

Stem Cell Biology and Regenerative Medicine

Kristina Hug
Göran Hermerén
Editors

Translational Stem Cell Research

Issues Beyond the Debate
on the Moral Status of the Human
Embryo

 Humana Press

Stem Cell Biology and Regenerative Medicine

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Preface

For many years, the discussion of the ethical aspects of human embryonic stem cell research focused on only one question: the moral status of the embryo. It soon became clear that there were three or four different basic positions, the arguments became well-known and were discussed over and over again, and the likelihood that any interesting new arguments would appear decreased over time.

In this book, we want to show that research on human embryonic stem cells, as well as research on stem cells of other kinds, also raise other issues that deserve to be discussed, over and above the issue on the moral status of the embryo, where little progress has been made during the last decade. The various parts of this book identify such issues and discuss ways of dealing with them.

The focus of this book, as indicated by its title, is on translational stem cell research, that is, not in the first place on stem cell research aiming at new, basic knowledge of stem cell biology. Instead, the focus is on ethical, legal, and social aspects of research, which aims at paving the way for clinical applications and translating the results of stem cell research into diagnostic and therapeutic applications.

It has become increasingly clear that different diseases raise different problems and offer challenges which are not identical. The book therefore opens with a part describing the state of the art in stem cell research focusing on a number of specific diseases such as diabetes; neurodegenerative, cardiovascular, and muscular disorders; oncologic and genetic diseases; as well as treating burn victims. How far have we arrived today, and what remains still to be achieved? Important aspects include the severity of the disease, whether alternative treatments exist, and how common the disease is.

The traditional way from bench to bedside involves a number of steps: first research in vitro, then research on small animals, then on large animals, then trials of unproved treatments in emergency situations, and finally small-scale trials – and later (we are not there yet) randomized clinical trials. What do we have to have demonstrated on each of these steps in order to proceed to the next one? Some of these steps raise ethical issues that are discussed in the latter half of the first part of this book. Children, of course, raise special problems since their capacity of giving a free and informed consent is limited. These issues are discussed in Part II of this book.

In the next part, some scientific, regulatory, and ethical challenges to basic research are discussed. Human eggs are required to produce human embryonic stem cell lines, and women can be exploited or put under pressure to deliver eggs. If eggs are collected in the course of IVF treatments, problems of gratitude and psychological pressure cannot be dismissed; so the forms of obtaining informed consent become an important ethical issue. To avoid some of these problems, and diminish the demand for human ova, some scientists have made experiments by using ova from cows or rabbits to create human–animal entities for translational research. This research raises other issues that are discussed in Part III.

Stem cell banks are becoming an increasingly important resource for research. Therapeutic cloning is emerging as a costly and unlikely way to achieve clinical progress on a large scale. Against that background, stem cell banks, repositories of stem cell lines, and registries are likely to become important in the future if and when stem-cell-based therapies exist. These banks raise issues about the procurement of the tissues (information, consent, etc.); about the processing and testing necessary for safety, as well as standardization; and finally about access: who is going to have access to the samples and the information collected, on what conditions, and who is going to decide about this? Such issues are discussed in Part IV of this book.

The long and winding road from bench to bedside, via the first idea, the first experiments, via proof of concept, and proof of principle, contains many steps, requires considerable economic resources, and many things can go wrong. No university institution by itself has the resources required to develop research results into commercially viable products. Collaboration with industry is necessary. Such collaboration is not always unproblematic, as a number of disputes between scientists and industrial sponsors have indicated, and it raises also ethical and strategic issues, which are discussed in Part V of this book.

To scale up and succeed on a competitive market, first rate science and economic resources are required. But in addition to that, also intellectual property rights. Industry is not likely to be interested in investing large amounts of money in a project if there is no protection of intellectual property, and their competitors can use the results of their investments for free. The possibility to patent methods and products based on stem cell research then becomes an important issue. Controversies have surrounded a number of patent applications, particularly involving human embryonic stem cells. Praxis in different parts of the world is not the same, the US Patent and Trademark Office being more liberal than its European counterpart, the European Patent Office.

In Part VI, the legal problems raised by patents on human stem-cell-based inventions are discussed, followed by a discussion of the extent to which there can be technological solutions to a moral dilemma. Finally, in this part, ethical issues raised by stem cell patent applications including and beyond the so-called morality clause in the European Patent Convention are discussed.

Many stakeholders are involved in the future of stem cell research, not just politicians and regulators, doctors, researchers, present and future patients, and their organizations. The stakeholders also include health-care providers, research-funding

organizations, pharmaceutical industry, and taxpayers. A broad and constructive debate on the development of this rapidly developing research area is essential, particularly since recent research results (*Cell Stem Cell*, May 2010) have indicated important differences between human embryonic stem cells and induced pluripotent stem cells, suggesting that one type of cell may not in all contexts be able to replace the other.

Accordingly, communicating results and concerns has become a crucial issue, especially in research involving human embryonic stem cells. Transparency and openness have proved to be successful, and “hype” creates problems. Imaginative ways of communicating research to the general public and creating conditions for a constructive dialogue have been tried successfully and are described in Part VII.

There are a number of psychosocial and cultural factors affecting judgment and decisions about translational stem cell research. Age, gender, and culture are such factors, and it has become increasingly clear that they play a role in decision making. To neglect them would be to give a distorted picture of the complex background and would make it difficult to understand why people’s views can differ so sharply. This is discussed in Part VIII of this book.

One stumbling block on the road from bench to bedside can be the evaluation of stem cell research projects in research ethics committees. Since this research is rather new and rapidly developing, it also presents challenges to the members of the research ethics committees. The systems of research ethics committee examination is not exactly the same, but international guidelines are used as a basis, like the Declaration of Helsinki and the Oviedo Convention and its protocols. The problems and procedures raised by this examination are discussed in Part IX.

In the final part, we take a look at the future of the translational stem cell research and stem-cell-based therapeutic applications. Which ethical issues are then likely to emerge? Risks, long-term effects, priority setting and social justice are such issues discussed in this concluding part.

During many years, both editors were involved in several EU-funded research projects: EuroStemCell, ESTOOLS, NeuroStemCell, Eurostemcell CA, and others. Over the years, we also learned something about the scientific aspects of the stem cell research, and we got to know many of the leading experts in the field. Finally, it is a pleasure to express our thanks to them and to all others who have contributed to this book. We also want to thank the editors at Springer for excellent collaboration in this project.

Lund, 15 May 2010

Kristina Hug
Göran Hermerén

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Editors

Göran Hermerén, Ph.D., is professor emeritus of medical ethics at the faculty of medicine, Lund University, Sweden. His current research interests and publications include priorities and allocation of resources in health care, as well as ethical aspects of genetic testing, care at the end of life, nanotechnologies, and stem cell research. Prof. Hermerén is President of the European Group on Ethics in Science and New Technologies since 2002 and the chair of the advisory board of the German Reference Center for Ethics in the Life Sciences. He is a member of the Swedish National Council on Medical Ethics and has served on many governmental and parliamentary commissions, as well as a referee for international journals. In addition, he has served as external examiner in bioethics at University College, Dublin, as a coordinator of the EU-funded research project “Euro-priorities,” and is a partner in several ongoing EU-funded research projects. From 2011 she will be an external ethics advisor for the European Network “ScreenTox” (Stem Cells for Reliable, Efficient, Extended and Normalized Toxicology).

Kristina Hug, M.A., is a Ph.D. student in the Department of Medical Ethics at Lund University, Sweden. She has studied Human Rights at the Central European University in Budapest and Medical Law at the University of Essex, UK. Her current research interests include biomedical research ethics in general and, more specifically, ethical and legal aspects of stem cell research, research on vulnerable groups, as well as models and functioning of Research Ethics Committees. Kristina teaches courses in Biomedical Research Ethics at Lund University and is a faculty member in Research Ethics Advanced Certificate Program (net-based course coordinated by Vilnius University, Lithuania, Albany Medical College, and the Graduate College of Union University, USA). Kristina’s working experience also includes teaching Health Law at Kaunas Medical University, Lithuania, as well as a position of a specialist in the Lithuanian Bioethics Committee. Since 2004, she has been a partner in several recent and ongoing EU-funded research projects, such as EuroStemCell, ESTOOLS and NeuroStemCell. Since May 2009, she is a member of the Editorial board of the journal “Stem Cell Reviews and Reports”. From 2011 she will be an external ethics advisor for the European Network “ScreenTox” (Stem Cells for Reliable, Efficient, Extended and Normalized Toxicology).

Contributors

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Peter W. Andrews, D.Phil., is the Arthur Jackson Professor of Biomedical Science and co-director of the Centre for Stem Cell Biology in the University of Sheffield. Previously, he was at the Wistar Institute of Anatomy in Philadelphia where his research focused upon the biology of human embryonal carcinoma (EC) cells, the malignant counterpart of human embryonic stem (ES) cells. His current work concerns the mechanisms by which human ES cells choose between self renewal and differentiation, and the nature of ES cell culture adaptation by which they acquire malignant characteristics reminiscent of EC cells.

Harold Ayetey, MB, BChir (MD)., after obtaining his medical and surgical degrees from the University of Cambridge in 2004, went on to pursue postgraduate training in General Internal Medicine in Cambridge, London, and Oxford, during which he developed a subspecialty interest in cardiology and the molecular basis of congenital cardiac arrhythmias in particular. In 2008, Dr Ayetey was appointed Wellcome Trust Clinical Research Fellow at the University of Cambridge and Honorary Specialty Registrar in Cardiology at Addenbrooke's Hospital in Cambridge, giving him the opportunity to combine clinical practice with a longstanding interest in stem cell biology and the concept of pluripotency. Currently, a PhD candidate in Professor Austin Smith's group at the Wellcome Trust Centre for Stem Cell Research in Cambridge, Harold's research focuses on the derivation and use of patient-specific induced pluripotent stem (iPS) cells for the study of congenital cardiac arrhythmias.

Gisela Badura-Lotter, Ph.D., is a biologist and ethicist in the field of biomedical ethics and philosophy of science. She worked as junior scientist at the Chair of Ethics in the Biological Sciences and the International Centre for Ethics in the Sciences and Humanities, both at the University of Tübingen, Germany, where she received her PhD with a dissertation on biological, medical, and ethical aspects of embryonic stem cell research. After a postdoc period at the Faculty of Medicine, at the University of Brest (France), where she worked within the EU-project

“Chimeras and hybrids in comparative European and international research – scientific, ethical, philosophical, and legal aspects,” she is now assistant professor at the Institute of the History, Philosophy and Ethics of Medicine at Ulm University, Germany. She has three beloved children.

Mary Baker, MBE, is patron and immediate past president of the European Parkinson’s Disease Association (EPDA), a position she was elected to in 1992 when the EPDA was first formed. Mary retired as Chief Executive of the Parkinson’s Disease Society of the United Kingdom in 2001 where she had worked for 18 years. Mary is also president of the European Federation of Neurological Associations, vice president of the European Brain Council, consultant to the World Health Organization (WHO) and chair of the Working Group on Parkinson’s Disease formed by the WHO in May 1997. In 2008, the Council of Europe reappointed Mary for a second term as one of the patient representatives to serve on the Management Board of the EMA, and in the same year she was appointed to the IMI JU Scientific Committee. In 2007, Mary was appointed to the Council of the ABPI and she is also a member of the ABPI Code of Practice. Other appointments include director-at-large for the World Stroke Association, former patient editor of the BMJ (now chair of the BMJ Patient Advisory Group). In 2009, Mary received the British Neuroscience Association Award for *Outstanding Contribution to British Neuroscience and for Public Service* and in 2003, an Honorary Doctorate from the University of Surrey was conferred upon her in recognition of work within the world of Parkinson’s disease.

Yann Barrandon, M.D., Ph.D., is joint professor in Stem Cell Dynamics at the Ecole Polytechnique Fédérale de Lausanne (EPFL) and Lausanne University (UNIL), and head of Experimental Surgery at the Lausanne University Hospital (CHUV). He graduated as a dermatologist in Paris and obtained his Ph.D. on the long-term cultivation of human hematopoietic stem cells. He then moved to Stanford Medical School (1982–1983) as a postdoctoral fellow and then to Harvard Medical School University where he trained with Prof. Howard Green, a pioneer in epidermal stem cell biology and cell therapy (1983–1990). During this period, Yann Barrandon participated in the world’s first transplantations of epidermal stem cells on extensive third-degree wounds and contributed several seminal findings, including the demonstration of stem cells in cultures of human keratinocytes. From 1990 to 2001, he was Director of Research at the INSERM and Head of Lab at the Ecole Normale Supérieure, Paris a period during which he demonstrated the presence of multipotent clonogenic stem cells in hair follicles and successfully brought epidermal stem cells from bench to bedside. Following his move to Lausanne in 2002, Yann Barrandon has explored the potency of stem cells of stratified epithelia and showed that oligopotent stem cells are present in the mammalian cornea, challenging previous dogma. He has also contributed to the characterization of several skin diseases and towards gene therapy of dystrophic epidermolysis bullosa. Yann Barrandon’s present research aims (1) at understanding stem cell fate, (2) at manipulating stem cell fate, (3) to translate cell and gene therapy from the bench to bedside. He was a PI in EuroStemCell, Therapeuskin (FP6), and is a partner in several

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Oliver Brüstle, M.D. Ph.D., is professor of Reconstructive Neurobiology at the University of Bonn. He is also co-founder and scientific director of LIFE & BRAIN GmbH, a biomedical enterprise serving as translational hub of the University of Bonn Medical Center. Trained as an M.D., he conducted research and clinical work in neuropathology and neurosurgery at the universities of Zurich and Erlangen, respectively. In 1993 he joined the laboratory of Ron McKay at the National Institutes of Neurological Disorders and Stroke in Bethesda, MD, USA to study neural stem cells. Upon his return to Germany in 1997, he started his own lab and, in 2002, became director of the newly founded Institute of Reconstructive Neurobiology. His field of interest is stem cell research, with a focus on stem-cell-based brain repair. The Brüstle lab has particular expertise in the generation of neural cells from pluripotent stem cells and their application in models of neurological disease. Having been the first researcher working on human embryonic stem cells in Germany, he was instrumental in shaping the public debate around this sensitive topic and became a fierce political advocate of stem cell research. In 2000, Oliver Brüstle received the Bennisen-Foerder Award. Since 2002, he serves as chair of the Steering Committee and board member of the Stem Cell Network North Rhine Westphalia. He is editorial board member and referee for several scientific journals and reviewer for numerous funding agencies. Since 2008, he also serves on the boards of directors of the multinational European research consortia ESTOOLS and NEuroStemCell.

