance of finding match samples for patients. In 2013, one of these UCB samples was transplanted into a patient with Aplastic Anemia (data not published). The Royan public cord blood bank processes its samples manually in a closed system. Both Royan's private and public cord blood banks act under a unique management as a hybrid bank and are member of Bone Marrow Donors Worldwide (BMDW) organization.

Furthermore, the public bank processes amniotic membranes (AM) in order to provide sterile dried or cryopreserved AM to Iranian Ocular transplantation centers and hospitals for treating corneal and skin injuries. It also creates AM sheets for transferring cells from bench to bed. Other activities of Royan public cord blood bank consists of obtaining fibrin glue from cord blood plasma and platelet lysate for research and therapy.

In 2010, the Iranian National Cord Blood Bank was also established as a part of the Iran Blood Transfusion Organization (http://incbb.ibto.ir). This bank processes its UCB samples utilizing the Sepax system. Currently, 4,500 samples, half of which have identified HLA, have been deposited there.

All these banks follow the rules codified by NetCord-FACT International Standards for Cord Blood Collection, Banking, and Release for Administration. Samples are carefully collected and the family history of the donors will be attentively studied to prevent transmission of hereditary disorders. The purity from infectious disease (e.g., HIV, hepatitis) is accurately checked and all other factors which can affect the quality of the cord blood (such as complications during pregnancy, perinatal asphyxia, and lower than normal birth weight) are carefully controlled [20].

Collecting samples from non-relative donors officially started in 2010 when the Iranian Stem Cell Donor Program (ISCDP, http://iscdp.tums.ac.ir/) commenced its activities as a new section in HORCSCT. In this program recruiting, registering, and maintaining the bone marrow and/or hematopoietic stem cells of voluntary relative and non-relative donors are performed without making any charges. By introducing the benefits of stem cell donation, this program encourages individuals to donate their bone marrow or peripheral blood stem cells to matched patients. Up to 2013, more than 40,000 volunteer donors have been enrolled in this program. However, the HLA of 3,000 of them was determined up to now. ISCDP is the only member of the BMDW organization in the Middle East.

5 The Future Landscape of Stem Cell Banking in Iran

Most of the banks were established and/or supported by Iranian Council of Stem Cell Research and Therapy (ICSCRT). The council was established in 2009 under the auspices of the Vice President for Science and Technology with the idea of keeping the country up to date with advances in the stem cell research and cell-based therapy.

One of the most important landscapes in hPSCs and adult stem cell research is their usage in cell therapy. However, problems such as transplantation rejection, risk of teratoma formation, and ethical issues have suspended their possible applications. Although adult stem cells are well known for being safe, their resources in adult tissues are limited and their in vitro propagation is very low. Regarding the nature of these common problems, the future of stem cell research will eventually overcome such obstacles. Establishing a safe hiPSC line bank in good manufacturing practice (GMP) condition with more matched HLA to the Iranian population is the next goal for Iranian stem cell industry.

Among the future programs of stem cell banking in Iran is the derivation of hESCs in GMP condition. Although these cells carry the risks of immune rejection and teratoma formation, they can be applied to immune-privileged regions such as subretinal space [21]. The transplantation of purified cells with a desired phenotype from differentiated hESCs and/or hiPSCs can overcome the risk of teratoma formation. Thus, the inauguration of GMP laboratories and stem cell banks for clinical grade pluripotent stem cell lines is on the way.

Another plan for the future is to establish a human NSCs bank. These cells, whether derived from hESCs or hiPSCs as well as fetal or adult brains, are appropriate candidates for curing diseases such as Parkinson's disease, Huntington's disease, Multiple Sclerosis, and spinal cord injuries [22–24]. After obtaining clinical grade human NSC lines and scaling them up in bioreactors [25], the foundation of this bank will be inevitable.

On the other hand, improving the levels of UCB processing can lead to obtaining more secure sources for cell therapy. Studying UCB samples has shown that HLA of Zoroastrians and Parses can match most Iranians except those in minority ethnic groups. Therefore, lower cord blood units can cover most Pars recipients. Increasing the number of inaugurating hematology-oncology and stem cell transplantation centers in the country is a great step towards obtaining more success in the field of clinical trials and treatments with these cells.

Furthermore, due to the important role of MSCs in regenerative medicine [26], it would be extremely useful to deposit these cells in large numbers. Also providing other appropriate sources such as multipotent dental pulp stem cells [27] or adipose-derived stem cells [28] for these cells and discovering more efficient methods for their maintenance and cryopreservation [29] and scalable expansion of stem cells are among Iran's future plans in the area of therapeutic stem cell banking.

It is important to acknowledge that stem cell field is still in infancy; however the initial studies are promising and this play a key role in supporting the forward movement of Iranian researchers.

Acknowledgment This study was funded by a grant provided from Royan Institute and Iranian Council of Stem Cell Research and Technology.

6 Appendix 1

6.1 RSCB Software

Because of having deposited the multiple cell lines, their variability and different passage numbers, with the help of Royan Institute bioinformatics experts RSCB designed and set up software for organizing this information. Right now this software is being used regularly for storage, retrieval, and sorting reports of the available cell lines in RSCB.

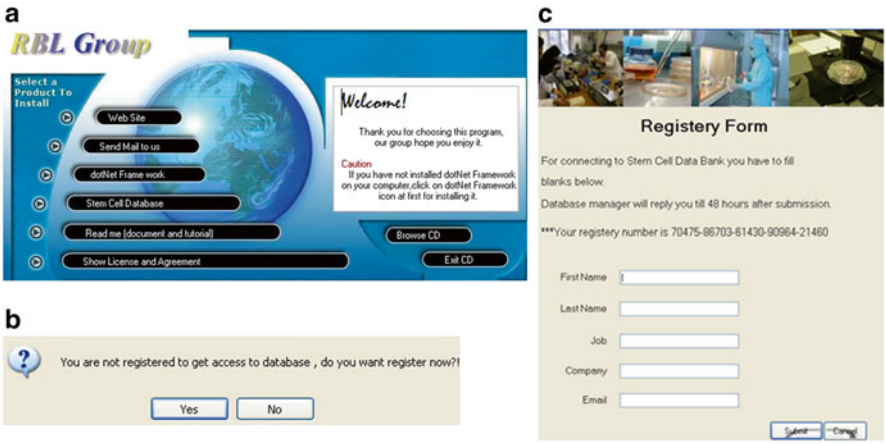


Fig. 12.5 Software installation. (**a**) Installation menu. (**b**) At first installation step this box appears. (**c**) Registry form. In this form, a unique registry number will be shown and asked you to fill your information fields. After filling related form and submitting it, database manager will send you an email that shows manager's decision. If you are permitted to access to the database you will gain a serial number

The system required for RSCB software installation:

- Windows 8, Windows 7, Windows 2000, Windows XP, Windows Vista, or other NT windows.
- Microsoft Office Excel and Microsoft Office Word 2003 or upper.
- Microsoft SQL Server 2000 or upper.
- Microsoft .NET Framework 3.5 or upper.

6.1.1 Software Installation

If the system is compatible with the software, a page will automatically appear (Fig. 12.5a) prompting the user to install the software in the desired location of computer's hardware. After installation and for its first time application, user form requiring personal information must be filled (as an admin, administrator or public user) and sent to the bioinformatics group for receiving an activation code (each user and system will receive the code only once) (Fig. 12.5b, c).

6.1.2 Application of the Software

After software activation, there are three types of connection for different servers (which can be defined) for using the program (Fig. 12.6a):

- *Internet connection* (wherever the Internet is accessible, connection to the software is possible).
- *Local connection* (which is applicable in the interior network of the defined server).
- *This computer*, the computer which is applicable only for the main server.

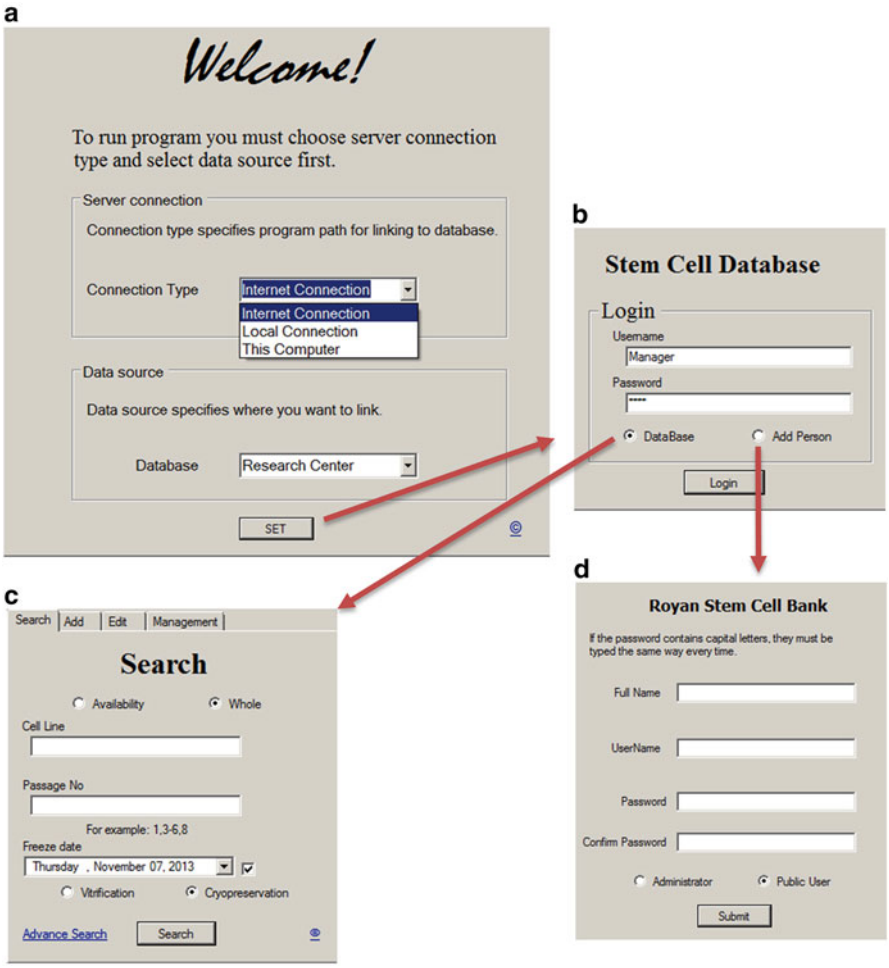


Fig. 12.6 Software entrance. (**a**) Welcome form. You can choose server connection type and database source. (**b**) Login form. In login form there are two radio buttons. The members can enter to the database by choosing "Database" icon or can add others and dedicate special usernames and passwords to new users by choosing "Add person" icon. (**c**) Database menu. Tabs of this menu could be changed based on dedicated user level. (**d**) Add person form. In this section new users are dedicated to access to the database

The next opened page requires user name and password received from the administrator (Fig. 12.6b).

In this step, the user can enter the data center (Fig. 12.6c) or, if the user is a program administrator, he or she can add other people as users (Fig. 12.6d).

After choosing data center, for admin users this page has searching, adding and editing tabs, which will be described later. For public users, only the search tab is active, whereas the management tab is only active for the manager.

6.1.3 Search in RSCB Software

Each user can access the RSCB information according to their needs. For example, choosing the "Availability" option will prompt the user to entering cell line specific code. As a result, the user will be informed about the number of available vials/straws for the particular cell line of interest (straw number is only for samples which have been frozen using vitrification method) (Fig. 12.7a). Furthermore, it is possible to define the minimum number of cryovials and straws. If their available number reduced drastically, the user would be informed by software alarm (Fig. 12.7b).

For more information about the status of a cell line (e.g., establishment date, passage number, and cryopreservation method), the user should choose the "Whole" option. The user can narrow the search results by choosing different options cited on the "Search" page (Fig. 12.8a). On the result page, the user may narrow the results by choosing column option (Fig. 12.8b, c) and save them in an Excel format by choosing the print option (Fig. 12.8d).

By choosing catalog options, the user may access the cell line certificate file, which is in PDF format (the administrator can upload new cell lines certificates or update the previous ones). By choosing the "edit" option the user can view the cell line information and/or edit it. Apart from these methods, the user can use advanced search options (Fig. 12.9).

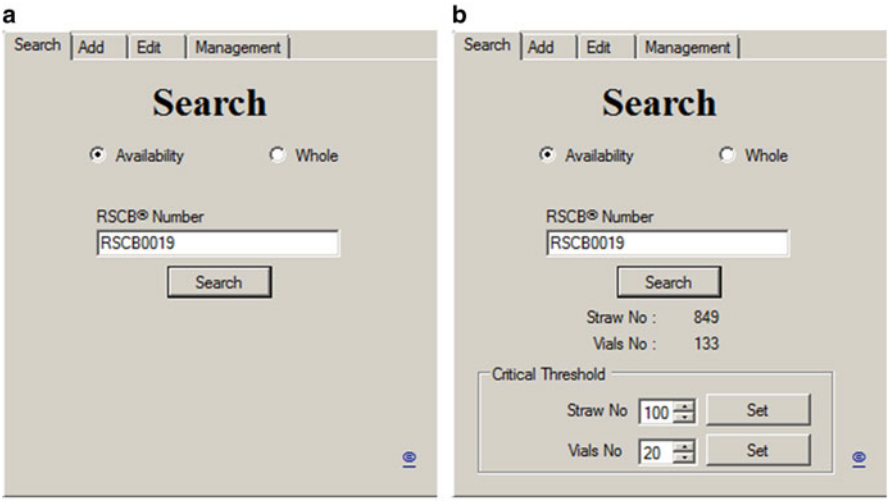


Fig. 12.7 Software searching. Interface of the program can do searching in two procedures: availability or whole search. By choosing availability search mode (**a**) for specific cell lines, result will appear (**b**)

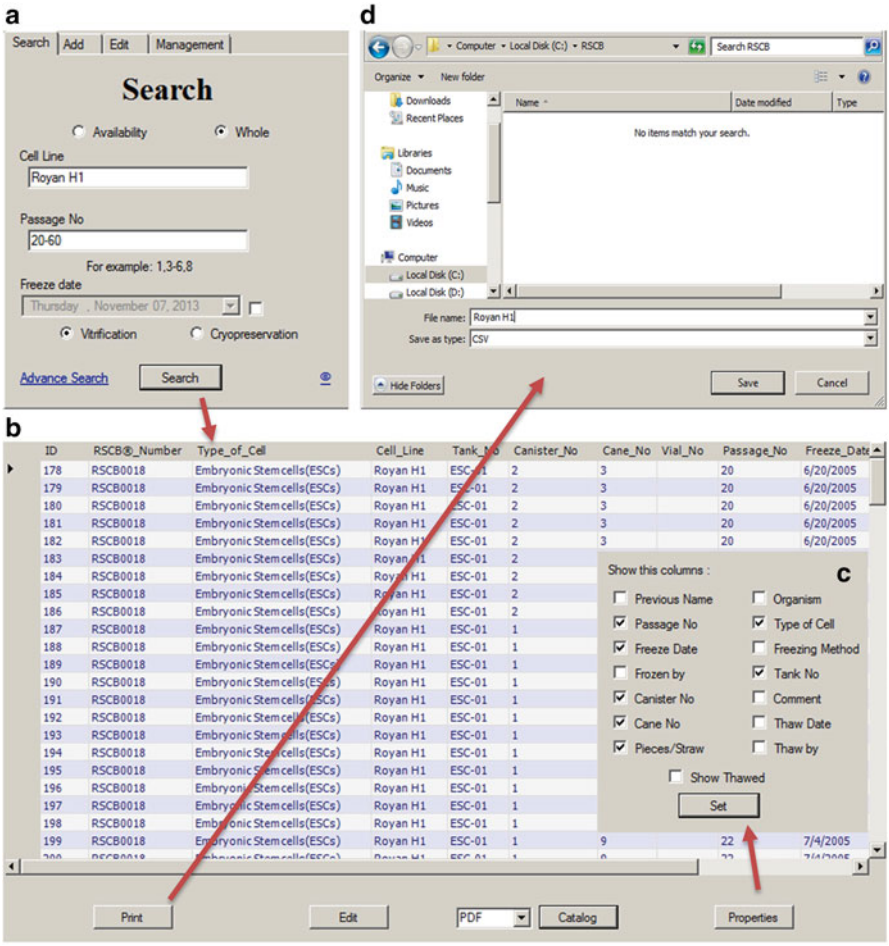


Fig. 12.8 Whole software searching mode. By choosing whole search mode (**a**) several text boxes are shown and also you are asked to enter your cell line, passage number, and freeze date. Based on filled boxes, result will appear (**b**), through the "Properties" button you can limit the options (**c**), using "Print" button you can save the result (**d**)

6.1.4 Adding Cryopreserved Cell Lines Information

To add new information on crypopreservation of cells, the user should first choose the organism type, then choose the applied cryopreservation method, and finally fill in the cryopreservation form and save the data (Fig. 12.10).

6.1.5 Editing Saved Information

The user can edit the data independently by entering each cell line ID and choosing its cryopreservation method (Fig. 12.11).

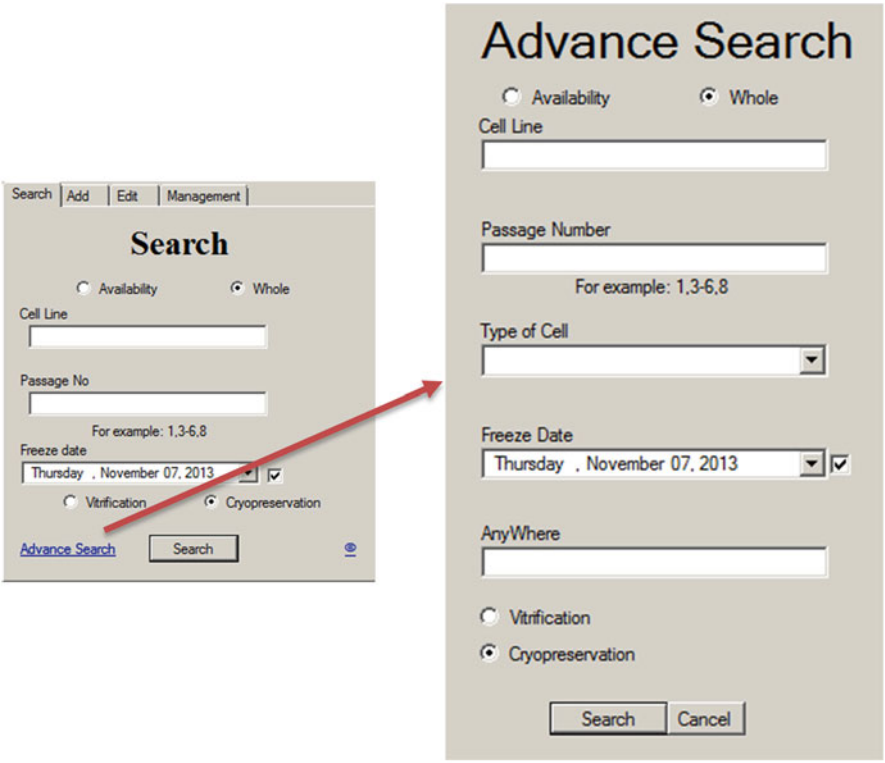


Fig. 12.9 Advanced search option

6.1.6 Overall Management of the Program

The software is managed by an administrator, who has been defined and, among other, has also the following "privileges" (Fig. 12.12):

1. *Witnessing events*: To view users' activity with exact date and time.
2. *Observing user names*: To check the activity or lack of activity of each user.
3. *Entering data*: To enter text data.
4. *Elimination of data*: To delete part of a data or all of it.
5. *Editing data*: To edit saved data.
6. *Recovery*: To take a backup from data or recover them.
7. *Data center configuration*: To change data center names and send messages to users.
8. *Blocking data center*: To block the data center whenever alterations have to be done in order to prevent unpredictable errors.
9. *Changing password*.