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Neville Cobbe, Ph.D., is currently a research fellow at the University of Liverpool, having previously worked for several years at the University of Edinburgh. His main research interests have been the evolutionary and functional analysis of proteins that contribute to chromosome behavior, cell division, and cell migration. Aside from research publications in genetics and cell biology, Neville has been interested in various aspects of communicating science and its relevance to society, organizing exhibitions for the Edinburgh International Science Festival over successive years and participating in workshops for young people or adults on various bioethical issues (ranging from genetic testing to cloning). This has led to invitations to give public presentations on stem cell research to diverse audiences on behalf of the UK Research Councils, the Scottish Council on Human Bioethics, and the Royal Society of Edinburgh, as well as contributing oral and written evidence to the House of Commons Science and Technology Select Committee.

Giulio Cossu, M.D. Ph.D., Giulio Cossu has a long lasting interest in the field of muscle cell and developmental biology and in the cell therapy of muscular dystrophies. He received his MD degree from the University of Rome in 1997. He trained as a Fogarty postdoctoral at the Wistar Institute, University of Pennsylvania (1980–1983), and then became associate and then full professor at the Dept. of Histology and Medical Embryology of the University of Rome “La Sapienza.” In 2000 he was appointed director of the “Stem Cell Research Institute” of the Hospital San Raffaele in Milan. Since 2005 he is professor of histology and embryology at the University of Milan. In 2008, he was appointed director of the newly created San Raffaele Division of Regenerative Medicine. Since 1997 Giulio Cossu is EMBO Member; he has been president of the Italian Association of Cell and Developmental Biology (1998–2001) and member of the Directory Board of the International Society for Stem Cell Research (2003–2005). Giulio Cossu is currently a member of the Directory Board of the International Society for Differentiation and senior editor of EMBO Molecular Medicine. He is currently serving as chairperson for Panel LS7 (Molecular Medicine) for the European Research Council. He is also member of the ISSCR Task force for Clinical Translation of Stem Cell Research.

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Petr Dvořák, Ph.D., is professor of molecular biology and genetics at Masaryk University, Brno, Czech Republic. He is the head of Department of Biology, one of the key research departments of Faculty of Medicine where he also serves as the vice dean for research. He has been involved in the Czech dialogue on embryonic stem cell policy since 2003 when his group derived several lines of human embryonic stem cells. Petr Dvořák is interested in growth factor signaling in human embryonic cells, as well as induced pluripotent stem cells and several specific topics related to their differentiation, genomic stability, and use for drug development. He has published many research articles and reviews in the biology of embryonic stem cells and has worked on several national and international projects focused on development of tools for medical application of stem cell research and contextual regulatory issues.

Melissa L. Finucane, Ph.D., is a senior fellow at the East-West Center in Honolulu, Hawaii. Dr Finucane conducts empirical research to clarify the mechanisms underlying human judgment and decision processes and their implications for public policy making. Her work focuses on the interplay of affect and cognition and the role of sociocultural factors in judgment and decision processes under conditions of uncertainty. Dr. Finucane received the Australian Skeptics Eureka Prize for Critical Thinking in 1999 and has received funding for her research from the National Science Foundation, the National Institutes of Health, and other organizations. She has published in numerous peer-reviewed journals, including *Journal of Behavioral Decision Making*, *Risk Analysis*, and *Social Science and Medicine*. Dr. Finucane is a member of the Society for Judgment and Decision Making.

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Nicolas Grasset, M.D., Ph.D., graduated from Lausanne Medical School in 1999 and was a surgical resident before joining the Barrandon's laboratory in 2003. He obtained his PhD in Life Sciences and Technology at the Ecole Polytechnique

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Manal Hadenfeld, Ph.D., is a biologist, currently working at LIFE&BRAIN GmbH, which is a technology transfer platform for the University of Bonn in the field of Biomedicine. At LIFE&BRAIN, she is perusing different projects on industrial applications of stem cells as well as public outreach projects related to stem cell technologies. Earlier, she worked and published as a scientist in the fields of stem cell engineering and protein biochemistry at the Universities of Bonn and Cologne, Germany.

Mattias Hansson, Ph.D., is head of the department of Stem Cell Biology at the Hagedorn Research Institute, which is an integrated R&D component of the global diabetes healthcare company Novo Nordisk. His research is focused on the development of cell replacement therapy for the treatment of diabetes mellitus with particular interest in translational stem cell research. Dr. Hansson has a M.Sc. in chemical engineering from Lund University, Sweden, and a Ph.D. from the University of Copenhagen, Denmark, where he was a Marie Curie fellow.

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Part I:
Translational Stem Cell Research:
What is Possible Today and What Still
Remains to be Achieved?

Chapter 1

Towards Clinical Application of Stem Cells in Neurodegenerative Disorders

Olle Lindvall and Zaal Kokaia

Abstract Stem cells have the capacity to generate neurons and glia cells which are lost in neurodegenerative diseases such as Parkinson's disease and stroke. The adult brain's own neural stem cells are potential novel therapeutic targets because they produce neurons and glia in response to injury and could become affected by the degenerative process. Besides cell replacement, stem cell-based approaches can also improve function by modulating inflammation, preventing neurons from dying, and increasing angiogenesis. These exciting laboratory findings should now be responsibly translated to the clinic. However, the development of stem cell-based therapies for human neurodegenerative diseases will require major research efforts so that the mechanisms regulating the proliferation, migration, differentiation, survival and function of stem cells are much better understood and can be effectively controlled. Strategies to prevent tumor formation must be developed. Finally, the functional efficacy of stem cells or their derivatives, their mechanisms of action, and absence of significant adverse effects should be demonstrated in animal models with pathology and symptomatology resembling the human disease.

Keywords Parkinson's disease • Alzheimer's disease • Stroke • Neurogenesis • Transplantation

1.1 Introduction

Neurodegenerative diseases comprise a wide range of human conditions in which neurons and glial cells in the brain and spinal cord are lost. In acute neurodegenerative diseases, different types of neurons and glial cells die within a restricted brain area

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over a short period of time, e.g., in response to ischemic stroke or spinal cord injury. In chronic neurodegenerative diseases, there is over several years either a rather selective loss of a specific cell population, such as dopamine (DA) neurons in Parkinson's disease (PD) or motor neurons in amyotrophic lateral sclerosis (ALS), or a widespread degeneration of many neuron types, such as in Alzheimer's disease (AD). Stem cells may preserve and restore function in several different ways, e.g., by releasing trophic molecules or modulating inflammation. However, the most attractive possibility would be if they could be used to replace the cells that have died. This could be achieved either by transplantation of stem cell-derived cells predifferentiated in vitro to various stages of maturation, e.g., to neuroblasts, or by stimulation of the formation of neurons and glia from the adult brain's own neural stem cells (NSCs). No stem cell-based treatment has yet been established for any neurodegenerative disorder. Despite this, "treatments" for several of these diseases are offered at "clinics" around the world, without rationale and with poor scientific and clinical basis. The vast majority of these sites over-promised the results and gravely underestimated the potential risks [1]. "Stem cell tourism" is a major problem in the field of neurodegenerative diseases that damages patients and their relatives and slows down the serious development of effective stem cell-based therapies [2]. For the responsible application of stem cell therapies in patients with neurodegenerative diseases, it is now important to define clinical road maps, i.e., the major milestones in basic and clinical research that need to be reached, and the ethical, regulatory, societal and economical issues that need to be addressed. In this chapter, we will discuss some general issues related to the clinical translation of stem cells. We will also describe the current status of stem cell-based approaches and define the critical milestones that remain to reach the clinic in three neurodegenerative disorders – PD, AD, and stroke.

1.2 Moving Stem Cells to the Clinic in Neurodegenerative Disorders: General Aspects

When considering the application of stem cells in patients with a specific neurodegenerative disorder, it must be understood what the acceptable risks are and what will be necessary for this approach to be clinically competitive. Stem cells and their derivatives represent, in most cases, entirely novel products. Proliferation and maturation of stem cells are difficult to control. Animal models may not fully predict their toxicity, occurrence of immune and other biologic responses, and risk for tumor formation after implantation in patients. It should also be underscored that the degree of disability and available therapeutic options differ widely between various neurodegenerative diseases. Patients with PD have virtually normal life expectancy, motor symptoms are effectively reversed during the first years by several drugs, and valuable improvement can be obtained in advanced stages as well. In contrast, for patients with ALS, which is a fatal disorder, there is currently no effective treatment. If efficacious therapy already exists, as in PD, the risk must be low and the stem cell-based approach must offer an advantage (e.g., better functional outcome and single procedure versus life-long drug therapy with associated side effects). If effica-

cious therapy is lacking, the severity of a disease like ALS might justify the risks of a stem cell-based experimental intervention in patients. However, no stem cell-based approach can be justified in diseases simply because there is no alternative effective therapy if that new experimental treatment has no proven efficacy in preclinical animal models, nor any evidence to show a mechanism of action.

The stem cell-derived cells which are going to replace those cells that have died differ between different diseases based on their specific pathology. In PD and ALS, these cells would have to exhibit the properties of DA and motor neurons, respectively, whereas in stroke and AD, several cell types would be needed for efficacy. However, also the transplanted stem cells may be affected by the disease process similar to what has been observed in grafts of embryonic mesencephalic tissue more than a decade after intrastriatal implantation in PD patients [3, 4]. The risk seems to be higher when patient-specific cells are produced by therapeutic cloning or induced pluripotent stem (iPS) cell technology, possibly leading to increased susceptibility to the disease process.

Not any type or magnitude of functional improvement in animal models is sufficient to justify clinical application. The stem cell-based approach must have been shown to induce substantial recovery of functional deficits that resemble the patients' symptoms. However, the behavior of stem cells and their derivatives after transplantation in animal models may only partly reflect how these cells will behave in patients. The animal model may not mimic all aspects of the pathology of the human condition, which can explain lack of efficacy in the clinical trial of the stem cell-derived product.

Finally, the biological mechanism underlying the observed functional improvements following a stem cell-based treatment must be determined in an animal model. Stem cells can lead to recovery, which could be clinically valuable, through several different mechanisms, such as cell replacement, trophic support, modulation of inflammation, stimulation of angiogenesis, and neuroprotection. Any stem cell-based approach for a neurodegenerative disorder must explicitly be proven to work through one or more of these mechanisms before clinical application.

1.3 Stem Cell Therapy for PD

In PD, deficits in the control of movement are caused by degeneration of the dopaminergic nigrostriatal pathway (Fig. 1.1), but other dopaminergic and nondopaminergic systems are also affected. The cardinal symptoms in PD are rigidity, hypokinesia, tremor, and postural instability. Whereas motor symptoms can be treated rather well with L-dopa, DA agonists, enzyme inhibitors, and deep brain stimulation, effective therapies for non-motor symptoms, such as dementia, are lacking, and it is currently not possible to counteract the progression of the disease. Using intrastriatal transplantation of human embryonic mesencephalic tissue, which contains large numbers of immature DA neurons, it has been shown that neuronal replacement can work in PD patients (Fig. 1.1) [5]. The implanted DA neurons can reinnervate and normalize DA release in the denervated striatum, and give rise to

major improvement of motor symptoms in some patients. A small fraction of the grafted DA neurons contain Lewy bodies (the hallmark of PD) more than 10 years after transplantation [3, 4], suggesting that they can become affected by the disease. However, cell replacement remains a viable therapeutic option for PD because the progression of the pathological changes from the patient's brain to the grafted cell grafts is slow and the DA neurons are still functional after a decade [6].