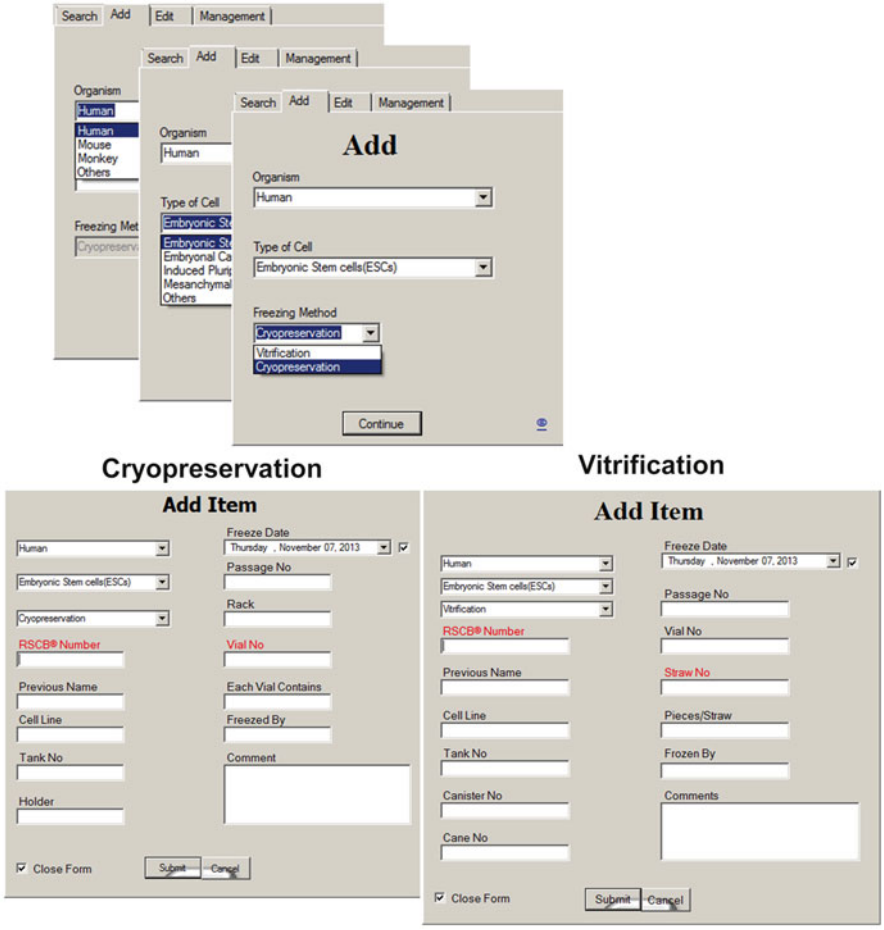


Fig. 12.10 Addition of frozen cell lines information. Based on filled boxes the user lead to "Cryopreservation" or "Vitrification" form to fill out

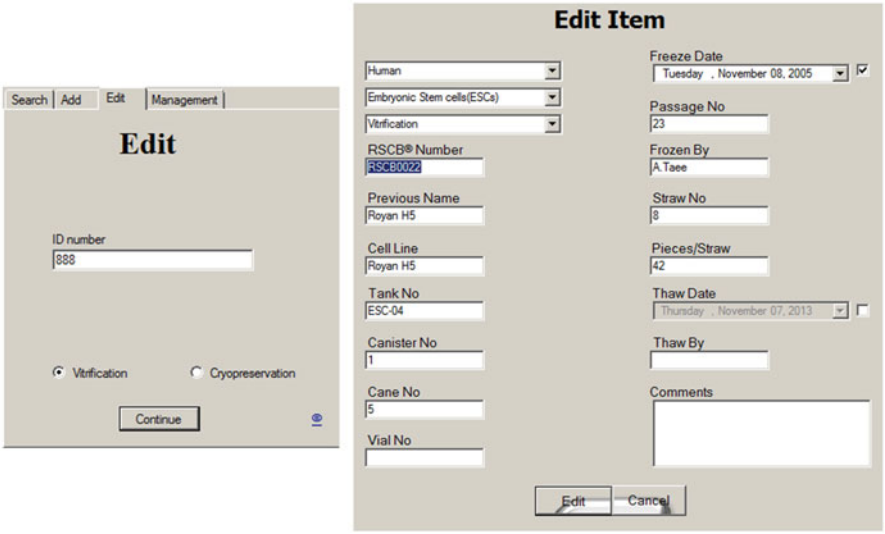


Fig. 12.11 Editing tab. Direct editing option via the ID. Each record according to its freezing method has a unique ID

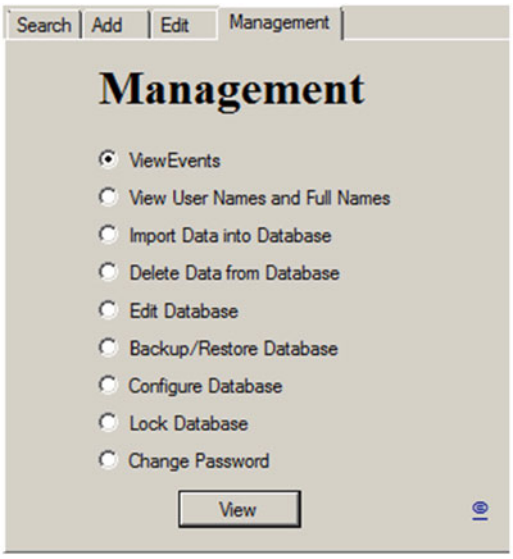


Fig. 12.12 Managing tab. Users with manager privilege have a high control on all data of the database and members who link to the database

References

1. Miremadi T, Salekdeh GH, Aghdami N, Gharanfoli M, Vasei M, Kouhkan A, Baharvand H. Stem cell research and therapy in the Islamic republic of Iran: pioneering in the Islamic world. Stem Cells Dev. 2013;22:51–7.
2. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. Science. 1998;282:1145–7.
3. Isasi RM, Knoppers BM. Governing stem cell banks and registries: emerging issues. Stem Cell Res. 2009;3:96–105.
4. Stacey GN, Masters JR. Cryopreservation and banking of mammalian cell lines. Nat Protoc. 2008;3:1981–9.
5. Baharvand H, Ashtiani SK, Valojerdi MR, Shahverdi A, Taee A, Sabour D. Establishment and in vitro differentiation of a new embryonic stem cell line from human blastocyst. Differentiation. 2004;72:224–9.
6. Totonchi M, Taei A, Seifinejad A, Tabebordbar M, Rassouli H, Farrokhi A, et al. Feeder- and serum-free establishment and expansion of human induced pluripotent stem cells. Int J Dev Biol. 2010;54:877–86.
7. Hassani SN, Totonchi M, Farrokhi A, Taei A, Larijani MR, Gourabi H, Baharvand H. Simultaneous suppression of TGF-beta and ERK signaling contributes to the highly efficient and reproducible generation of mouse embryonic stem cells from previously considered refractory and non-permissive strains. Stem Cell Rev. 2012;8:472–81.
8. Hassani SN, Totonchi M, Sharifi-Zarchi A, Mollamohammadi S, Pakzad M, et al. Inhibition of TGFbeta signaling promotes ground state pluripotency. Stem Cell Rev. 2014;10(1):16–30.
9. Baharvand H, Ashtiani SK, Taee A, Massumi M, Valojerdi MR, Yazdi P, et al. Generation of new human embryonic stem cell lines with diploid and triploid karyotypes. Dev Growth Differ. 2006;48:117–28.
10. Taei A, Gourabi H, Seifinejad A, Totonchi M, Shahbazi E, Valojerdi MR, et al. Derivation of new human embryonic stem cell lines from preimplantation genetic screening and diagnosis-analyzed embryos. In Vitro Cell Dev Biol Anim. 2010;46:395–402.
11. Taei A, Hassani SN, Eftekhari-Yazdi P, Rezazadeh Valojerdi M, Nokhbatolfoghahai M, Masoudi NS, et al. Enhanced generation of human embryonic stem cells from single blastomeres of fair and poor-quality cleavage embryos via inhibition of glycogen synthase kinase beta and Rho-associated kinase signaling. Hum Reprod. 2013;28:2661–71.
12. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006;126:663–76.
13. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007; 131:861–72.
14. Ghodsizadeh A, Taei A, Totonchi M, Seifinejad A, Gourabi H, Pournasr B, et al. Generation of liver disease-specific induced pluripotent stem cells along with efficient differentiation to functional hepatocyte-like cells. Stem Cell Rev. 2010;6:622–32.
15. Seifinejad A, Taei A, Totonchi M, Vazirinasab H, Hassani SN, Aghdami N, et al. Generation of human induced pluripotent stem cells from a Bombay individual: moving towards "universal-donor" red blood cells. Biochem Biophys Res Commun. 2010;391:329–34.
16. Totonchi M, Taei A, Seifinejad A, Tabebordbar M, Rassouli H, Farrokhi A, et al. Feeder- and serum-free establishment and expansion of human induced pluripotent stem cells. Int J Dev Biol. 2010;54:877–86.
17. Bacigalupo A, Hows J, Gluckman E, Nissen C, Marsh J, Van Lint MT, et al. Bone marrow transplantation (BMT) versus immunosuppression for the treatment of severe aplastic anaemia (SAA): a report of the EBMT SAA working party. Br J Haematol. 1988;70:177–82.
18. Bacigalupo A, Hows J, Gordon-Smith EC, Gluckman E, Van Lint MT, Congiu M, et al. Bone marrow transplantation for severe aplastic anemia from donors other than HLA identical siblings: a report of the BMT working party. Bone Marrow Transplant. 1988;3:531–5.

19. Gluckman E, Broxmeyer HA, Auerbach AD, Friedman HS, Douglas GW, Devergie A, et al. Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical-cord blood from an HLA-identical sibling. N Engl J Med. 1989;321:1174–8.
20. Butler MG, Menitove E. Umbilical cord blood banking: an update. J Assist Reprod Genet. 2011;28:669–76.
21. Schwartz SD, Hubschman JP, Heilwell G, Franco-Cardenas V, Pan CK, Ostrick RM, et al. Embryonic stem cell trials for macular degeneration: a preliminary report. Lancet. 2012;379:713–20.
22. Dunnett SB, Rosser AE. Cell transplantation for Huntington's disease should we continue? Brain Res Bull. 2007;72:132–47.
23. Lunn JS, Sakowski SA, Hur J, Feldman EL. Stem cell technology for neurodegenerative diseases. Ann Neurol. 2011;70:353–61.
24. Mothe AJ, Tator CH. Advances in stem cell therapy for spinal cord injury. J Clin Invest. 2012;122:3824–34.
25. Baghbaderani BA, Mukhida K, Sen A, Kallos MS, Hong M, Mendez I, Behie LA. Bioreactor expansion of human neural precursor cells in serum-free media retains neurogenic potential. Biotechnol Bioeng. 2010;105:823–33.
26. Moroni L, Fornasari PM. Human mesenchymal stem cells: a bank perspective on the isolation, characterization and potential of alternative sources for the regeneration of musculoskeletal tissues. J Cell Physiol. 2013;228:680–7.
27. Smith AJ, Smith JG, Shelton RM, Cooper PR. Harnessing the natural regenerative potential of the dental pulp. Dent Clin North Am. 2012;56:589–601.
28. Konno M, Hamabe A, Hasegawa S, Ogawa H, Fukusumi T, Nishikawa S, et al. Adipose-derived mesenchymal stem cells and regenerative medicine. Dev Growth Differ. 2013;55:309–18.
29. Thirumala S, Goebel WS, Woods EJ. Manufacturing and banking of mesenchymal stem cells. Expert Opin Biol Ther. 2013;13:673–91.