The further development of a clinically useful cell replacement therapy for PD requires other sources of DA neurons. The availability of human embryonic mesencephalic tissue is limited, and since tissue from several donors is needed for each patient, transplantation can only be performed in very few cases. Moreover, the variability of the functional outcome after transplantation is high. Some patients have improved to the extent that L-dopa could be withdrawn for several years, whereas others have exhibited modest clinical benefit, if any. Poor standardization of the cell material probably plays a major role for the high variability, but this problem, as well as the lack of cells for transplantation, could be solved if large numbers of standardized and quality-controlled DA neuroblasts were generated from stem cells. Observations in animal experiments and clinical trials have indicated that the transplanted stem cell-derived DA neurons (Fig. 1.1) must exhibit the properties of *substantia nigra* neurons, i.e., those neurons that have died in the

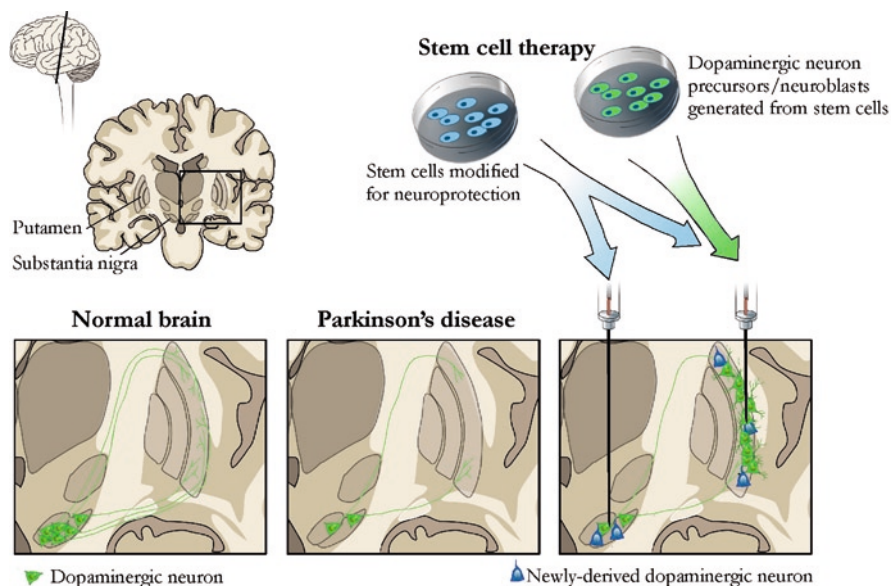


Fig. 1.1 Stem cell-based therapies for PD. PD leads to the progressive death of DA neurons in the *substantia nigra* and decreased DA innervation of the putamen. Stem cell therapy could be used to prevent progression of disease in two ways – first, by implanting stem cells modified to release growth factors, which would protect existing neurons and/or neurons derived from other stem cells treatments; and second, by transplanting stem cell-derived DA neuron precursors/neuroblasts into the putamen where they would generate new neurons to ameliorate disease-induced motor impairments

patient's brain, in order to induce substantial, clinically useful improvement in PD. Cells with properties of DA neurons have been generated *in vitro* from stem cells of several different sources and species, e.g., ES cells, NSCs from embryonic ventral mesencephalon, adult NSCs from the subventricular zone, bone marrow stem cells, and fibroblast-derived iPS cells (for references see, e.g., [7]). In a clinical setting, the DA neurons that are implanted have to be of human origin. It is well established that human stem cell-derived DA neurons can survive in animal models of PD and after maturation exert functional effects. However, some properties that are fundamental for successful clinical translation have not yet been demonstrated for stem cell-derived DA neurons (for references see, e.g., [7]). For example, it has not been shown that they can substantially reinnervate striatum, restore DA release *in vivo*, and markedly improve deficits resembling the symptoms experienced by patients with PD. Experimental work establishing these properties remains to be performed before a human stem cell-derived DA neuroblast can be selected as a candidate cell for patient application. Patient-specific DA neuroblasts can now be made from iPS cells [8, 9]. If such cells are used for transplantation, the ethical concerns associated with ES cells are eliminated and, as with DA neurons derived through therapeutic cloning [10], immune reactions would be avoided. It remains to be demonstrated, though, that the DA neurons generated from the PD patient's iPS cells do not exhibit deficits in growth capacity and functional properties. The patient's gene profile may also make the patient-specific DA cells particularly susceptible to PD pathology after transplantation. A major concern when transplanting ES or iPS cell-derived DA neuroblasts is the risk for tumor formation, which has been observed in animal models [11]. With normal life expectancy, even a minor risk of tumor formation is unacceptable in PD patients. Therefore, before application of stem cell-derived cells can be considered in patients with PD, the tumor risk has to be eliminated. Engineering stem cells with regulatable suicide genes or use of cell sorting to eliminate tumor-forming cells are currently being considered to improve safety. Because PD patients already have several therapeutic options, a stem cell-based DA cell replacement therapy has to induce a major reduction of motor symptoms without significant side effects if it should be clinically competitive. This could be achieved if patients are carefully selected and the dose and site of implantation of the DA cells are determined based on preoperative imaging using, for example, positron emission tomography of the patient's DA system. Troublesome involuntary movements, so-called off-medication dyskinesias, which have been observed in a subgroup of PD patients receiving embryonic mesencephalic grafts (for references see, e.g., [7]), have to be avoided following transplantation of stem cell-derived neurons. Recent experimental and clinical data indicate that off-medication dyskinesias could be prevented by minimizing the number of serotonergic neuroblasts in the transplant material [12], and by distributing the DA neuroblasts evenly over the putamen. Finally, it must be remembered that PD is a multisystem disorder and that symptoms that are caused by pathology in non-dopaminergic systems will not be improved by DA grafts placed in the striatum. Also, in most cases, DA cell replacement has to be combined with a neuroprotective therapy to hinder disease progression if the functional recovery after transplantation

should be long-lasting. One possible strategy to counteract the degeneration of DA neurons could be to deliver a trophic factor into the striatum and substantia nigra, either by direct injection of the gene coupled to a viral vector or by transplantation of stem cells genetically modified to secrete the trophic factor (Fig. 1.1).

1.4 Stem Cell Therapy for AD

In AD, patients develop memory impairment, cognitive decline, and dementia due to widespread and progressive pathological changes (Fig. 1.2). Many areas in the patients' brains, such as the basal forebrain cholinergic system, amygdala, hippocampus, and cerebral cortex, exhibit neuronal and synaptic loss, neurofibrillary tangles, and deposits of β -amyloid protein in senile plaques. Although some degree of symptomatic improvement can be obtained in AD patients following administration of drugs, effective treatment or prevention of the disease remains elusive.

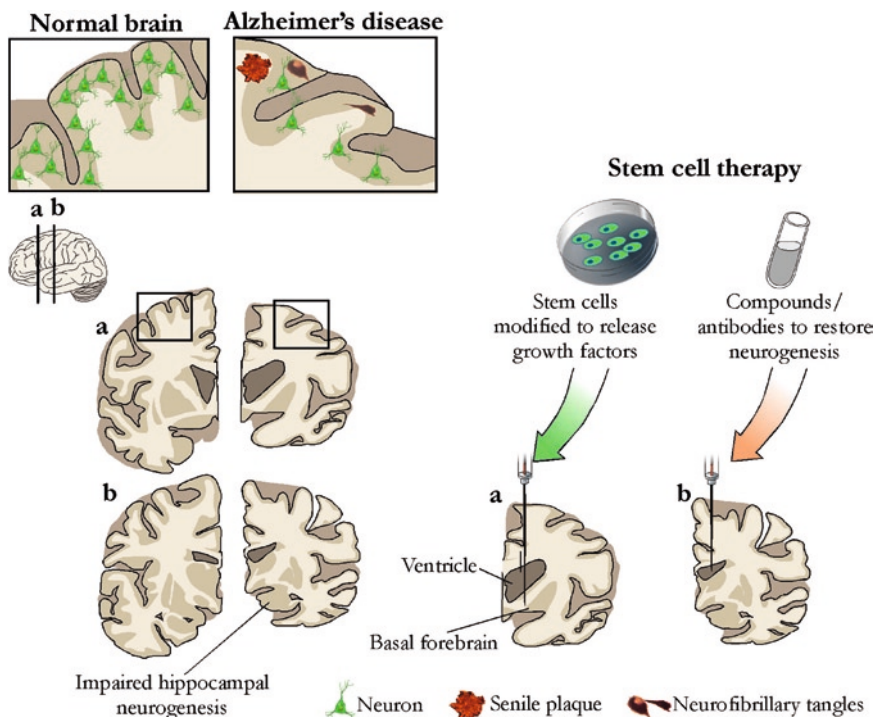


Fig. 1.2 Stem cell-based therapies for AD. AD leads to neuronal loss in the basal forebrain cholinergic system, amygdala, hippocampus, and cortical areas of the brain; formation of neurofibrillary tangles; and β -amyloid protein accumulation in senile plaques. Stem cell therapy could be used to prevent progression of the disease by transplanting stem cells modified to release growth factors. Alternatively, compounds and/or antibodies could be infused to restore impaired hippocampal neurogenesis

The widespread distribution of the pathological changes creates an extremely complex situation for a neuronal replacement strategy aiming at functional restoration in AD. In order for such an approach to be successful in AD, the stem cells would have to be pre-differentiated to many different types of neuroblasts in culture prior to subsequent implantation in a large number of brain areas. The cognitive symptoms could hypothetically be improved by transplantation of basal forebrain cholinergic neurons because acetylcholinesterase inhibitors, which enhance cholinergic function, induce some temporary improvement in AD patients. Cholinergic neurons can be generated from stem cells [13]. However, the host neurons that the new cholinergic neurons should act on are probably damaged in AD, and it is therefore inconceivable that transplantation of stem cell-derived cholinergic neurons would induce long-lasting symptomatic benefit. Stem cell-based cell replacement strategies are very far from clinical application in AD.

The formation of new hippocampal neurons from the adult brain's own NSCs located in the dentate gyrus contributes to mood regulation, learning and memory [14] (Fig. 1.2). It has therefore been proposed that the cognitive symptoms in AD might be partly caused by impairment of hippocampal neurogenesis, but whether this is indeed the case is not known. Transgenic mouse models of AD and analyses of brains from AD patients have provided evidence of deficits in neurogenesis and maturation of the new neurons [15–20]. Thus, there is a rationale for the development of stem cell therapies that aim at enhancing hippocampal neurogenesis and/or improving maturation of the new neurons. In support of such an approach, active β -amyloid vaccination in young AD mice decreased β -amyloid burden and increased hippocampal neurogenesis [21]. Moreover, passive β -amyloid immunotherapy with an antibody specific for aggregated β -amyloid restored neurogenesis and morphological maturation of new hippocampal neurons in aged transgenic mice with β -amyloid-related impairments of neurogenesis [15].

Transplantation of genetically modified stem cells could be used to deliver factors modifying the course of AD. An advantage with stem cells is their capacity to migrate and reach large areas of the brain. In support of this strategy, basal forebrain grafts of fibroblasts producing NGF, which counteracts cholinergic neuronal death, stimulates cell function and improves memory in animal models, have given some benefits in AD patients in an open-label trial [22]. Stem cells could also carry other genes such as BDNF, which has substantial neuroprotective effects in AD models [23].

1.5 Stem Cell-Based Therapies for Stroke

Following an ischemic stroke, caused by occlusion of a cerebral artery, there is focal tissue loss and death of multiple neuron types as well as oligodendrocytes, astrocytes, and endothelial cells (Fig. 1.3). Apart from thrombolysis during the first hours after the injury, which is available only for a minority of cases, there is no effective treatment to promote recovery. Most patients exhibit varying degrees of permanent disability with motor, sensory, or cognitive impairments.

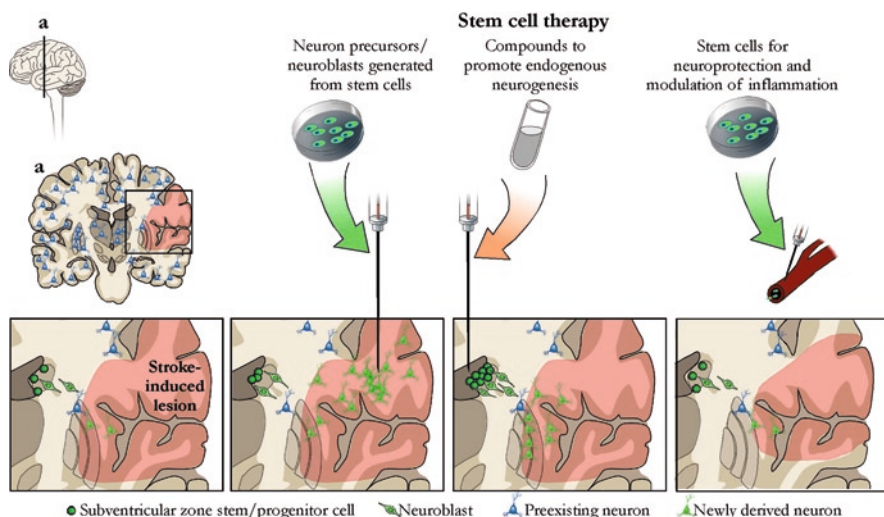


Fig. 1.3 Stem cell-based therapies for stroke. Ischemic stroke leads to death of multiple neuronal types and astrocytes, oligodendrocytes, and endothelial cells in the cortex and subcortical regions. Stem cell therapy could be used to restore damaged neural circuitry by transplanting stem cell-derived neuron precursors/neuroblasts. Also, compounds could be infused that would promote neurogenesis from endogenous subventricular zone progenitors or stem cells could be injected systemically for neuroprotection and modulation of inflammation

Three different stem cell-based approaches are currently being explored for promoting functional recovery after stroke. In the first one, neuron precursors or neuroblasts generated from stem cells are transplanted into the brain (Fig. 1.3). In the second one, compounds that promote the formation of new neurons from the adult brain's NSCs are delivered either into the brain or systemically. In the third one, stem cells are administered systemically for neuroprotection, modulation of inflammation, and stimulation of angiogenesis.

Experiments in animal models of stroke have provided evidence that replacement of functional neurons by stem cell grafts is possible in the stroke-damaged brain, and suggest that this mechanism contributes to behavioral improvements (Fig. 1.3). For example, human ES cell-derived NSCs, implanted into the ischemic boundary zone in rats subjected to stroke, migrated towards the lesion and improved forelimb performance [24]. Electrophysiological recordings showed functional neuronal properties in the grafted cells and synaptic input from host neurons [25]. Also, human NSCs derived from embryonic striatum and cortex generated morphologically mature neurons after transplantation into stroke-damaged rat striatum [26]. Although promising, much work remains before neuronal replacement by stem cells could become a viable therapeutic strategy for stroke patients. Procedures to generate large numbers of neurons with correct phenotype from stem cells, in particular cortical neurons, should be developed. It has to be shown that the grafted stem cell-derived neurons establish connections with the host brain and become functionally integrated into its neural circuitries, and that they give rise to substantial behavioral recovery.

Recovery after stroke could potentially be induced by stimulating endogenous neurogenesis (Fig. 1.3). Following stroke in rodents, NSCs in the subventricular zone (SVZ) increase their proliferation and generate neuroblasts, which migrate to the damaged area in the striatum during the following months, are morphologically integrated, and seem to become functional mature neurons (for references see, e.g., [27, 28]). Since stroke mainly affects older people, it is of major clinical importance that studies in rats indicate that stroke-induced neurogenesis is maintained in the aged brain [29]. Neurogenesis following stroke has also been detected in humans, where there is evidence for enhanced SVZ cell proliferation and neuroblast formation [30–32]. It is clear, however, that in order to become of real clinical value, neurogenesis must be optimized (Fig. 1.3), which potentially could be accomplished using three different strategies: (1) By increasing the survival of the new neuroblasts or mature neurons. Approximately 80% of neuroblasts and neurons die during the first 2 weeks after their formation and only a fraction survive long-term after stroke in rats [33]. (2) By promoting the migration of the new neurons to the damaged area. Delivery of molecules that regulate migration in the vicinity of the damage could be a useful approach to attract stroke-generated neuroblasts. (3) By stimulating the differentiation of the endogenous NSCs to cortical neurons, which are formed only in limited numbers after stroke.

Various types of stem cells, such as NSCs and mesenchymal stem cells (MSCs), can ameliorate functional impairments after stroke by other mechanisms. These stem cell-based approaches could become of clinical value, but it is important that their efficacy and risks be compared to those of other treatments aiming at the same therapeutic targets. For example, intravenously administered human MSCs reduced stroke-induced deficits in rats, most likely by inducing angiogenesis and improving cerebral blood flow [34]. If these human MSCs were genetically modified to express various growth factors, neuroprotection and functional improvement were enhanced [34, 35]. Importantly, mouse NSCs delivered intravenously 3 days after stroke in mice, despite survival of only a small percentage of the cells in the infarct boundary zone, suppressed inflammation and glial scar formation, and gave rise to delayed neuroprotection and improved functional recovery [36]. This finding suggests an extended time window for neuroprotection using NSCs.

Some clinical trials with delivery of stem cells in stroke have already been completed. For example, an immortalized human teratocarcinoma cell line, implanted into infarcts affecting the basal ganglia and in some cases also the cerebral cortex, induced slight improvements in some patients [37]. No significant clinical recovery was detected following intravenous injection of autologous MSCs in stroke patients [38]. Several clinical studies using intravenous or intraarterial (into damaged territory) infusion of autologous bone marrow-derived stem cells and one trial involving transplantation of immortalized NSCs isolated from human fetal cortex in stroke patients are ongoing or planned.

Many issues remain before stem cell-based therapy can advance to full-scale clinical trials for the treatment of stroke. For example, it is necessary to determine the type of cells suitable for transplantation; to learn how to control the proliferation, survival, migration, differentiation, and functional integration of endogenous and grafted stem cells and their progeny in the stroke-damaged brain; and to develop procedures for cell delivery, scaling-up, optimum functional recovery,

and patient selection and assessment. Development of cell replacement strategies for stroke is much more challenging than for PD, for example, and should be a long-term goal. Stem cell-based treatments that act by neuroprotection, modulation of inflammation, and enhancement of angiogenesis seem closer to application in patients. However, even if some stem cells may be easily accessible for this purpose, such as autologous bone marrow-derived MSCs, each new approach has to show preclinical evidence of efficacy and safety and its mechanisms of action in the stroke-damaged brain have to be understood prior to trials in patients.

1.6 Perspectives

Stem cell research holds promise to lead to the development of novel therapies for several neurodegenerative diseases that currently lack effective treatments. Over the past few years there has been continuous progress in developing procedures to generate the types of neurons and glial cells that are needed to replace those cells that have died due to these diseases. Also, it is now well established that the NSCs in the adult brain produce new neurons and glial cells in response to neurodegeneration. Furthermore, besides cell replacement, stem cells have been shown to lead to improvements in models of neurodegenerative diseases that could become of clinical value also through modulation of inflammation, neuroprotection and stimulation of angiogenesis. It is inconceivable, though, that these advancements in stem cell research will lead to a large number of scientifically justified clinical trials in neurodegenerative diseases within the next couple of years. Transplantation of human stem cell-derived DA neurons are likely to be implanted in PD patients within 5 years. Clinical attempts at neuronal replacement for stroke and AD are more distant. Therapeutic approaches using stem cells mainly for neuroprotection or modulating inflammation will most likely be applied sooner in these disorders. In our view, the further development of stem cell-based therapies for human neurodegenerative diseases will require major research efforts in basal stem cell biology so that the mechanisms regulating the proliferation, migration, differentiation, survival and function of stem cells and their derivatives are much better understood and can be effectively controlled. Strategies to prevent tumor formation from pluripotent stem cells (ES cells and iPS cells) must be developed. Functional efficacy of stem cells and their mechanisms of action should be demonstrated in animal models with pathology and symptomatology resembling the human disease. Most patients with neurodegenerative disorders have few or no therapeutic options and are prepared to test any new approach. Scientists, clinicians, regulators, and ethicists must act together for the responsible clinical translation of stem cell research into appropriate applications for patients with these diseases. Finally, it must be remembered that even if the biology of stem cells is exciting, the clinical usefulness of stem cell-based therapies for neurodegenerative diseases will be determined by their ability to provide patients with safe, long-lasting and more substantial improvement in the quality of life as compared to other treatments.

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Chapter 2

Treating Cardiac Disorders with Stem Cells

Christine Mummery

Abstract Heart failure is one of the leading causes of death in the western world and its incidence is increasing in the east. One of its causes is myocardial infarction which results in loss of muscle mass through death of cardiomyocytes. Replacing these by transplanting stem cells or encouraging cells in the heart itself to multiply are among the ways being investigated to prevent heart failure developing. The only stem cells which can form cardiomyocytes though are pluripotent stem cells, until recently only available from human embryos. Other types of (adult) stem are not able to form cardiomyocytes but, if transplanted, may help the heart recover and be of short term benefit through other mechanisms. One area using human embryonic stem cells is controversial because of its ethics, the other because of its sometimes disputed clinical outcome. Here, a critical overview of the issues is presented.

Keywords Cell therapy • Stem cells • Cardiomyocytes • Heart infarct • Clinical trials

2.1 Introduction

Among the ailments discussed in the context of cell transplantation therapy, those of the heart feature most prominently because of the large numbers of patients in the prime of life with cardiac disease. Cardiac failure almost inevitably follows myocardial infarction because the remaining healthy heart tissue attempts to compensate for the loss of heart cells by working harder. This leads to hypertrophy (swelling) followed by thinning of the heart wall and eventually its collapse. Cardiac failure and hypertrophy may also result from other (life-style) conditions independent of myocardial infarction, such as high blood pressure, obesity, or diabetes. The clinical

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cardiologist's interest in using cell therapy is based on the assumption that dysfunction of the left ventricular chamber of the heart, which pumps oxygenated blood around the body, is largely due to the loss of a critical number of cardiomyocytes. Implanting new contractile heart cells into the regions of scarring or wall thinning could inhibit or even reverse the process. Scientists have been searching for the best cellular sources on which to base therapies and have included cells derived both from embryonic stem or progenitor cells and adult tissues. Some patients have already been treated with the safest option: cells from their own bodies. These are not rejected by the immune system as being "foreign." These adult stem cells are most commonly derived from the bone marrow and other tissues, such as fat. Benefits so far seem modest and temporary and the mechanisms are poorly understood. Contrary to initial studies suggesting that bone marrow cells might be "plastic" and turn into heart cells once injected into the heart, this turns out not to be the case. The present assumption is that any benefit may derive from limiting ischemic damage after myocardial infarction or creating new blood vessels that improve blood supply to the heart muscle. On the other hand, evidence is unequivocal that human embryonic stem cells (hESC) can become cardiomyocytes. These could perhaps be used to repair heart tissue by contributing contractile force, but they have other associated risks specifically in humans, namely the ability to beat spontaneously in the absence of any pacemaker cells that could cause lethal arrhythmias. Unlike bone marrow cells, they would not be autologous and so would be rejected by the body unless immunosuppressive drugs were used, and could form tumors (teratomas) if rogue undifferentiated cells remained present in the transplanted cell preparations. The issue of rejection could possibly be addressed by using induced pluripotency stem cells (iPSCs) derived from the patient's skin as a source of cardiomyocytes; this approach would, however, likely be too expensive for individualized therapy. These issues aside, the greatest obstacle is still likely to be proper and stabilized integration of the grafted cells from whatever (pluripotent or progenitor) cellular source into the host tissue. Will it be possible to align new heart cells so that there is more contractile force? Will there be sufficient blood supply to the graft to support survival? Will grafts and any associated scar tissue interrupt the electrical circuitry of the heart and interfere with sequential contraction of the chambers? How will we repair the tiny muscles that open and close the valves if these have also been damaged by ischemia? These are all questions that need addressing as basic research advances towards clinical practice. It is, however, clear that restoring function of failing hearts by replacing damaged cardiomyocytes is straightforward in principle but probably among the most challenging paradigms of regenerative medicine.

2.2 Adult Stem Cells

Bone marrow cells (BMCs) were among the first non-cardiomyocyte sources of regenerative cells described for the heart. Genetically tagged BMCs expressing green fluorescent protein (GFP) as a visible marker were transplanted to the

experimentally infarcted hearts of normal mice [1]. The general approach in pre-clinical studies for determining the effects of stem cell transplantation on heart function after myocardial infarction are shown in Fig. 2.1. Initially, transdifferentiation of the transplanted BMCs to cardiomyocyte was thought to take place since GFP coincided with cardiomyocytes marker expression, explaining functional improvement to the heart compared to controls. This later turned out to be autofluorescence from scar tissue or dead cells with fusion between injected and host cells possibly contributing to co-expression of markers. After 4–6 weeks, no injected cells were ever recovered [2–5]. Nevertheless, these early results evoked an unprecedented progression to completion of the first randomized clinical trials within 5 years. Early reports of non-controlled pilot studies were unanimously positive but in later randomized (placebo-) controlled trials, effects were more modest, especially after longer follow-up times. Most researchers now agree that if BMCs improve cardiac function after myocardial infarction, it is more likely to result from

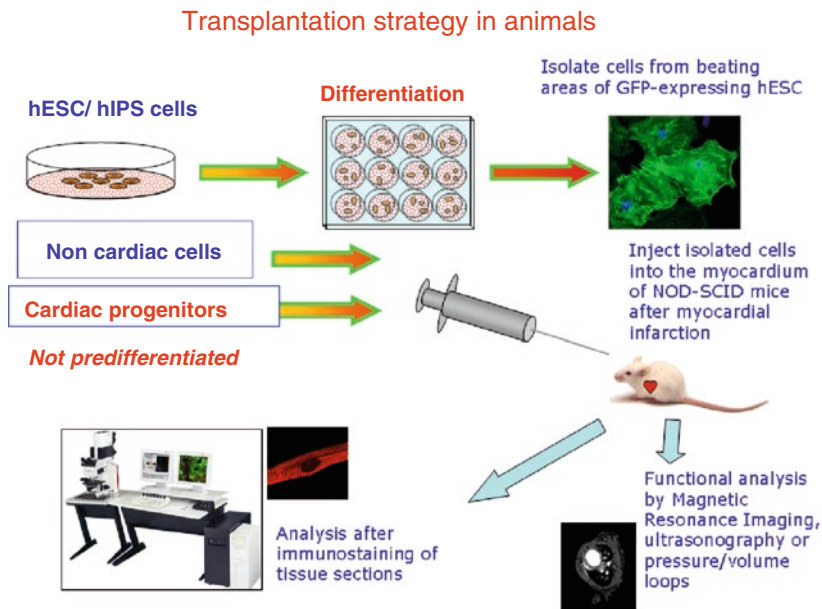


Fig. 2.1 Transplantation of stem cells or their derivatives to a mouse heart after myocardial infarction. The schematic diagram shows how these experiments are generally carried out. The cell of interest is usually labeled in some way (for example, genetically, fluorescently, or using iron particles) and injected into the (anaesthetized) mouse either through the tail vein or directly into the heart muscle. The mouse is usually immunodeficient to prevent cell rejection and a suture used to tie off one of the blood vessels of the heart. The cells are usually introduced at the same time as the surgery for myocardial infarction. Variable numbers of cells may be used. One group of mice usually functions as a sham control. Heart function in the mouse is determined over time after infarction and cell delivery. Non-invasive magnetic resonance imaging, echography, or invasive “pressure-volume loop” are the most common ways of determining cardiac function. The fate of the transplanted cells over time is monitored by immunohistochemistry or the like

early salvage of ischemic myocardium by some kind of paracrine action from the transplanted cells (reviewed in [6]). This does not mean the concept of cardiac regeneration (intrinsic repair) or repair (building tissue from an external source) should be abandoned. For instance, the underlying causes of cardiovascular disease affect BMC function; it may be of value to select subgroups of patients without BMC dysfunction for inclusion in trials. Parameters such as infarct remodeling or exercise capacity did appear to improve after BMC treatment, at least for up to 4–6 months, patients with the largest infarcts generally benefiting most [7]. Moreover, a small group of patients with angina pectoris (chronic chest pain) caused by underlying cardiac hypertrophy rather than acute myocardial infarction, have reported significant quality of life and modest functional improvements when bone marrow cells were injected into the heart [8]. Further basic research on the mechanism underlying any benefit will be required before the best cell type, intrinsic or extrinsic, can be mobilized or isolated for transplantation based therapy.