Chapter 13
Stem Cell Biobanks and Long-Term Sustainability: A Swiss Working Model

Silvana Bardelli and Tiziano Moccetti

1 Cord Blood Transplants

Stem cell biobanks are established worldwide in order to provide high-quality units and their associated data to transplant centers or for research purposes. Potentially, every type of stem cell can be stored for later use. Our own experience arises from the clinical application of cord blood transplants. The first successful umbilical cord blood (UCB) transplant was performed by the group led by Eliane Gluckman on a patient affected by Fanconi anemia in the 1980s [1, 2]. The very first blood stem cell transplant was actually reported in 1957 by Edward Donnall Thomas, who later received the Nobel Prize for his pioneering research [3, 4]. Since then this alternative source of hematopoietic stem cells (HSCs) has been applied to patients suffering from hematological malignancies, bone marrow failures, and metabolic disorders [5]. The greatest advantage of using UCB for cell therapy lies on the fact that it needs to be matched at four of six human leukocyte antigen (HLA) class I and II molecules. This clearly reduces the incidence of graft-versus-host disease (GVHD) in transplants most likely due to the immunological naïve status of lymphocytes in UCB units and average lower number of T cells. Notably, one of the biggest issues to be faced in UCB transplant is the efficient engraftment in adults. In order to make UCB units more suitable for transplants in adults, ex vivo expansion of HSCs of cord blood samples has been engaged as a resolving approach. Although very challenging, experimental efforts to expand UCB cells led to the production of

S. Bardelli (✉)
Human Cardiac Stem Cell Unit, Cardiocentro Ticino Foundation,
Via Tesserete 48, Lugano 6900, Switzerland
e-mail: silvana.bardelli@cardiocentro.org

T. Moccetti
Human Cardiac Stem Cell Unit and Cardiology Unit, Cardiocentro Ticino Foundation,
Via Tesserete 48, Lugano 6900, Switzerland

D. Ilic (ed.), *Stem Cell Banking*, Stem Cell Biology and Regenerative Medicine,
DOI 10.1007/978-1-4939-0585-0_13, © Springer Science+Business Media New York 2014

hematopoietic progenitor cells that improve short-term engraftment of myeloid cell lineages [6–9]. Nonetheless, long-term engraftment of HSCs remains elusive so far. "Double UCB transplants" (DUCBTs), namely the infusion of two units of UCB in one patient, is being used as an alternative approach to increase cell dose related to patient weight. The two UCB units used for this type of clinical practice have to be at least four of six HLA matched to the patient and each other [10]. This approach has led to remarkable clinical success [11, 12]. Since the first DUCBT in 1999 in Europe on two adults with acute lymphoid and chronic myelogenous leukemia, this procedure has been used in adult patients with hematologic diseases according to Eurocord Registry data. In 2005, Barker et al. published the safety and feasibility data and encouraging results of 23 adults affected by malignant diseases who had received a DUCBT as a strategy for no adequately sized units [13]. Since 2005 this number has surpassed the number of adults receiving single cord blood units mainly for hematologic malignancies. Early post-transplant, a sustained dominance of one unit is apparent from which long-term hematopoiesis is obtained [14, 15]. The advantages of double transplant are manifold: even though the engraftment rate is equal for both single and double UCB transplantation, the latter approach leads to shorter periods of neutropenia due to early engraftment of the mobilized HSCs, lower incidence of GVHD, and lower leukemia relapse for patients in first complete remission [16]. Moreover, the combination of reduced intensity conditioning and double transplant has widened the opportunity of the application of this approach to older or intensively treated patients who share a higher risk of treatment-related mortality [17]. Even though the biology underlying DUCBT is not fully understood, this strategy is very promising in a clinical setting although somehow limited by the cost of manipulating and processing two units. Ongoing randomized clinical trials on DUCBT or comparing double and single UCB transplants in patients affected by hematological malignancies will shed light on their relative role in restoring hematological capacity.

2 Cord Blood Banking

To favor transplantation, repositories of frozen cord blood units have been established throughout the world and hundreds of thousands of units are currently available for transplant [18]. Today, on the one millionth blood stem cell transplant, this quite recent source of stem cells is gaining importance on a clinical perspective [19]. Beyond discussing issues of ethical or commercial considerations on public or private banking, the rationale for storing UCB stem cells is primarily scientific [20–23]. Since organ transplant shortage is a considerable reality, any alternative and qualified source of human tissues is emerging as fundamental [24–27]. Many attempts are being performed to employ the self-renewing capacity of our tissue cells. Accordingly, cord blood repositories have been established to make samples easily and efficiently available for transplantation. Given the need to store these units for later use, many quality issues have been improved over the years

to guarantee their proper application in patients [28–30]. To standardize the methodology and operative procedures the Foundation for the Accreditation of Cellular Therapy (FACT)-NetCord international standards are being issued on a regular basis. FACT-NetCord has significantly increased the regulatory aspects covering all the activities of cord blood banks (CBBs) in recent years. These standards are applied by CBBs pursuing high-quality products to provide an optimal cell source for the safe exchange of cellular products across different countries [31–33]. The establishment and evolution of quality procedures, and the management of banking facilities have been deeply influenced by the clinical outcomes of cord blood transplantations during the past years [34, 35]. The success in cord blood transplantation led to the establishment of the first unrelated CBB in New York in 1991, which gave donor units for the first two unrelated UCB transplants in 1993 [36, 37]. Nowadays, an international network of CBBs throughout the world has been achieved through FACT-NetCord since 1998. From our perspective, being compliant to these quality standards represents a fundamental prerequisite for a qualified banking facility [38, 39].

3 Stem Cell Repositories

In addition to cord blood stem cells, many other stem cell sources have drawn the attention of the scientific community in recent years [40–44]. Considering the advances in science and technology, we have the opportunity of storing virtually every type of stem cell nowadays. Biobanks, intended as comprehensive and well-organized collections of human biological samples and associated clinical and research data, constitute remarkable platforms within the public health system [45, 46]. It is a fact that healthcare systems worldwide are severely challenged by the exponential growth of aging populations, and the widespread incidence of chronic diseases.

The newly developed technologies are improving the diagnosis, prevention, treatment, and management of the disease towards an emerging path to personalized medicine in the immediate future [47]. Biobanks in this scenario provide the raw material for the advance of scientific and medical research, including a real opportunity to improve public health and individual care. The field of biobanking has evolved tremendously over the past 30 years addressing regulatory and serious ethical and legal issues. Biorepositories have actually started as a practical response to investigators' needs of banking specimens for their specific projects, and have further extended including newly derived information obtained from biological sciences advancements, and specifically from the more recent fields of proteomics, genomics, and other "omics" platforms which eventually converge to the idea of personalized medicine. It is easy to recognize a strong parallel link between the advancements in the field of science and the development of biorepositories. Many types of biobanks can be identified: disease-centric, population-based, containing genetic material (DNA/RNA), project-driven, collecting one or more tissue types

(tissue-specific), until virtual biobanks [48]. Most recently virtual biobanks have developed to help investigators locate biospecimens for data mining from multiple biobanks in different locations. These biobanks utilize special software designed to connect biobanks throughout the world. Clearly, virtual biobanks are electronic databases of biological specimens and their related information, regardless of where the actual storage location of the sample is.

One such successful example is the University College of London (UCL) biobanks, which act as physical repositories for collection of biological samples and data from patients who consented the collection at UCL Hospitals (UCLH), Partners hospitals and external resources [49, 50]. Software allows one to view information stored across all collections. Within this merging picture, stem cell biobanks play a key role in the present health system as they can be conceived as representing the basis of alternative solutions to direct transplants. By definition, adult stem cells are normally quite rare in most tissues compared numerically to their differentiated counterparts. As a consequence, specific methods for their isolation have developed and suitable long-term freezing techniques and storage methods currently exist. These techniques, along with international collaborations, shared high-quality standards, and flexibility, are the principal characteristics of the design and management of modern biobanks, which essentially follow the fast growing development of scientific knowledge [46, 51]. The scientific frameworks within which biobanks evolve, newly established stem cell biobanks in particular, fuel their potential and keep the pace in the advancement of new technologies and continued innovation. This in turn improves the quality of the banking facility itself [29, 47].

The establishment of stem cell biobanks is eventually potentiated by the convergence of large-scale genomic studies and next-generation stem cell technologies. As a consequence, future uses of samples and data, operations, design, and utilization of stem cell biobanks cannot be fully anticipated when the infrastructure is first established. Application of next-generation stem cell technologies to existing biobanks will include applications beyond the original intent of the biobank. As per the evolving and revolutionary nature of science, the design of future studies cannot be anticipated at the time samples are collected. All future uses cannot be anticipated or imagined; for this reason bank-specific review committees with a sound background in science possibly including genetics and stem cell research will be vital and in this view integrated professional development is largely needed. Future centers of excellence for biobanking will employ a heterogeneous group made up of cell biologists, bioinformaticians, clinicians, ethics advisors, and dedicated administrative staff. In our own experience, thinking far ahead, i.e., not only of the banking facility itself, is mandatory. Biobanks have to be imagined nowadays as core infrastructures to generate stem cells on a large population scale [52, 53]. The most recent example would be the Japanese bank of HLA-homologous induced pluripotent stem (iPS) cells for regenerative medicine (see Chap. 7 by Saito et al. in this book). To briefly summarize this section, the key relationship is the direct and powerful link of science to biobanks.