2.3 Pluripotent (Embryonic) Stem Cells and Cardiac Progenitors: Sources of Cardiomyocytes

This group of cells will be considered together because all have the capacity to generate the most important cellular components of the heart (reviewed in [9, 10]). Human embryonic stem cells (hESCs) are pluripotent cells derived from blastocyst-stage embryos. They proliferate indefinitely in vitro in an undifferentiated state and have the potential to differentiate into derivatives of all three primary germ layers (ectoderm, endoderm and mesoderm) and thus all 220 cell types of the adult individual. Mesoderm is the embryonic origin of the four major cell types of the heart: cardiomyocytes, vascular smooth muscle cells, endothelial cells and cardiac fibroblasts. HESCs are therefore a potential cell source for tissue regeneration including that necessary in the heart following myocardial infarction (MI). Likewise, human induced pluripotent stem cells (hiPSCs), derived by reactivating a set of pluripotency genes in somatic cells, can also self-renew indefinitely and differentiate to the same spectrum of cell types as hESC. Finally, cardiac progenitors endogenous to the heart could be a source of different cardiac cell types. These have been isolated from human fetal and adult hearts, shown to divide in culture to some extent but not indefinitely and to differentiate to one or more of the cell types in the heart. Their identity, however, is still a matter of discussion [11].

Multiple methods have been described to induce differentiation of stem and progenitor cells to cardiovascular derivatives. These range from methods that attempt to recapitulate normal development, such as growth as aggregates in suspension called “embryoid bodies,” and/or addition of specific growth factors, to more artificial methods, such as addition of demethylating agents that alter gene expression through epigenetic mechanisms. The cardiomyocytes obtained beat spontaneously and, in contrast to adult cardiomyocytes, express sarcomeric proteins,

cardiac transcription factors and multiple cardiac ion channel genes. They may have ventricular, atrial or pacemaker action potentials, respond as expected to positive and negative chronotropic agents, and form gap-junctions. Differentiated derivatives of stem and progenitor cells have an immature or fetal phenotype. Differentiated cell populations from pluripotent stem cells rarely exhibit a single phenotype and selection is necessary to obtain more pure populations. Much research today is focused on how best to do this; genetic marking, cell surface antibodies and physical methods based on differences in cell size or elasticity are all methods being tested. Independent of whether drug discovery and toxicity studies or regenerative medicine are the goals, obtaining well-defined homogeneous cell populations under defined conditions will be essential.

The first transplantation of hESC-derived cardiomyocytes demonstrated their potential to act as biological pacemakers in electrically silenced pig hearts, where the intrinsic pacemaker activity had been blocked by destroying the small region of pacemaker cells. At the same time, these studies demonstrated the risk of injecting immature (beating) cardiomyocytes into the heart – their potential to induce local arrhythmias. Transplantation studies were rapidly extended to regenerating the working myocardium. After transplantation into the healthy myocardium of immunodeficient rats or mice [12–14], hESC-derived cardiomyocytes were described as surviving and maturing for between 4 and 12 weeks [13, 14]. Although mixed cell populations were injected, preferential survival of cardiomyocytes was reported, with non-cardiac elements lost over time. Disconcertingly, however, grafted human cells formed a syncytium with each other but were largely separated from the rodent myocardium by a layer of fibrotic tissue. When transplanted into infarcted hearts in rodents, cardiomyocytes formed considerable grafts, with one study showing that addition of a pro-survival cocktail increased graft size. Cardiac function in animals receiving cardiomyocyte-containing populations was better during the first few weeks following transplantation than when non-cardiomyocyte derivatives were injected, and this was in turn better than when vehicle alone was injected, that is it seems any cell type is better than nothing, at least in mice and rats, cardiomyocytes work best. Hence, there were cardiomyocyte-specific benefits; these were quantitatively correlated in one study with the degree of neovasculture derived from the host in the border zone of the infarct. Only one study so far has, however, extended functional follow-up to 12 weeks after transplantation. At this point in time, the advantage of cardiomyocytes over non-cardiomyocyte-containing populations was no longer present, even when the number of cells transplanted or the number of injections was increased. “Priming” hESC with a growth factor called Bone Morphogenetic Protein (BMP) has been suggested as an alternative therapeutic option [15]. Primary fetal human cardiac progenitors, although difficult to obtain routinely, have also been described to differentiate in the rodent heart after MI and improve cardiac function, perhaps for even longer periods than hESC-CM [16]. Overall, though, functional enhancement by stem- or progenitor-derived cardiomyocytes even when neovasculture also forms, appears limited to mid-term at most. The therapeutic benefit of the cell therapy may be prolonged when using a pro-survival cocktail or different timing of injection, for example after the initial inflammatory phase when the environment may be hostile to the donor cells, but this remains to be

proven. A fibrotic layer can develop between injected and host cells that may or may not impede transduction of electrophysiological signals. The question arises of whether rodents are the most useful model animals to address this potential safety issue in humans. Rodent hearts beat at 400–600 times per minute, those of humans at 60–100. Injection of human cardiomyocytes with intrinsic different electrical properties into rodent hearts is therefore unlikely to contribute to cardiac function. The more slowly beating human cardiomyocytes would likely die from tachycardia if they really coupled to rodent host cardiomyocytes. Transplantation in the rodent myocardium is less likely to create arrhythmic substrates than the same procedure in humans. Transplantation of new cardiomyocytes from any stem or progenitor cells source into the human heart is thus likely to be fraught with safety and efficacy issues at the outset.

2.4 Summary and Conclusions

Almost any cell will cause functional improvement in the heart of a mouse with a myocardial infarction, independent of whether it is transplanted as or can become a cardiomyocyte or not. The only published exception so far appears to be skin fibroblasts (reference in [9]) which have no effect. Short term effective cell therapy in animals or humans does not appear to require that the cells survive permanently or even long-term in the heart. Current evidence suggests that any (transient) benefit to heart function and/or quality of life derives from new vessel formation or an as-yet undefined paracrine mechanism. Although ethically acceptable, likely safe and even commercially available as an unproven “treatment,” the long-term benefit of adult non-cardiac stem cells from any source, however, remains an open question. “Stem Cell Tourism,” a serious concern of the International Society of Stem Cell Research (www.isscr.org), which recently issued a recommendation on this subject, reflects the ethical and health concerns on excessively positive information on the benefits of stem cell treatment that are not provided as evidence-based therapies. Co-funding of basic and preclinical research through private and public partnerships may, however, accelerate clinical introduction of validated therapies. Animal studies and models could certainly be improved. Here exists the greatest disparity between basic scientists and cardiologists. Basic scientists make very clean “wounds” in the hearts of otherwise healthy mice; a normal heart patient would be stented (a small tube inserted) very quickly after myocardial infarction so the vessels would be reopened. In mice, the vessel is usually tied off permanently and cells transplanted immediately. In the case of heart failure in patients, the disparity with mouse models is even greater: large areas of hypertrophy interspersed with areas of necrosis and scar tissue and brittle vessels, small but crucial muscles operating valves inside the heart chambers damaged and dysfunctional. A large animal, such as a pig or sheep, could model aspects of human disease more effectively because of their greater physiological similarity to humans than rodents. For example, vessels could be tied off temporarily, then released and stents inserted as is done in patients. The timing of cell transplantation after infarction could be

varied, and different numbers and types of cells could be compared directly and simultaneously. The best delivery method could be identified. Should cells be injected deeply, or more towards the outer surface? Should a liquid matrix or gel be used, and would the presence of additional growth or survival factors be beneficial?

So far, these experiments are only being done on a limited scale, in part because they are very expensive. A pig can cost 10,000–20,000 euros compared to, say, 50 euros or less for a mouse. Housing of pigs during follow-up is significantly more costly than for mice. In Europe, preclinical studies of this kind in non-human primates, arguably the best animal model, are largely precluded by ethical considerations although a few large centers in the USA and Russia do carry out preclinical studies in these animals. Tissue engineering may also represent a way forward, particularly when the goal is to use contracting (immature) cardiomyocytes for transplantation, since these would allow prealignment of cardiomyocytes into myocardial “patches” for repair. If biodegradable scaffolds were used, then over time only the transplanted cardiac and vascular cells would remain.

In summary, there is clearly much basic preclinical research to be done. If cell therapy for the heart had already been proven, then the justification for using embryonic stem cells as a potential cell source for repair may be less controversial on ethical grounds. However, to close this avenue of research prematurely may deprive patients of potential therapies in the future, given the experimental options that remain to be explored. At present, it remains essential to carry out these studies in parallel to identify those with the most robust clinical potential in the shortest time frame. On the other hand, properly controlled clinical trials are imperative to combat the epidemic of “stem cell tourism” [17, 18].

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Chapter 3

Treating Diabetes

Mattias Hansson and Ole Dragsbæk Madsen

Abstract Diabetes mellitus is a group of metabolic disorders characterized by chronic hyperglycaemia and perturbed carbohydrate, fat and protein metabolism due to inadequate production of the peptide hormone insulin. More than 200 million people worldwide suffer from diabetes. Diabetes mellitus and the micro- and macrovascular complications associated with the disease cause immense distress for the patients and impose an enormous economical burden on society. The development of complications in patients is tightly associated with poor glucose regulation, as hyperglycaemia has direct and indirect detrimental effects on the vascular system. Although intensive diabetes treatment can reduce the risk for developing vascular complications in some instances, it is also associated with an increased risk of hyperglycaemia. Hence, there is an unmet need for improved glucose regulation in diabetes patients. Although the reconstitution of a functional beta cell mass by transplantation of isolated islets can restore tight blood glucose control and thereby minimizes the risk of developing severe complications, a shortage of donor material is one of the factors preventing the general use of cell replacement therapy for the treatment of type 1 diabetes mellitus. Advances in the directed differentiation of pluripotent stem cells toward beta cells via the stepwise recapitulation of embryonic development have generated proof of concept demonstrating that stem cells may be an appropriate source of cells for the generation of therapeutic beta cells. However, progress toward a clinical application of this technology is slow and challenging.

Keywords Diabetes mellitus • Stem cell • Cell replacement therapy • Beta cell • Islet

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3.1 Introduction

More than 200 million people worldwide suffer from diabetes mellitus (DM) and it is estimated that this number will rise to 366 million by 2030 [1]. DM and the complications associated with the disease cause immense distress for the patients and their families, and they impose an enormous economical burden on society. In particular, the late-stage complications are debilitating and related to increased hospital in-patient care. It is estimated that about 77% of the cost of DM in the US is associated with treatment of diabetes-related chronic complications and excess general medical costs, whereas the medical care to directly treat diabetes constitutes 23% of the total cost [2]. Generally, the complications are separated into macrovascular complications, including coronary artery disease, peripheral arterial disease, and stroke, and microvascular complications, such as nephropathy, neuropathy, and retinopathy. The development of complications in diabetes patients is tightly associated with poor glucose regulation, as hyperglycaemia has direct and indirect detrimental effects on the vascular system. Although intensive diabetes treatment can reduce the risk for developing vascular complications in some instances, it is also associated with an increased risk of hypoglycaemia [3–5]. Hence, there is an unmet need for improved glucose regulation in DM patients. The potential to reconstitute a functional beta cell mass through transplantation of stem cell-derived beta cells has raised the hope for a future treatment that restores a tight blood glucose control and thereby minimizes the risk of developing severe complications.

3.2 Insulin, the Beta Cell and Two Major Types of Diabetes

Diabetes mellitus is a group of metabolic disorders characterized by chronic hyperglycaemia and perturbed carbohydrate, fat and protein metabolism due to inadequate production of insulin. Type 1 DM (T1D) is characterized by an absolute lack of insulin due to an autoimmune destruction of the insulin-producing beta cell, whereas the relative insulin-deficiency of Type 2 DM (T2D) in part is a result of impaired sensitivity in the peripheral tissues normally responsive to the effects of insulin. Obesity is invariably linked to an increase in peripheral insulin resistance, thereby explaining the global increase in the prevalence of diabetes. Direct failure to up-regulate functional beta cell mass in obesity thus appears to explain onset of T2D [6, 7].

Insulin is a peptide hormone produced in the pancreas, a glandular organ located below the stomach and opposite the liver along the gastrointestinal tract. The pancreas's main function is to regulate the nutritional homeostasis in the body. This is achieved by two diverse mechanisms. The exocrine function of the pancreas is carried out by acinar and duct cells, which secrete and transfer digestive enzymes and other components into the duodenum to facilitate food digestion. The endocrine part of the pancreas controls glucose homeostasis through the cells in the islets of Langerhans. These are clusters of endocrine cells dispersed in the exocrine tissue. Insulin is produced in the beta cells, one of the four hormone-producing cells in the adult islets. The beta cells respond to elevated blood glucose levels and secrete insulin into the blood through the

capillary system that penetrates the cell clusters. The secreted insulin stimulates glucose utilization in several insulin-dependent tissues, such as muscle and fat. Conversely, low blood glucose stimulates the release of the hormone glucagon (the antidote of insulin) from the islet alpha cells. Glucagon primarily targets the liver to signal glycogen breakdown and glucose-release to the blood.

3.3 Cell Therapy of Diabetes to Normalize Blood Sugar Levels

The idea of replacing the insulin-producing cells for treating DM is not new. In fact, one of the first documented attempts was reported in 1894 [8], long before the discovery of insulin and the full understanding of the pancreas's function. The first successful islet transplantation in diabetic rats was reported in 1972 by Ballinger and Lacy [9]. The team used isogenic islets from non-diabetic animals to restore the beta cell mass in diabetic rats. This was followed by reports of successful auto- and allografts in human subjects [10–12]. However, the overall success rate of islet transplantations was generally low [13]. A breakthrough was reported in 2000 when Shapiro and co-workers reported improved success rate in islet transplantation using a glucocorticoid-free immunosuppressive regimen combined with infusion of a sufficient mass of freshly prepared islets from two or more pancreata from deceased donors [14]. These findings initiated new interest in islet transplantations and similar results have now been reported from several international transplantation centers [15]. Although the initial success rate is high, the majority of the patients revert back to insulin dependence within a 5 year period [16]. However, about 80% of the patients have residual function of the transplanted beta cell measured by circulating C-peptide levels. The surviving beta cells provide clear benefits in terms of improved euglycaemic control and protection from severe hypoglycaemia. Although the majority of the transplanted patients lose insulin independence over time, there are remarkable examples of long-term (>10 years) survival and function of allogeneic islets that restore normoglycaemia [17]. The reasons for success are not resolved but may include the choice of immunosuppression, low metabolic demand and low immune responsiveness. This demonstrates that long-term insulin independence after allogeneic islet transplantation is an achievable target. However, the lack of donors is one of the limiting factors for developing islet transplantation into a viable diabetes therapy. This has stimulated intense research to identify alternative sources of beta cells for transplantation, such as utilizing porcine islets in xenotransplantations [18] or the generation of functional beta cells from stem cells.

3.4 Therapeutic Beta Cells from Stem cells?

Stem cells are defined by two basic characteristics: (1) they have the capacity to self-renew, and (2) they can generate committed progeny [19, 20]. These unique features have identified stem cells as a potential unlimited source of somatic cell

types for cell replacement therapies of degenerative diseases such as diabetes. Stem cells are commonly classified by their developmental potential, ranging from the totipotent to the unipotent stem cell. The totipotent stem cell has the potential to generate a complete organism, including all somatic-, germline- and extraembryonic tissues. The zygote and the early blastomeres in mammals are examples of totipotent cells. Pluripotent stem cells, a category that includes embryonic stem (ES) cells and induced pluripotent stem (iPS) cells, can generate all cell types in the body but are unable to independently develop into a complete organism. ES cells are derived from the inner cell mass (ICM) of the expanded blastocyst by culturing the intact blastocyst or the isolated ICM [19]. iPS cells are generated from somatic cells by reprogramming through the forced expression of a specific set of transcription factors [21, 22]. The expression of these factors revert the cell towards a pluripotent stage similar to ES cells [23]. Multipotent stem cells (e.g., haematopoietic or neuronal stem cells) have the capacity to generate a limited set of terminally differentiated cell types, whereas unipotent stem cells only give rise to one cell lineage. Stem cells can also be classified according to their origin – embryonic, fetal or adult, which in most instances reflect their developmental potential.

Several groups have investigated the generation of pancreatic beta cells from different multipotent adult stem cells such as hepatic cells [24–28], stem-like cells from the small intestine [29–31] or salivary glands [32], and adipose-derived mesenchymal stem cells [33]. Although these reports contain promising data, several issues remain to be addressed before any of these cells can be considered as a potential source of beta cells for replacement therapy, including the ability to achieve fully functional beta cells as well as the expandability and stability of the cells. Furthermore, several of these studies are based on transgenic overexpression of key transcription factors in target cells, which will further complicate the translation to the clinic. Bone marrow-derived haematopoietic stem cells (HSC) have been reported to differentiate to functional beta cells *in vivo* [34], but subsequent studies indicate that the HSC facilitate beta cell regeneration indirectly by forming endothelial cells or inhibiting the inflammatory response rather than the direct conversion of HSC to beta cells [35–37]. Of special interest are the recent reports where improved metabolic control was achieved in patients with T1D [38, 39] or T2D after autologous HSC transplantation [40].

The generation of beta cells from pluripotent ES cells has been addressed in several studies. Early reports suggested that beta cells were generated by directed differentiation via neural progenitors [41–43]. However, subsequent studies failed to show *bona fide* insulin biosynthesis in cells generated from ES cell-derived neural progenitors, but rather that the insulin found in these cells was due to its uptake from the surrounding medium [44–48]. A more successful approach has been to recapitulate beta cell development *in vitro* and establish ES cell differentiation strategies on the knowledge of normal beta cell development. All gastrointestinal organs, including the pancreas, are derived from the definitive endoderm (DE), one of the three germ layers formed during gastrulation (reviewed in [49]).

Several recent studies have addressed the generation of DE from ES cells [50–56] and its subsequent differentiation to pancreatic progenitors and insulin-producing beta-like cells [57–70] by recapitulating this progressive developmental process *in vitro*. Although the endpoint cells derived *in vitro* are distinctly different from fully functional beta cells [61, 63, 68], glucose-responsive “beta-like” cells can be generated by *in vivo* maturation of ES cell-derived progenitors [66]. Together, these studies suggest that pancreatic endocrine cells can be derived from ES cells.

The generation of insulin-producing “beta-like” cells from iPS cells has also recently been reported [70–72]. The accessibility to iPS cells from any individual opens up the possibility towards autologous patient-specific cell therapy without the need for continuous immunosuppression. However, for this to become reality in a broader sense, it requires the development of highly reproducible and standardized methods for iPS cell derivation and differentiation, but immunosuppression would likely still be required in the context of autoimmune diseases, such as T1D.

3.5 Tackling Immunological Compatibility and Autoimmunity

Allo-immunoreactivity in man (as in any mammal) will result in tissue rejection following transplantation of organ donor material from the same species. Future stem cell-based therapies can thus be viewed in two scenarios:

1. Physical encapsulation of therapeutic cells to allow nutrient exchange as well as appropriate release of the secreted biological factors in question (such as insulin for diabetes treatment). Encapsulation of islet cells for transplantation has been investigated for a number of years, and a multitude of approaches are being pursued (reviewed in [73]). Effective encapsulation could potentially also allow efficient use of xenogeneic donor islet (such as pig islets) and trials are at present running to explore this further (<http://www.clinicaltrials.gov/ct2/show/NCT00940173?term=pig+AND+islet&rank=1>)
2. Direct transplantation combined with immune intervention strategy to prevent rejection. Immunosuppression combined with matched tissue-types is thus a prerequisite for successful transplantation therapy. Lifelong education of emerging lymphocytes occurs centrally (thymic epithelium) and peripherally to maintain an active and fully operational immune system. HSC residing in the bone marrow are responsible for the continued production and replenishment of red and white blood cells. Malignancies in the immune system are effectively treated with radiation- and chemo-therapy (to eradicate the lymphoid cancer stem cells) combined with donor-matched bone marrow transplantation (to replenish the functional immune system and capacity to produce red blood cells). As a consequence, such patients evolve immune chimerism that with a certain frequency can trigger graft-versus-host disease (GVHD) but may also lead to true functional chimerism where two immune systems cooperate peacefully [74]. As a curiosity, such patients would in theory be tolerant towards additional organ

transplantation from the same original bone marrow donor – without the necessity for life-long immunosuppression to prevent graft rejection. Establishment of mixed immune chimerism is thus an explanation as well as a goal for transplantation tolerance in general [75, 76].

3.5.1 Adult Stem Cell-Mediated Correction of Autoimmunity?

Failure to eliminate or control autoreactive B- and T-lymphocytes during immune repertoire maturation may lead to autoimmunity. T1D is the result of autoimmune destruction of the beta cells selectively within the islet of Langerhans. Only ~15% of new T1D occur in families with affected first-degree relatives and the concordance rate among identical twins is as low as 35%, indicating that the heredity component of T1D is rather low. Prevalence of autoimmunity appears to increase worldwide and could possibly be linked to the “hygiene-hypothesis” [77], explaining the general increase in allergy [78].

Recently, a controversial study in Brazil reported remission and possibly cure of newly-diagnosed young T1D by combined chemotherapy, and autotransplant of the patient’s own bone marrow cells [38, 79]. A potential explanation could be the reset towards a “normal” immune repertoire mediated by the knock-down or elimination of the autoimmune lymphocytes followed by replenishment of new lymphoid development through the re-infused stem cells. If autoimmunity towards beta cells has been corrected by this treatment, it is highly interesting that several of the patients show improved beta cell function over time [38] (<http://www.clinicaltrials.gov/ct2/show/NCT00315133?term=NCT00315133&rank=1>).

Animal studies show that functional beta cells can regenerate following severe or complete non-immune mediated beta-cell ablation [80, 81]. Other studies also suggest that the human pancreas may attempt life-long beta cell regeneration in T1D [82, 83], suggesting that future correction of autoimmunity could lead to spontaneous normalization of blood glucose regulation.

3.5.2 Pluripotent Stem Cell-Mediated Tolerization Towards Therapeutic Cells

The pluripotent stem cell is the “mother of all stem cells” and HSC can thus be derived from mouse (or human) ES or iPS cells [84, 85]. The ability of HSC to induce tolerance by immune chimerism following allogeneic bone-marrow transplantation predicts that ES cell-derived HSC can serve to establish tolerance towards a subsequent therapy using cells derived from the same ES cell origin, as recently demonstrated in mice [84]. The future scenario of this could revolutionize stem cell-based therapies by transplantation without the need for lifelong immunosuppression therapy – and thus also with no need to encapsulate cells to prevent rejection. The

question remains whether the autoimmune restricted beta cell killing in T1D patients will allow therapeutic beta cells to remain protected by the chimera immune cells. Ultimately, if such a strategy works out, it requires at the same time a highly stringent quality of the ES cell-derived therapeutic cells to avoid tumor formation.

3.5.3 iPS Derived Beta Cells from Patients to Devise New Strategies to Understand and Prevent Autoimmune Diabetes

With the discovery of iPS cell technology, a novel tool in disease modulation/modeling has appeared [86] – and with great potential to further our understanding of the nature of autoimmune T1D. Pluripotent iPS cells derived from fibroblasts from a T1D patient may allow the generation of fully functional beta cells *in vitro* [71]. These beta cells would in theory be identical to the antigenic target of the autoimmune *in vivo* destruction of the particular patient. By harvesting lymphocytes from the patient, the killing process can for the first time be reconstituted *in vitro* – and such experimental conditions may allow for the design of drugs to specifically prevent the immune mediated killing (*in vitro*). Furthermore, using iPS cells from a T1D patient identified in diabetes prevention trials could provide access to cryopreserved lymphocyte preparations prior to onset of disease – and might allow *in vitro* studies of the nature of autoimmune development, including the acquisition of the “licence to kill” beta cells by the immune cells.

3.6 Future Outlook

Cell therapy of diabetes is a highly constructive future avenue to potentially reduce or eliminate the risk of developing life-threatening late complications without increasing the risk of fatal hyperglycaemic episodes. In order to reach this goal we need:

- Efficient differentiation protocols to generate therapeutic cells
- Technological breakthroughs in encapsulation technology and/or swift and safe methods to establish mixed chimerism to generate tolerance towards the therapeutic cells.