4 Clinical Translation—From Theory to Practice

As might be understood from previous statements and a former publication [32] our experience goes beyond the banking activity. It is apparent that the peculiarity of our model goes further. As independent writers not currently directly involved in the activity of the banking facility, we would like to describe our working model, which we would like to present as an effective and valuable source for anyone pursuing the transfer of knowledge and technology to the therapeutic perspective, with the intent to make this move straightforward.

We actually started as a cord blood stem cell bank in summer 2005. The idea of establishing "Swiss Stem Cell Bank," a private CBB in Lugano, simply came out of the opportunity of offering an autologous stem cell repository for cord blood samples in Southern Switzerland as no other similar facilities were to be found at that time. As for the original design, the peculiarity we were offering as a CBB with respect to others in Europe included the fact that every single step of the cord blood unit processing, freezing, and final storage would be performed directly in situ, at our own facility without relocating the process or parts of it. In other words, cord blood units are delivered at our facility, and processed immediately afterwards without shipping them elsewhere. Additionally, our processing laboratory allows us to process the cord blood units, and freeze them by controlled freezing rates, and store them right away in our own temperature-controlled freezers. Notably, this all happens within the same institution. Collection of cord blood samples could be performed at the adjacent hospital or elsewhere and units will be transported by a dedicated courier to our institution, where the final storage is achieved. Given these introductory ideas, a huge initial investment effort was employed to establish the CBB within the structure of a high-standard private hospital "Cardiocentro Ticino," a renowned center of excellence for the treatment of cardiovascular diseases.

With this set-up opportunity and the quite fortunate location close to Italy's border, the banking activity rapidly developed. As was decided when the CBB was founded, the income generated by the banking activity is used to fund our own research laboratory. Thanks to this intercommunication within these two linked modules, research becomes self-funded. As per an equal exchange, the research laboratory works with the purpose of developing new technologies and methodologies to further improve and expand the banking activity. For example, we are on the verge of being able to bank other tissues aside from cord blood stem cells.

After a significant initial investment effort on new equipment and high-standard instrumentation, shortly after the establishment of the banking facility, and according to the requests of a highly motivated and very forward-looking medical direction and administration, a clean room for cell therapy products (CTPs) was eventually built. With this latter module we could close the circle. And proudly, the first clean room for the production of cell-based products in Switzerland was established at our facility. Now, given the infrastructure, highly qualified personnel were hired. People who had acquired international experience in their professional fields were much requested to develop a stimulating background, mostly dedicated to the

backbone of the translational research unit. Since the opening, the pursuit of quality management has been the main target of the structure design. In light of this, accreditation by FACT-NetCord was successfully obtained by the staff of the Swiss Stem Cell Bank in 2012.

Cell manufacturing for clinical application is a form of manufacturing that relies on respecting stringent work practices designed to ensure product consistency and prevent contamination by microorganisms or by another patient's cells. Adhering to international quality standards is critical to minimize the risks associated with cell manufacturing. Current good tissue practice (cGTP) and current good manufacturing practice (cGMP) are general standards that draw a guideline for cell manufacturing facilities. According to this scenario, scientists can now broaden their roles as translational researchers in the manufacturing of cell- and tissue-engineered products for therapeutic use. They also have the opportunity of establishing cell processing laboratories to develop standard operating procedures (SOPs), implement quality management systems, and design cGMP facilities. In our own words, collaboration is fundamental: the immediacy of being located on the site of a top-quality hospital gives the unique opportunity of exchanging views with clinicians and directly receiving samples from the clinical departments into the research laboratory. This situation allows scientists to work more closely with physicians, and vice versa. The research lab represents the ideal place of convergence between new methodologies and sample manipulation with the purpose of transferring this know-how to the clean room manufacturing activity. The translational research laboratory is situated right between hospital and banking facility, and CTP GMP manufacturing. It goes without saying that in our workflow the existence of a clean room accredited by Swissmedic (the Swiss Regulatory Authority for Healthcare) completes the circle. Long-term existence of such a global infrastructure representing high-quality specimens relies on stable funding and highly supportive administration.

5 The Method of Return of Investment

Maintaining a global biobanking activity is expensive and long-term sustainability is one of the major challenges for the existence of biobanks, in the highly dynamic environment of stem cell research and application. For this purpose it is apparent that grant mechanisms with a limited time of funding are clearly not sufficient for its sustainability [38, 46, 54]. On a conceptual basis, preservation of today's biobanks is critical for realizing science and medicine of tomorrow, as it was clearly demonstrated by a distinguished successful biobanking initiative started long ago and still producing valuable results, the Framingham study [55–58]. For this reason, a method of return of investment was implied into our model (Fig. 13.1).

Specifically, when the CBB was established almost a decade ago, it was decided from the very beginning that the income generated by the bank was to fund the activities of the research laboratory. The return of investment obtained by the banking activity is turned directly into funding clinical research activity making research self-funded. The research lab in its background was thought to be translational and

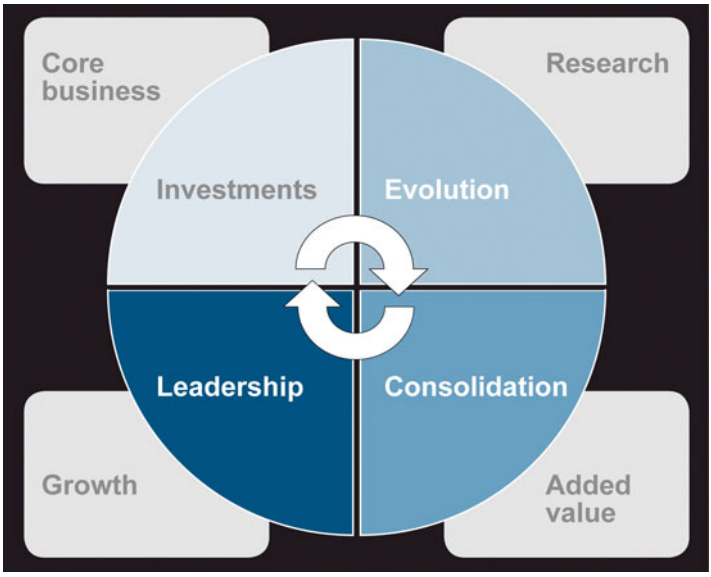


Fig. 13.1 The working model of the Swiss Stem Cell Bank. Core business funds research which creates an added value and a comprehensive growth for the biobanking, and cell therapy facility

not to be focused only on basic research. As a consequence, the research studies undertaken in our laboratories are specifically designed to fuel the clean room facility with new products in order to produce a direct benefit for the patients. The working model of interplay between these functional modules is mutual. It has been applied, for example, to the clean room staff taking care of monitoring the microbiological tests for the quality management of banking facility or the research lab bringing new protocols for tissues to be stored and developing banking methodologies, just to mention a few of the possible interactions. All is focused on clinical application, as was planned from the beginning.

One striking example of the strength of collaborative interaction within our institution was the successful STIM study [59] or the involvement of Cardiocentro Ticino in the Phase I/II multicentric SWISS-AMI clinical trial which included the whole of Switzerland, and evaluated the safety and feasibility of intracoronary injection of bone marrow mononuclear cells in patients affected by acute myocardial infarction in multiple hospitals in the country [60]. Our facility undertook the role of manufacturing site for the study. This innovative approach turned out to be a very successful idea since our laboratories are evolving into the constitution of a much broader institution named the "Swiss Institute for Regenerative Medicine" which will include the banking facility and the new biotech campus further sustained by the Foundation for Cardiological Research and Education (FCRE) (Fig. 13.2). Having described this comprehensive scenario, it is apparent that modern biobanks are linked to the advancements of science and are now recognized as important institutional platforms for samples and data sharing, therapeutic application, and knowledge acquisition.

Fig. 13.2 The Swiss Institute for Regenerative Medicine (SIRM). The new biotech campus will include the banking facility and it will be further sustained by the Foundation for Cardiological Research and Education (FCRE)

6 Conclusions

Stem cell biobanking has the potential to be a very powerful platform for health innovation and knowledge generation. Its main characteristics are flexibility, quality, and international harmonization. Having this banking entwined with other main infrastructures makes the model self-funded, highly and efficiently focused towards clinical application. That was the main idea from which our institution started its activity almost a decade ago. Looking back, the most important choice taken at the beginning was the idea of closing the circle, making translational research self-funded and strongly entwined to banking activities, and therapeutic application.

Conflicts of Interest There are no conflicts of interest.

References

1. Gluckman E, Broxmeyer HA, Auerbach AD, Friedman HS, Douglas GW, et al. Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical-cord blood from an HLA-identical sibling. N Engl J Med. 1989;321:1174–8.
2. Wagner JE, Gluckman E. Umbilical cord blood transplantation: the first 20 years. Semin Hematol. 2010;47:3–12.
3. Thomas ED. A history of haemopoietic cell transplantation. Br J Haematol. 1999;105:330–9.
4. Wagner JE. Umbilical cord transplantation. Leukemia. 1998;12:S30–2.
5. Gluckman E, Ruggeri A, Volt F, Cunha R, Boudjedir K, Rocha V. Milestones in umbilical cord blood transplantation. Br J Haematol. 2011;154:441–7.
6. Macmillan ML, Blazar BR, DeFor TE, Wagner JE. Transplantation of ex-vivo culture-expanded parental haploidentical mesenchymal stem cells to promote engraftment in pediatric recipients of unrelated donor umbilical cord blood: results of a phase I–II clinical trial. Bone Marrow Transplant. 2009;43:447–54.