3.6.1 Improved In Vitro Differentiation Protocols for ES Cells and iPS Cells

Much effort needs to be put into the generation of improved (robust) protocols for differentiating ES cells towards beta cells. A first roadblock is to generate or

evolve the entirely defined culture conditions for maintaining pluripotent human ES cells. Multiple human ES cells (and even iPS) lines adapted to proliferate long-term in such a medium have a greater likelihood to behave similarly towards subsequent differentiation signals. Such defined differentiation media provide value to continuously stimulate biotech investment towards making cell therapy commercially viable.

3.6.2 Improved Transplantation, Including Encapsulation or Immunomodulation (Non-myeloablative Induced Tolerance by Mixed Chimerism)

Encapsulation technologies may evolve into sophisticated “artificial endocrine pancreas chambers/incubators,” where beta cell replenishment can be performed swiftly when necessary (e.g., once every 1–2 years). However, the optimal scenario includes a two-step therapy based on human ES cells:

Step 1 is the induction of tolerance towards the tissue-haplotype of the human ES cell source by deriving haematopoietic progenitor or stem cells (HSCs) for a non-myeloablative procedure to establish mixed immune chimerism. Step 2 includes the subsequent therapy with fully functional beta cells derived from the same ES cell source.

Finally, analogous to the reprogramming of somatic cells to iPS cells, recent breakthroughs in mouse studies suggest that existing pancreatic cells can be directly reprogrammed into functional beta cells. Targeting the simultaneous expression of three transcription factors (TFs) into the exocrine acinar cell, or just a single factor into the glucagon producing islet alpha cell, is enough to fully reprogram such cells into functional beta cells. Future cell-specific targeting technologies need to be developed to move such technology to the clinic, and immunosuppression might be required to prevent autoimmune-mediated killing of reprogrammed beta cells.

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Chapter 4

Treating Oncologic Disease

Peter W. Andrews

Abstract The cancer stem cell hypothesis has become popular in the past few years, in part to provide an explanation of the recurrence of the disease following initial remission and apparent cure. Nevertheless, the concept that cancer is a developmental disease resulting from aberrant control of cellular differentiation is old and is epitomized by teratocarcinomas, a form of testicular germ cell tumor, and by the hematological malignancies. These ideas have developed in parallel with the notion of tissue stem cells that provide for the replacement of functional cells in adult tissues, especially in those tissues subject to continual ‘wear and tear’ throughout adult life. Although a simple view might hold that cancers contain a small population of cancer initiating stem cells equivalent to and, perhaps, derived from the stem cells of the tissue from which they arise, the circumstances of such cancer stem cells is inevitably very different from those of tissue stem cells. Consequently the possibility that a tumor can be composed entirely of oxymoronic nullipotent stem cells is still compatible with a stem cell view of cancer initiation and progression. This review considers the origins of the cancer stem cell concept, and issues that need to be addressed to enhance its utility for developing methods for preventing and treating cancer.

Keywords Cancer • Teratocarcinomas • Germ cell tumors • Embryonal carcinoma • Cancer stem cells

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4.1 Introduction

A common feature of cancer is that it is difficult to treat once it has metastasized and spread to sites far removed from its site of origin. Before that stage, surgery is the usual and very effective treatment option, but after that, surgery is of limited value and chemo- and radiotherapy are the approaches of choice. In some cases, for example in testicular cancer, the results are spectacularly successful; cure rates in excess of 90% may be achieved even in moderately advanced cases of testicular germ cell tumors, the most common cancer in young men. However, in other cases, results are dismal; for example, cures of pancreatic cancer are rare once the tumor has spread.

One aspect of treating metastatic cancer is that apparent initial success, with the patient entering remission, may be followed by relapse as the cancer recurs, sometimes after quite considerable time. Why should this be? In some cases, it may be because a genetic susceptibility to the cancer results in multiple origins from different cells. This is most easily recognizable in cases of cancer of paired organs where the inherited loss of a specific tumor-suppressor gene results in clearly separate cancers occurring independently in both organs. The paradigm is the inherited susceptibility to retinoblastoma, in which a dominant susceptibility to developing this cancer of the eye results from inheritance of a loss of function of one allele of the gene encoding the cell cycle regulatory protein, RB [1]. Loss of the remaining normal allele of the *RB* gene may then occur randomly in different retinal cells, which consequently separately acquire a cancer phenotype. Reversing the argument, the occurrence of apparently independent cancers in paired organs may be used to suggest an underlying inherited susceptibility to the cancer. For example, having had testicular cancer is one of the strongest risk factors for developing another cancer in the contra-lateral testis, and there are other arguments that strong genetic factors play a key role in the etiology of testicular germ cell tumors: for example, in the laboratory mouse these tumors only occur spontaneously in one inbred strain named “129”.

However, most cancers are sporadic, without an obvious familial susceptibility. In such cases, recurrence probably implies that a small population of cancer cells has escaped the chemotherapy. It is to explain this that the cancer stem cell hypothesis has become popular in the past few years [2, 3]. The ideas are not new but have developed over the past 50 or more years in parallel with the notion of stem cells providing for the replacement of normal tissues subject to continual turnover throughout life – notably, the blood, the skin and the lining of the gut. In the simplest version of stem cell concept for tissue turnover, it is proposed that in such tissues most of the functional cells have a limited life span and eventually die or are lost. To replenish these lost cells, the tissues also contain small populations of unspecialized cells, the stem cells that continue to divide throughout the life of the organism. After cell division, these stem cells can generate either another stem cell, or a different type of cell, a “transit amplifying cell,” that can continue dividing for a limited period, but is destined eventually to cease division

and acquire a functional phenotype appropriate to that tissue [4, 5]. In one version of the cancer stem cell hypothesis, these tissue stem cells are the targets for carcinogenesis. Since the transit amplifying cells and terminally differentiated cells of tissues subject to turnover will eventually die, according to this view, it is only in the stem cells that mutations could affect cells that persist and so be capable of developing into a cancer. Indeed, Cairns [6] suggested that the stem cell architecture of tissues provides one line of defense against cancer since it minimizes the target for mutations that could cause cancer.

By analogy with the stem cell concept of normal adult tissues, the cancer stem cell concept typically envisages that a cancer contains only a small population of cells that are capable of forming the cancer – the cancer initiating cells: most of the bulk of the cancer is assumed to be composed of cells that are derived from these cancer stem cells, but are themselves not capable of forming a tumor *de novo*. If true, this can immediately provide an explanation for the recurrence of a cancer in a relapsing patient: the chemotherapy is proposed to eliminate these bulk tumor cells but leave the cancer initiating cells, or cancer stem cells, untouched. In turn, this leads to the notion that most efforts in developing chemotherapy may have been directed to the wrong targets, and that future efforts should be directed to identifying agents that specifically target the cancer stem cells [7].

These ideas are superficially attractive since they lead to hypotheses that can be tested and they indicate a new direction for searching for new cancer treatments. However, they are also controversial, and evidence has been advanced that in some cancers, the proportion of cancer-initiating cells is much higher than the cancer stem cell hypothesis would suggest [8]. Also, it is not clear whether proposed cancer stem cells can be simply equated to altered versions of the normal stem cells of the tissues from which the cancers arise, or whether they are derived from other cells that have acquired the characteristic stem cell features. On the other hand, such criticisms may not take into account more sophisticated versions of the cancer stem cell idea. Certainly, the concepts of stem cells in adult tissues are evolving and the notions of discrete compartments of stem cell, transit amplifying cells and terminally differentiated may prove to be an oversimplification. For example, it may be that cells thought to have committed to a transit amplifying state, might reverse that decision under some circumstances [9, 10].

4.2 Developmental Cancers

4.2.1 *Teratocarcinomas and Germ Cell Tumors*

The cancer stem cell concept can be traced back to ideas that cancers involve defects in the mechanisms that control cell differentiation. In turn, these ideas were stimulated by studies of two very different types of cancer – teratomas and leukemia. Teratomas are peculiar and rare tumors that typically occur in the ovary or

testis, and derive from germ cells, so they are classed as germ cell tumors [11]. These tumors have fascinated medical science for centuries since they seem to present gross distortions of embryogenesis. They contain a haphazard array of cells and tissues, sometimes formed into recognizable organs – hair and teeth are commonly found in ovarian teratomas. Mostly, the ovarian forms of these cancers are benign, but the testicular forms are always malignant and are consequently known as teratocarcinomas. Experimental study of teratocarcinomas was initiated with the discovery, by Stevens, working at the Jackson Laboratory in Bar Harbor, Maine, in 1954, that male mice of the “129” strain produce spontaneous testicular teratocarcinomas [12]. An important subsequent early study was the demonstration by Kleinsmith and Pierce [13] that a single cell from these tumors is capable of generating a complete teratocarcinoma containing the whole range of differentiated cell types characterizing the cancer. Arguably, this was one of the first experimental demonstrations of a cancer stem cell.

Further extensive studies over the following decades demonstrated that the crucial malignant cell of these teratocarcinomas, both in the laboratory mouse and in humans, is the embryonal carcinoma (EC) cell [14]. EC cells are the stem cells of these cancers: they are both capable of differentiating to produce all of the wide array of differentiated cells found in these curious tumors, and of dividing indefinitely to produce identical EC cells that also retain the capacity for differentiation; such proliferation is known as “self-renewal,” a characteristic feature of stem cells. Indeed, consistent with the cancer stem cell notion, the EC cells are capable of generating a complete teratocarcinoma, whereas their differentiated derivatives, which make up the bulk of the tumor, do not have that capacity.

While the teratocarcinomas provide a paradigm for a basic cancer stem cell model in which cancer stem cells may make up a minority of the tumor mass, they also provide insights into those situations where most of the cells in a cancer may be capable of cancer initiation. On a moment’s reflection it will be evident that if a stem cell can both self-renew and differentiate, whereas differentiation results in cells with limited future proliferative capacity and no tumor-forming ability, then there will be a strong selective advantage for a stem cell that acquires, through mutation, a greater chance of self-renewal than of differentiation. One might then anticipate that, in the absence of any regulatory activity to prevent it, any stem cell system will evolve gradually to a state in which mutant stem cells have completely lost the capacity to differentiate. Such “nullipotent” stem cells might appear to be an oxymoron, but indeed nullipotent EC cells have long been recognized in teratocarcinomas of the laboratory mouse [15], while it is recognized that a significant proportion of human testicular germ cell tumors are composed exclusively of EC cells alone – presumably “nullipotent” EC cells [16]. Typically, cancers progress over many years from an initially indolent state to a highly aggressive invasive and metastatic state. The evolution of stem cells by mutation resulting in the slow loss of differentiation capacity could be one factor in this cancer progression, and could explain how a stem cell model of cancer development could still be compatible with a situation in which most of a tumor is composed of cancer initiating cells.

4.2.2 *Leukemia*

The stem cell concept of tissue architecture owes much to studies in the hematopoietic system. It is often traced back to the findings of Till and McCullough [17, 18] that cells able to form hematopoietic colonies in the spleens of lethally irradiated mice can be found in the bone marrow. These stem cells are multipotent and can both self-renew and differentiate to produce colonies containing a range of different hematopoietic cells. The results, complemented by those with other systems such as the intestinal mucosa, led to the development of the idea that tissues may be divided into compartments containing, on the one hand, the stem cells, and on the other, the “transit amplifying” cells committed to differentiate further to a restricted range of terminal cell types responsible for the principal functions of the tissue [4, 5]. The wide array of functionally distinct cells in the hematopoietic system are organized into a well-characterized hierarchy linked to the hematopoietic stem cell through a series of progressively more lineage-restricted intermediate progenitor cells. Of these cells, only a small subset are capable of fully rescuing a mouse that has been lethally irradiated; this assay defines the most primitive stem cells capable of regenerating the whole hematopoietic system, which can, in turn, be used as a source of stem cells to reconstitute the hematopoietic system of second lethally irradiated mouse. This serial, long-term reconstitution assay provides the definitive functional test to define the hematopoietic stem cell. Other cells in the system can also rescue such a mouse but only for short periods; such cells do not meet the stem cell criterion of indefinite self-renewal.

Like the hematopoietic system, leukemias are commonly heterogeneous, containing a variety of different cell types, only a small subset of which are capable of initiating a new leukemia when transferred to another animal [19, 20]. Such results are understood to indicate the presence of a leukemic stem cell. Different forms of leukemia may be characterized by the extent to which the leukemic cells are phenotypically related to different cell types along the normal lineages of hematopoiesis. Thus, some leukemias are composed of cells with phenotypic characteristics of T or B lymphocytes, whereas as others appear more closely related to cells of the myeloid lineage, at different levels of lineage restriction. This classification of leukemias could be consistent with the notion that they arise from different stages in the hematopoietic lineages. On the other hand, that is not necessarily consistent with the notion that the leukemic cells were initiated by a mutation in the most primitive, pluripotent hematopoietic stem cell. Since only those most primitive cells exhibit true stem cell properties, any leukemic stem cell that arises from a later stage must necessarily acquire the capacity for self-renewal. This interpretation highlights the possibility that putative cancer stem cells do not necessarily have to be derived from a pre-existing stem cell: a stem cell must ultimately be defined, not in terms of its cell of origin or expression of particular phenotypic markers, but rather in terms of function, the dual capacities for self-renewal and differentiation, notwithstanding the possibility, discussed above, that progressive mutation and selection might generate a nullipotent stem cell that

retains the phenotypic markers of a corresponding multipotent stem cell, but has lost the capacity to differentiate.