7. Delaney C, Heimfeld S, Brashem-Stein C, Voorhies H, Manger RL, Bernstein ID. Notch-mediated expansion of human cord blood progenitor cells capable of rapid myeloid reconstitution. Nat Med. 2010;16:232–6.
8. Brunstein CG, Miller JS, Cao Q, McKenna DH, Hippen KL, Curtsinger J, et al. Infusion of ex vivo expanded T regulatory cells in adults transplanted with umbilical cord blood: safety profile and detection kinetics. Blood. 2011;20(117):1061–70.
9. De Lima M, McNiece I, Robinson SN, Munsell M, Eapen M, Horowitz M, et al. Cord-blood engraftment with ex vivo mesenchymal-cell coculture. N Engl J Med. 2012;367:2305–15.
10. Scaradavou A, Brunstein CG, Eapen M, Le-Rademacher J, Barker JN, Chao N, Cutler C, et al. Double unit grafts successfully extend the application of umbilical cord blood transplantation in adults with acute leukemia. Blood. 2013;121:752–8.
11. Brunstein CG, Gutman JA, Weisdorf DJ, Woolfrey AE, Defor TE, Gooley TA, et al. Allogeneic hematopoietic cell transplantation for hematologic malignancy: relative risks and benefits of double umbilical cord blood. Blood. 2010;116:4693–9.
12. Sideri A, Neokleous N, Brunet De La Grange P, Guerton B, Le Bousse Kerdilles MC, Uzan G, et al. An overview of the progress on double umbilical cord blood transplantation. Haematologica. 2011;96:1213–20.
13. Barker JN, Weisdorf DJ, DeFor TE, Blazar BR, McGlave PB, Miller JS, et al. Transplantation of 2 partially HLA-matched umbilical cord blood units to enhance engraftment in adults with hematologic malignancy. Blood. 2005;105:1343–7.
14. Ramirez P, Wagner JE, Defor TE, Blazar BR, Verneris MR, Miller JS, et al. Factors predicting single-unit predominance after double umbilical cord blood transplantation. Bone Marrow Transplant. 2012;47:799–803.
15. Ramirez P, Wagner JE, Defor TE, Eide CR, Miller JS, Weisdorf DJ, Brunstein CG. CXCR4 expression in CD34+ cells and unit predominance after double umbilical cord blood transplantation. Leukemia. 2013;27:1181–3.
16. Verneris MR, Brunstein CG, Barker J, MacMillan ML, DeFor T, McKenna DH, et al. Relapse risk after umbilical cord blood transplantation: enhanced graft-versus-leukemia effect in recipients of 2 units. Blood. 2009;114:4293–9.
17. Majhail NS, Brunstein CG, Shanley R, Sandhu K, McClune B, Oran B, et al. Reduced-intensity hematopoietic cell transplantation in older patients with AML/MDS: umbilical cord blood is a feasible option for patients without HLA-matched sibling donors. Bone Marrow Transplant. 2012;47:494–8.
18. Bart T. Cord blood banking in Europe and worldwide: factors of organization and sustainability. ISBT Sci Ser. 2013;8:109–10.
19. Smith AR, Wagner JE. Alternative haematopoietic stem cell sources for transplantation: place of umbilical cord blood. Br J Haematol. 2009;147:246–61.
20. Harris DT, Schumacher MJ, Rychlik S, Booth A, Acevedo A, Rubinstein P, et al. Collection, separation and cryopreservation of umbilical cord blood for use in transplantation. Bone Marrow Transplant. 1994;13:135–43.
21. Almici C, Carlo-Stella C, Wagner JE, Mangoni L, Garau D, Re A, et al. Clonogenic capacity and ex vivo expansion potential of umbilical cord blood progenitor cells are not impaired by cryopreservation. Bone Marrow Transplant. 1997;19:1079–84.
22. Burke MJ, Vogel RI, Janardan SK, Brunstein C, Smith AR, Miller JS, et al. Early lymphocyte recovery and outcomes after umbilical cord blood transplantation (UCBT) for hematologic malignancies. Biol Blood Marrow Transplant. 2011;17:831–40.
23. Webb S. Banking on cord blood stem cells. Nat Biotechnol. 2013;31:585–8.
24. Cambon-Thomsen A. Assessing the impact of biobanks. Nat Genet. 2003;34:25–6.
25. Harris JR, Burton P, Knoppers BM, Lindpaintner K, Bledsoe M, Brookes AJ, et al. Toward a roadmap in global biobanking for health. Eur J Hum Genet. 2012;20:1105–11.
26. Rao M, Ahrlund-Richter L, Kaufman DS. Concise review: cord blood banking, transplantation and induced pluripotent stem cell: success and opportunities. Stem Cells. 2012;30:55–60.

27. Su RJ, Yang Y, Neises A, Payne KJ, Wang J, Viswanathan K, et al. Few single nucleotide variations in exomes of human cord blood induced pluripotent stem cells. PLoS One. 2013;8:e59908.
28. Knoppers BM, Isasi R. Stem cell banking: between traceability and identifiability. Genome Med. 2010;2:73.
29. Rebulla P, Lecchi L. Towards responsible cord blood banking models. Cell Prolif. 2011;44 Suppl 1:30–4.
30. Petrini C, Farisco M. Informed consent for cord blood donation. A theoretical and empirical study. Blood Transfus. 2011;9:292–300.
31. Brunstein CG, Wagner JE. Umbilical cord blood transplantation and banking. Annu Rev Med. 2006;57:403–17.
32. Bardelli S. Stem cell biobanks. J Cardiovasc Transl Res. 2010;3:128–34.
33. Gluckman E, Ruggeri A, Rocha V, Baudoux E, Boo M, Kurtzberg J, et al. Family-directed umbilical cord blood banking. Haematologica. 2011;96:1700–7.
34. Navarrete C, Contreras M. Cord blood banking: a historical perspective. Br J Haematol. 2009;147:236–45.
35. Arrojo IP, Lamas Mdel C, Verdugo LP, Alfaro PR, Pena RR, Gordo FS, et al. Trends in cord blood banking. Blood Transfus. 2012;10:95–100.
36. Rubinstein P, Taylor PE, Scaradavou A, Adamson JW, Migliaccio G, Emanuel D, Berkowitz RL, et al. Unrelated placental blood for bone marrow reconstitution: organization of the placental blood program. Blood Cells. 1994;20:587–96.
37. Kernan NA, Schroeder ML, Ciavarella D, Preti RA, Rubinstein P, O'Reilly RJ. Umbilical cord blood infusion in a patient for correction of Wiskott-Aldrich syndrome. Blood Cells. 1994;20:245–8.
38. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research. Guidance for industry: current good tissue practice (CGTP) and additional requirements for manufacturers of human cells, tissues, and cellular and tissue-based products (HCT/Ps). 2011.
39. Wall DA. Regulatory issues in cord blood banking and transplantation. Best Pract Res Clin Haematol. 2010;23:171–7.
40. Raval Z, Losordo DW. On the fabric of the human body. Circulation. 2012;126:1812–4.
41. Basik M, Aguilar-Mahecha A, Rousseau C, Diaz Z, Tejpar S, Spatz A, et al. Biopsies: next-generation biospecimens for tailoring therapy. Nat Rev Clin Oncol. 2013;10:437–50.
42. Anversa P, Leri A. Innate regeneration in the aging heart: healing from within. Mayo Clin Proc. 2013;88:871–83.
43. Anversa P, Kajstura J, Rota M, Leri A. Regenerating new heart with stem cells. J Clin Invest. 2013;123:62–70.
44. Di Trapani M, Bassi G, Ricciardi M, Fontana E, Bifari F, Pacelli L, et al. Comparative study of immune regulatory properties of stem cells derived from different tissues. Stem Cells Dev. 2013;22:2990–3002.
45. De Souza YG, Greenspan JS. Biobanking past, present and future: responsibilities and benefits. AIDS. 2013;27:303–12.
46. Kreiner T, Irion S. Whole-genome analysis, stem cell research, and the future of biobanks. Cell Stem Cell. 2013;12:513–6.
47. Terzic A, Waldman SA. Translational medicine: path to personalized and public health. Biomark Med. 2010;4:787–90.
48. Watson PH, Wilson-McManus JE, Barnes RO, Giesz SC, Png A, Hegele RG, et al. Evolutionary concepts in biobanking—the BC BioLibrary. J Transl Med. 2009;7:95.
49. Ollier W, Sprosen T, Peakman T. UK Biobank: from concept to reality. Pharmacogenomics. 2005;6:639–46.
50. Yuille M, Dixon K, Platt A, Pullum S, Lewis D, Hall A, Ollier W. The UK DNA banking network: a "fair access" biobank. Cell Tissue Bank. 2010;11:241–51.
51. Hansson EM, Lindsay ME, Chien KR. Regeneration next: toward heart stem cell therapeutics. Cell Stem Cell. 2009;5:364–77.

52. Kato S, Nishihira H, Hara H, Kato K, Takahashi T, Sato N, et al. Cord blood transplantation and cord blood bank in Japan. Bone Marrow Transplant. 2000;25 Suppl 2:S68–70.
53. Isoyama K, Ohnuma K, Kato K, Takahashi TA, Kai S, Kato S, et al. Japanese Cord Blood Bank Network: cord blood transplantation from unrelated donors: a preliminary report from the Japanese Cord Blood Bank Network. Leuk Lymphoma. 2003;44:429–38.
54. Schofield PN, Eppig J, Huala E, de Angelis MH, Harvey M, Davidson D, et al. Research funding. Sustaining the data and bioresource commons. Science. 2010;330:592–3.
55. Vasan RS. Biomarkers of cardiovascular disease: molecular basis and practical considerations. Circulation. 2006;113:2335–62.
56. Elosua R, Cupples LA, Fox CS, Polak JF, D'Agostino Sr RA, et al. Association between well-characterized lipoprotein-related genetic variants and carotid intimal medial thickness and stenosis: the Framingham Heart Study. Atherosclerosis. 2006;189:222–8.
57. Kabra A, Neri L, Weiner H, Khalil Y, Matsumura ME. Carotid intima-media thickness assessment in refinement of the Framingham risk score: can it predict ST-elevation myocardial infarction? A pilot study. Echocardiography. 13 Aug 2013. [Epub ahead of print].
58. Cheng S, Larson MG, McCabe EL, Osypiuk E, Lehman BT, Stanchev P, et al. Age- and sex-based reference limits and clinical correlates of myocardial strain and synchrony: the Framingham Heart Study. Circ Cardiovasc Imaging. 2013;6:692–9.
59. Moccetti T, Sürder D, Klersy C, Vassalli G, Crljenica C, Rossi MG, et al. Sustained improvement in left ventricular function after bone marrow derived cell therapy in patients with acute ST elevation myocardial infarction. A 5-year follow-up from the stem cell transplantation in ischaemic myocardium study. Swiss Med Wkly. 2012;25:142.
60. Sürder D, Schwitter J, Moccetti T, Astori G, Rufibach K, Plein S, et al. Cell-based therapy for myocardial repair in patients with acute myocardial infarction: rationale and study design of the SWiss multicenter Intracoronary Stem cells Study in Acute Myocardial Infarction (SWISS-AMI). Am Heart J. 2010;160:58–64.

Chapter 14
Establishing a Public Cord Blood Bank and Regulatory Framework in Parallel: A Serbian Example

Dragana Vujic, Emilija Lazic, Zeljko Zecevic, and Svetlana Vekic

1 Introduction

The first proposal for establishing a public cord blood bank (CBB) in Serbia was submitted to the Ministry of Health in 2001. At that time, transplantation, including haematopoietic stem cell transplantation (HSCT), was not considered a priority and the idea was put on hold till July 2007 when the Ministry of Health announced a call for projects that would facilitate further development and strengthening of the Serbian health care system. In response to this call, The Mother and Child Health Care Institute in Belgrade submitted the project "Providing the conditions for HSCT in children in Serbia". The main goal of this project was to enable further improvement of transplantation medicine in the country and make significant savings in the budget of the Health Care Fund through reducing the number of patients sent for treatment in foreign centres. The Institute was the only paediatric institution in the country with an HSCT programme for children and therefore suitable to coordinate and carry out such a project. By 2007, the transplantation team of the Institute was performing only autologous HSCT and allogeneic ones from identical sibling donors. Due to the need for introducing other forms of HSCT (from matched unrelated donors and from haploidentical donors), the project included establishing a public/family CBB.

D. Vujic (✉)
Mother and Child Health Care Institute of Serbia "Dr Vukan Čupic",
Radoja Dakica 6-8, 11070 Belgrade, Serbia

Medical School, University of Belgrade, Dr Subotica 8, 11000 Belgrade, Serbia
e-mail: vujicdbg@gmail.com

E. Lazic • Z. Zecevic
Mother and Child Health Care Institute of Serbia "Dr Vukan Čupic",
Radoja Dakica 6-8, 11070 Belgrade, Serbia

S. Vekic
Bar Association of Serbia, Decanska 13, 11000 Belgrade, Serbia

D. Ilic (ed.), *Stem Cell Banking*, Stem Cell Biology and Regenerative Medicine,
DOI 10.1007/978-1-4939-0585-0_14,

The proposal was accepted by the Ministry of Health in 2007 and marked as a project of national importance. The first funds were allocated in 2008. The project and its realization were expedited by the fact that development of transplantation medicine is one of the conditions for the EU integration of Serbia.

1.1 Writing the Project

In order to set the standard as high as possible and make subsequent accreditation by Foundation for the Accreditation of Cellular Therapy (FACT)-NetCord achievable, once the bank had been completed and made functional, we used the following documents to design the project:

- JACIE Standards for haematopoietic progenitor cell collection, processing and transplantation from the Joint Accreditation Committee of ISCT-EBMT, supported by the European Commission under the Public Health Care Programme 2003–2008 [1].
- Directive 2004/23/EC of the European Parliament and of the Council of 31st March 2004 on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells [2].
- International scientific cooperation in the 6th Framework Programme for Research and Technological Development which was in force from 2002 to 2006 [3].
- Third Edition NetCord-FACT International Standards for Cord Blood Collection, Processing, Testing, Banking, Selection and Release [4].

Experience acquired during the visits paid to the Milan Cord Blood Bank in Italy, and to Anthony Nolan Bank (Nottingham, UK), constituted a significant help in planning space, equipment and personnel (Tables 14.1 and 14.2 and Fig. 14.1).

1.2 Realization

According to the original proposal submitted in 2007, we planned that civil works should be completed within the first year of the project, and that CBB would become functional in next 6–12 months. However, project realization speed is dictated by allocated funds and we quickly realized that our original plan could not work. In order to bring the project to end, we would have to completely change the strategy and adapt to given circumstances.

The Ministry of Heath's financial allocation for the following year is not known before Parliament approves it at the end of the current year. After the amount of monetary allocation becomes known the Ministry of Heath makes budget corrections in the first quarter of every year in compliance with the funds allocated. If funds allocated to our project were not used, we would be unlikely to receive continual funding the next year. Due to the fact that obtaining necessary construction permits

Table 14.1 Gantt chart of establishing a public/family cord blood bank according to the project of 2007

Activities	Months[a] 1	2	3	4	5	6	7	8	9	10	11	12
In the first year of the project realization												
1 Civil works—a public/family cord blood bank	x	x	x	x	x	x	x	x	x	x	x	x
2 Inviting a tender and procuring equipment for the public/family cord blood bank									x	x	x	x
3 Project participant education						x	x	x	x	x	x	x
4 Promotion through media										x	x	x
5 Drawing up of standard operative procedures									x	x	x	x
6 Announcing of public competition and employing the new staff										x	x	x
In the second year of the project realization												
1 Getting an inspection certificate		x	x	x								
2 Inviting a tender and equipping of the public/family cord blood bank (equipment, furniture)	x	x	x									
3 Project participant education	x	x	x	x	x	x	x	x	x	x	x	x
4 Promotion through media	x	x	x	x	x	x	x	x	x	x	x	x
5 Drawing up of standard operative procedures and preparation for accreditation	x	x	x	x	x	x						
6 Accreditation of the public/family cord blood bank												x
7 Including maternity wards in the public/family cord blood bank	x	x	x	x	x	x	x	x	x	x	x	x
8 Getting an inspection certificate for the public/family cord blood bank and bank opening							x					

[a] Designate the month when a certain project activity is planned with x

Table 14.2 Staff, according to the project of 2007

Number of FTEs	Staff profile
1	Physician, paediatrician or internist with a speciality in haematology/oncology or transfusion medicine
2	Molecular/cell biologists
2	Laboratory technicians
2	Nurses
1	Administrator
1	Cleaner

in Serbia is a lengthy and laborious process, in order not to lose the budget allocated in 2008 and 2009, we focused on improvement of the Laboratory for cryobiology: purchasing the equipment and training of the transplantation team members.

It was not until 2010 that the planning of general, technical and main projects for the construction of the public/family CBB was taken up. Overcoming the problems

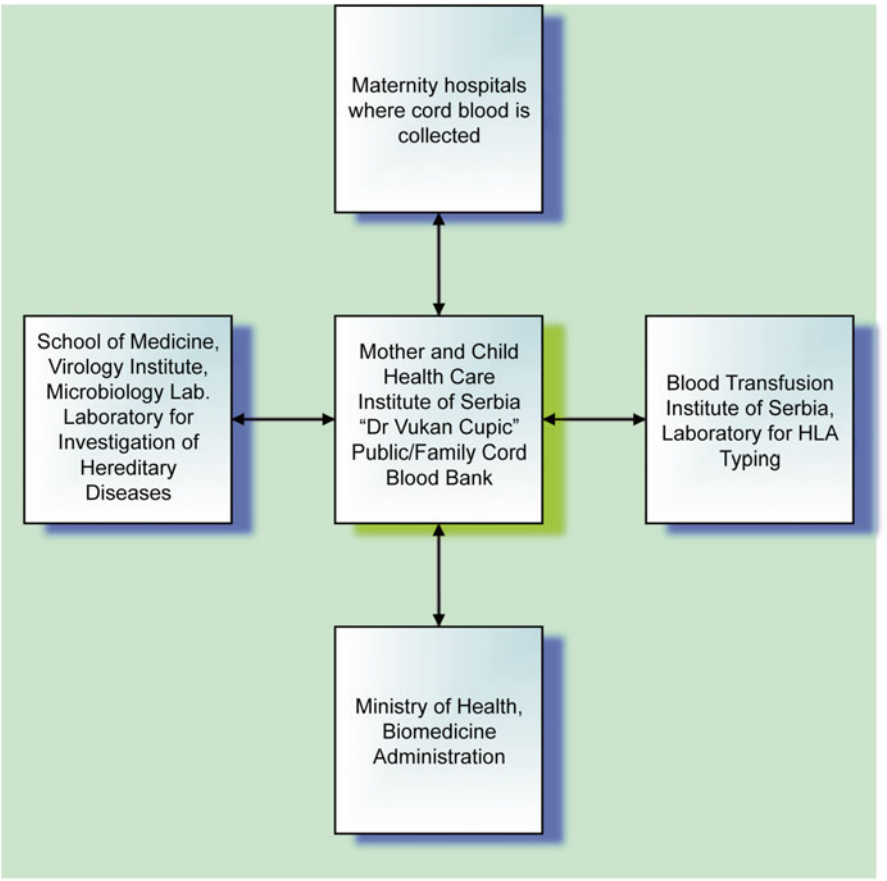


Fig. 14.1 Work organization of the public/family cord blood bank according to the project of 2007 scheme

such as contractor selection, works phasing in compliance with the funds available and preparing the site for the future bank took almost a year, which meant that the construction of the public/family CBB began in April 2013.

2 The History of CBB Establishment in Serbia

2.1 Collection and Storage of Cord Blood Samples

The first sample of sibling cord blood was stored in the Mother and Child Health Care Institute in 2002 in anticipation of further treatment for a boy suffering from high-risk acute lymphoblastic leukaemia. This denotes the starting point in establishing a sibling bank at the Institute, in the Bone Marrow Transplantation Department.

In compliance with the European Society for Blood and Marrow Transplantation (EBMT) recommendations for treatment of children, over the course of 11 years, we collected 51 samples of cord blood for allogeneic application within a family. We were freezing whole CB until 2009, when we bought a Sepax Cell Separation System with CoolMix. Since then volume reduction and separation of mononuclear cells from cord blood on Sepax became a standard procedure in the Institute. So far we have processed 19 samples in this way and stored them in liquid nitrogen.

2.2 Licensing Sibling Cord Blood Banking

From when we started collecting cord blood in 2002 up until the Law on Cell and Tissue Transplantation become effective in 2009 [5], only a written agreement from parents that cord blood could be collected and stored for the needs of the sick child was sufficient. Indications for collecting cord blood were in agreement with the indications of European Blood and Marrow Transplantation (EBMT) Group. After the Law on Cell and Tissue Transplantation became effective, we submitted an application to the Ministry of Health requesting permission to establish a sibling CBB within the Institute. The Ministry of Health granted a licence for establishing sibling CBB to the Institute in 2010 in compliance with Article 67 of the Law on Cell and Tissue Transplantation, with the aims of the project and with the document of the European Group on Ethics in Science and New Technologies to the European Commission, Ethical aspects of umbilical cord blood banking.

After we obtained the licence, we focused on the preparation of standard operating procedures (SOPs) for cord blood processing and regulations on giving opinion concerning the justifiability of leaving cord blood for the needs of treating the sick sibling [6, 7].

3 The Regulatory Landscape in Serbia

The Republic of Serbia's Law on Cell and Tissue Transplantation was approved in September 2009 [5], and has been applied since January 2010. At the time when the project was drawn up and accepted, the only existing legal provision was the Instruction on Cord Blood Taking and Transporting passed by the Minister of Health [8]. Coming into force on September 2008 this law made possible the unhindered work of foreign privately owned CBBs. In 2009, by passing the Law on Cell and Tissue Transplantation, the Minister of Health has enacted the instruction on alterations and amendments to the instruction on cord blood taking and transporting [9]. Adoption of these instructions, as well as Article 67 of the Law on Cell and Tissue Transplantation, made possible the opening and work of the representative offices

of foreign privately owned CBBs. By April 2013, ten representative offices of foreign privately owned CBBs were registered with the licence granted by the Biomedicine Administration of Ministry of Health. Due to the fact that there is still no public CBB in Serbia and that the law makes work of both public and privately owned banks possible, depositing cord blood in privately owned banks is on the increase. Only in 2012 more than 3,000 samples were exported to privately owned banks having seats not only in Europe but in the other continents as well.

3.1 New Regulations

The Republic of Serbia's Law on Cell and Tissue Transplantation defines rules for establishing cell and tissue banks, the issuing of work permits and their renewal, cancellation and supervision, record keeping, cell and tissue banks register, quality system control, appointing a person responsible for quality system control, staff, cell and tissue selection, estimation and obtaining, cell and tissue recipients, processing and storage conditions, designation, documenting, identification and distribution, as well as for the relationship between cell and tissue bank and the third person.

In the course of drawing up the law, the experience of member countries of European Union such as The Netherlands, Slovenia and Croatia was used and the Law was also harmonized with EU directives [2, 10, 11].

3.2 Amendments Still Needed

3.2.1 Consenting

To ensure the best quality and the best use of the products deposited in both public and privately owned banks, regulations should be amended. For example, the current Law on Cell and Tissue Transplantation does not address the problem of cryopreserved sibling cord blood samples that will not be used due to the death of the children whose further treatment they were intended for. A possibility should be provided that parents, when giving consent for a cord blood sample to be taken, also give consent that the sample can be used for the treatment of an unrelated person in cases where material has not been used for treatment of the family member for whom it was collected for and who suffers from a nonhereditary, acquired, disease which, according to existing medical indications, can be treated with HSCT. This type of consent is important due to the fact that the statistical probability of the donor or a member of the same family developing identical type of nonhereditary disease is negligible. When hereditary diseases are concerned, family members would have priority in using samples from the family bank, in compliance with the indications accepted in Europe. EU Directives leave this question to national legislations to regulate.

3.2.2 Mandatory Disposal of Samples That Do Not Meet Required Standards

It is essential that the method of destroying disposed cord blood samples that do not meet the conditions stipulated by Fact-NetCord standards should be regulated by the by-laws that would guarantee observation of legal, ethical and ecological rules and expressed will of the donor and donor guardians, respectively. In compliance with Directives EU 2006/17/EU and 2006/86/EU [10, 11], by-laws, statutes, regulating donor selection criteria, laboratory testing necessary to be carried out before cell storage, donating procedure, cell obtaining and receiving at the bank, accreditation requests, appointing, issuing licenses to the banks and requests for approving cell and tissue preparation procedures in tissue banks still need to be drawn up.

3.2.3 Reporting Adverse Events

The statute on reporting adverse events is to be harmonized with current practice in Europe and the Vigilance and Surveillance of Substances of Human Origin (SOHO V&S) Guidance for Competent Authorities communication and investigation of serious adverse events and reactions associated with human tissues and cells and with cell and tissue coding system and Eurocet 128 [12–14].

3.2.4 Defining Financial Obligations

To apply Italian legal provisions as a model [15, 16], in particular the provision by which the Italian National Health Service bears all expenses associated with cord blood donation, processing, storage and distribution, it is necessary to make an amendment to the Republic of Serbia's Law on Health Care, enabling all costs associated with the work of the national public CBB are covered by the resources of the Republic Health Care Fund. Since 2009, financial resources required for the work of the family CBB at the Mother and Child Health Care Institute have been obtained through the Project "Providing the conditions for HSCT in children in Serbia". How the public CBB will operate financially in the future, once when the project ends, has not been clearly planned.

3.2.5 Enforcing Dissemination of Information

For the time being, information on the significance of establishing public/family banks in Serbia is sporadic and mainly disseminated via internal professional meetings, primarily in paediatrics and haematology, with an occasional small amount of information appearing in scientific publications [17, 18]. In order to keep future Serbian parents better informed on the possibilities of cord blood donation or

depositing with the view to treating a family member, and prevent one-sided information coming from privately owned banks, it would be advisable to make it mandatory to provide future parents with a printed brochure explaining public and sibling banks.

4 Conclusion

Harmonizing legal provisions and by-laws of the Republic of Serbia with European Union regulations and standards in the shortest possible time constitutes the main prerequisite for further development in this field. CBBs, either public or privately owned, provide the best quality product intended for use in human medicine by adhering to legal provisions. Public and privately owned banks must be treated equally in the areas of technical conditions for work, qualification of both medical and other staff working in the banks, work quality and legality control (observation of ethical principles, preventing cell trade abuse) and existence of a national register of donors and recipients containing all the necessary data. It is essential to ensure availability of information based on relevant medical facts. There must be two types of information, one intended for future parents and another for health care staff acting as future educators. Primary health care doctors, who must be appropriately trained, would be acting as educators. Conditions are to be created for public CBB accreditation, enabling incorporation of Serbia into Eurocord and NetCord, as well as adoption of clear and precise provisions on cell import and export and on the control of their implementation.

References

1. JACIE Standards for haematopoietic progenitor cell collection, processing and transplantation from the Joint Accreditation Committee of ISCT-EBMT. 2nd ed. http://www.jacie.org/document-centre.
2. 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. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:102:0048:0058:EN:PDF.
3. The 6th framework programme for research and technological development (2002–2006). http://europa.eu/legislation_summaries/research_innovation/general_framework/i23012_en.htm.
4. 3rd edition FACT-JACIE international standards for cellular therapy product collection. http://www.factwebsite.org/Standards/.
5. Law on cell and tissue transplantation. Official gazette of RS, no. 72. 2009 [in Serbian]. www.slglasnik.com.
6. Ethical aspects of umbilical cord blood banking. The European Group on Ethics in Science and New Technologies (EGE). http://ec.europa.eu/bepa/european-group-ethics/docs/avis19_en.pdf.
7. Apperley J, Carreras E, Gluckman E, Gratwohl A, Masszi T. ESH-EMBT handbook on haemopoietic stem cell transplantation. ESH-EBMT. 2004.

8. Ministry of Health of Republic Serbia. Instructions on cord blood collection and transport, no. 011-00-131/2008-03. 2008. [In Serbian]. http://www.navidiku.rs/firme/upload/images/cryo/InstrukcijaMinistarstvazdravlja.pdf.
9. Ministry of Health of Republic Serbia. Instruction on alterations and amendments to the Instructions on cord blood collection and transport—amendments. No. 011-00-67/2011-06. 2011. [In Serbian]. http://www.navidiku.rs/firme/upload/images/cryo/DopunainstrukcijeMinistarstvazdravlja.pdf.
10. 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. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:038:0040:0052:EN:PDF.
11. 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. http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:294:0032:0050:EN:PDF.
12. Ljungman P, Bregni M, Brune M, Cornelissen J, de Witte T, Dini G, et al. Allogeneic and autologous transplantation for haematological diseases, solid tumours and immune disorders: current practice in Europe 2009. Bone Marrow Transplant. 2010;45:219–34.
13. SOHO V &S guidance for competent authorities: communication and investigation of serious adverse events and reactions associated with human tissues and cells. Vigilance and surveillance of substances of human origin (SOHO V&S). A project founded by the EU Public Health Programme (Project number: 20091110). 2013. http://www.sohovs.org/soho/mod/resource/view.php?id=347.
14. Eurocet 128. http://137.204.230.15/128/.
15. Capone F, Lombardini L, Pupella S, Grazzini G, Costa AN, Migliaccio G. Cord blood stem cell banking: a snapshot of the Italian situation. Transfusion. 2011;5:1985–94.
16. Petrini C. Cord blood banking: regulations, ethics and practice in a disputed Italian case. Clin Ter. 2012;163:e27–8.
17. Petrini C. A comparative analysis of the opinions from European national and international ethics committees regarding the collection, storage and use of umbilical cord blood. Blood Transfus. 2012;10:279–89.
18. Fox NS, Stevens C, Ciubotariu R, Rubinstein P, McCullough LB, Chervenak FA. Umbilical cord blood collection: do patient really understand. J Perinat Med. 2007;35:314–21.

About the Editor

Dr. Dusko Ilic obtained his M.D. degree and B.Sc. in Molecular Biology at the University of Belgrade and his Ph.D. at the Tokyo University, Japan; he completed postdoctoral training at the University of California, San Francisco. Before joining King's College School of Medicine in London, he held positions of Adjunct Associate Professor at the University of California, San Francisco, Consultant at the Veteran Affairs Medical Center, San Francisco, and as Director of R&D at StemLifeLine, a California-licensed stem cell company. Dr. Ilic's current research interests are human embryonic stem (hES) cells, induced pluripotent stem (iPS) cells, and mesenchymal stem cells and their potential in drug discovery and cell-based therapies.

D. Ilic (ed.), *Stem Cell Banking*, Stem Cell Biology and Regenerative Medicine,
DOI 10.1007/978-1-4939-0585-0, © Springer Science+Business Media New York 2014

Index

D. Ilic (ed.), *Stem Cell Banking,* Stem Cell Biology and Regenerative Medicine,
DOI 10.1007/978-1-4939-0585-0, © Springer Science+Business Media New York 2014