One aspect of stem cells that is apparent from the hematopoietic system, in contrast to the teratocarcinomas, is the issue of stem cell quiescence. It may be that the long-term repopulating hematopoietic stem cells are generally quiescent and divide rarely [21]. This may also be a common feature of many adult stem cells in other systems, including cancer stem cells, which could provide a mechanism whereby cancer stem cells escape chemotherapy. This situation contrasts markedly with that in teratocarcinomas in which EC cells seem to be actively dividing, and, interestingly, these tumors are particularly susceptible to cure by chemotherapy. This difference may reflect the fact that the normal counterparts of EC cells, the cells of the inner cell mass or primitive ectoderm of the early embryo, are properly not stem cells by the traditional definition; they are cells that exist transitorily in the embryo before generating later cells with more restricted potential, and might therefore more closely fit the definition of transit-amplifying cells. Embryonic stem (ES) cells derived by explanting the inner cell mass cells of an early embryo at the blastocyst stage appear to represent “normal” counterparts of EC cells, with which they share many characteristics. However, their acquisition of a capacity for indefinite self-renewal may be a consequence of their changed environment after growth *in vitro*. They might also be regarded as an instance of a “transit-amplifying” cell, in this case the inner cell mass cell, acquiring stem cell properties.

4.2.3 Other Developmental Tumors

Most cancers develop over long periods. In humans, the initiating event may often be separated from the appearance of a clinically apparent malignant tumor by many years. Pre-malignant changes to tissue histology can often be recognized in that intervening period, as the organization of the cells within the tissue, as well as the phenotypes of some cells, begin to show features atypical of that tissue – a facet exploited in screening for some cancers, such as for cervical cancer using the Pap smear. Yet even when a tumor first becomes invasive, and evidently malignant, it may still retain many differentiated characteristics of its tissue of origin [22, 23]. For example, a colon cancer may contain extensive glandular differentiation, the presence of structures resembling that of the normal colonic mucosa [23]. Subsequently, as a cancer becomes more aggressive, the proportion of differentiated cells may reduce, while undifferentiated, rapidly dividing cells begin to predominate. It appears that development of a tumor involves not only excessive cell proliferation but also distortions in the normal processes of cell differentiation. Pierce described cancers as “caricatures” of normal tissues – many of the normal processes of cell proliferation and differentiation occur, but in aberrant manner, with a slow loss of organization as the tumor evolves.

One necessary characteristic of the stem cell structure of tissues is that when a stem cell divides, at least on average, exactly half of all its progeny must retain a

stem cell phenotype, and half must initiate differentiation. Anything different from this will inevitably cause problems: if less than half stay as stem cells, then the stem cell pool will slowly become depleted and the tissue will lose its ability to be replaced. This, of course, could be one potential cause of aging. Alternatively, if more than half stay as stem cells, there will be an inevitable ever-increasing size of the stem cell pool – effectively an incipient cancer. One way in which an exact 50:50 split between a stem cell phenotype and differentiating cell can be produced is by asymmetric cell division, and this is certainly observed in some systems [24]. However, it is not clear that this is always the mechanism, in which case there must either be very close control of fate choice for dividing stem cells, or the existence of a system to ensure that excess stem cells do not survive. Such a regulative mechanism would have strong advantages over a rigid asymmetric cell division mechanism, by providing flexibility and a capacity to respond to different situations – for example, injury, albeit at the expense of cancer if the regulatory systems fail.

4.3 Prospective

The development of metastatic cancer almost always seems to involve a need for multiple heritable changes in gene expression and function, either as a result of mutation or gene rearrangement, or due to epigenetic changes. Cancer is also a progressive disease in which tumors gradually acquire more aggressive phenotypes, typically associated with the loss of differentiated features. Given our knowledge of the stem cell architecture of many tissues, and not necessarily only those most readily identified with a lifetime need for replacement, but also tissues such as the nervous system or the skeletal muscles, the possibility that at least some cancers arise from dysregulation of stem cell behavior is obvious. Nevertheless, it is important to recognize that cells with stem cell-like properties could in principle arise *de novo*, so that cancers containing stem cells could arise from transit-amplifying cells or more differentiated terminal cell types if they could acquire properties of self-renewal. Equally, as discussed above, stem cells might acquire mutations that minimize their propensity to differentiate, and that such mutations may confer a strong adaptive advantage that could contribute to the evolution of a more aggressive character.

By contrast to the genetically unstable situation prevailing in cancers, stem cells in normal tissues operate in a considerably more stable situation, containing multiple mechanisms to prevent the accumulation of genetic change and to eliminate potentially aberrant cells. The development of cancers is the result of a breakdown of these homeostatic mechanisms. Therefore, although our understanding of stem cells in normal tissues may provide insights into possible cancer biology, it is possible that cancer stem cells will exhibit behaviors not seen in tissue stem cells under normal circumstances.

It is against this background that discussion of the cancer stem cell concept must be considered. From the point of view of treatment, the key attraction of the cancer

stem cell idea is that these are the cells that must be targeted for therapy if a complete cure of a metastatic cancer is to be achieved. Certainly, examples are emerging where tumors are heterogeneous with respect to the presence of cancer-initiating cells, and other cells, and they provide situations where it is appropriate to use this notion in the development of new treatment regimens. That some cancers may not have a minority population of cancer-initiating cells does invalidate the argument for those cancers in which cancer stem cells can be identified. As discussed above, it is entirely possible that a cancer initially conforming to the cancer stem cell concept will evolve to be composed of cells phenotypically resembling the stem cells, but lacking the capacity for differentiation, as in the case of tumors composed of pure embryonal carcinoma. In terms of developing treatments, the arguments eventually devolve to pragmatism.

However, consideration of stem cells and the role of stem cells in cancer behavior is also pertinent to our understanding of the mechanisms by which cancers develop and evolve. In this context, identifying the cell of origin of a cancer is important: Which cancers arise from tissue stem cells? Do some cancers arise from transit amplifying/progenitor populations, and if so, how do these cells acquire the capacity for indefinite self-renewal? Do multipotent cancer stem cells commonly evolve to a nullipotent state as seen in some EC cells? If so, what are the mechanisms and to what extent do they contribute to cancer progression? Answers to none of these questions are currently clear, but addressing them will provide not only a better understanding of cancer itself, but also of the homeostatic mechanisms that operate normally to regulate embryonic development and to maintain tissue integrity in the adult.

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Chapter 5

Clinical Application of Autologous Epithelial Stem Cells in Disorders of Squamous Epithelia

Nicolas Grasset and Yann Barrandon

Abstract Epidermis and epithelia lining the ocular surface, the oral cavity, the pharynx, the oesophagus, the larynx, and the vagina, called stratified squamous epithelia (SSE), contain stem/progenitor cells that support renewal and repair. Under appropriate conditions, these cells can be massively expanded in culture. Restoration of the integrity and the function of SSE is obtained by transplantation and engraftment of the autologous cultivated stem cells in case of several severe clinical conditions (e.g., extensive third-degree burns, limbal deficiency). Successful gene therapy for hereditary SSE disorders has also been achieved by means of correction of the genetic defect in cultivated autologous keratinocyte stem cells. However, the mechanisms controlling engraftment of the transplanted stem cells remain poorly understood, leading to unpredictable clinical results. Further fundamental investigations to explore the behaviour of the transplanted stem cells and their plasticity, anticipating the regulatory affairs main concerns, are needed for successful cell and gene therapy.

Keywords Skin • Cornea • Engraftment • Cell therapy • Gene therapy

5.1 Introduction

Stratified squamous epithelia (SSE) are self-renewing and protect the body against environmental hazards. They include the epidermis, the outermost layer of the skin, the epithelia lining the ocular surface, and those epithelia lining the oral cavity, the

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pharynx, the esophagus, the larynx and the vagina. SSE, with the exception of skin appendages that contain multipotent stem cells, are thought to solely contain oligo or unipotent stem/progenitor cells, the multiplication of which nicely balances the loss of terminally differentiated cells continuously sloughing off the epithelial surface. Importantly, stem/progenitor cells from human SSE can be extensively cultured and transplanted to treat extensive burn wounds [1–4], disabling hereditary skin diseases [5], and corneal and other SSE deficiencies [6–8].

5.2 The Epidermis and the Cornea

SSE contains keratinocyte stem cells that are distributed throughout the basal layer of the epithelium. In the epidermis, each stem cell is supposedly in charge of a very small portion of epithelium; consequently, the epidermis appears to function as an assembly of independent columnar units [9, 10]. Keratinocyte stem cells are thought to divide infrequently and asymmetrically to generate daughter stem cells and transient amplifying cells (TA) with limited potential [11]. TA cells then actively divide to compensate for the loss of terminally differentiated cells at the epithelial surface [10, 12]. However, this notion has been recently challenged [13–15]. Epidermal appendages (hair follicles, sweat glands) contain stem cells that are crucial for appendage renewal [12]; for instance, hair follicle multipotent stem cells are important for the generation of a hair germ at the onset of a new hair cycle [16–20]. The bulge of the hair follicle has been regarded as the sole niche of stem cells in the skin [21, 22], but there is now compelling evidence that cells endowed with stem cell properties are present in the epidermis, in sweat glands and in other regions of the hair follicle [17, 20, 23–29]. Most importantly, stem cells located in epidermal appendages can be efficiently recruited to repair a cutaneous wound and restore the epidermal barrier.

Identification of slow-cycling cells (label-retaining cells or LRC) in the basal layer of the limbal epithelium of the mouse has led to the notion that the limbus is the niche for the stem cells responsible for the long-term renewal of the cornea [30]. In this model, corneal stem cells reside in the basal layer of the limbal epithelium and renew through asymmetrical divisions. A dividing limbal stem cell supposedly generates two daughter cells with differing fates; the stem daughter remains in the limbus, whereas the transient amplifying daughter exits it [31]. The TA cell then migrates centripetally to the central part of the cornea while dividing a few times during its journey. Hence, epithelial cells that are continuously shed from the corneal surface are efficiently replaced. However, it makes the cornea an exception among stratified epithelia that otherwise renew by resident stem cells. We have proposed that the cornea is self-maintained like other SSE and that the limbus is required only for major corneal repair and not for steady state renewal [32].

In the human, the epidermis [33], the hair follicles [20] and the limbus [34] contain keratinocyte stem cells that can be efficiently expanded *ex vivo*. Under appropriate culture conditions, these stem cells adopt the phenotype of a holoclone

[33]. Holoclones can undergo more than 180 doublings when serially subcultured, while generating progressively growing colonies constituted of small multiplying cells [20, 35]. When properly transplanted, holoclones retain the capacity to permanently reconstitute the epidermal barrier [2–5] or the corneal surface [8, 36]. Keratinocyte stem cells, like any stem cells, make choices that include self-renewal, quiescence, division, differentiation and death. Choices are influenced by intrinsic and extrinsic factors. Indeed, keratinocyte stem cells of the epidermis and of the ocular surface need to adjust to varying temperature, pH, nutrients, oxygen and shear stress because of their unique location at the interface of the body with the outside world. It is thus crucial to understand how stem cell behavior is regulated *in vivo* and *ex vivo*. The capacity to manipulate stem cell choice to produce enough differentiated cells while maintaining the pool of stem cells for proper tissue homeostasis, repair and regeneration is a major scientific challenge. One can predict important therapeutic applications, e.g., in wound healing – if successful.

The skin and the ocular surface are ideal for *ex vivo* cell and gene therapy because the epithelial and mesenchymal stem cells they contain can be extensively cultured and transplanted. Today, cell therapy is an option to restore tissue function and integrity when the stem cells of the skin (e.g., in a third-degree burn) and of the ocular surface, (e.g., in a limbal deficiency) are lost [37]. In 1983, Howard Green and colleagues in Boston performed the first transplantation of autologous keratinocyte stem cells onto extensively burned patients [2]. The technology is now part of the toolbox to treat third-degree burned patients worldwide [1, 3, 4, 38, 39] and several biotechnology companies (e.g., in the US, France, Italy, Australia, Japan, and South Korea) provide the service of cultivating autologous keratinocyte stem cells for cell therapy. Stem cell therapy is also used in reconstructive surgery to enhance healing of donor sites, to prevent cheloid recurrence, after excision of giant congenital nevi and tattoos, and in urology and oral pathology (reviewed in [6]). Nevertheless, transplanted cultured keratinocytes only regenerate epidermis, explaining why patients and surgeons demand that the functionality and the esthetics of the regenerated skin improve, which means reconstruction of sweat glands and hair follicles, a thick dermis and a homogeneous pigmentation. It is a challenge that necessitates a better comprehension of the developmental mechanisms involved in the morphogenesis of epidermal appendages; even if significant progress has been made in laboratory animals, thanks to long-term transplantation assays [17] and functional genomics [12], it will be a long time before functional hair follicles and sweat glands are reconstructed in humans.

Besides the treatment of extensive burns, the most impressive application of *ex vivo* cell therapy in SSE is the reconstruction of the corneal epithelium in severe corneal deficiencies [8, 36]. Limbal holoclones can regenerate a corneal epithelium when transplanted, a therapeutic gesture usually associated with a keratoplasty that can result in the dramatic improvement of vision in patients with severe sight deficiency [8, 36]. *Ex vivo* gene therapy for disabling hereditary skin diseases is also an impressive outcome of keratinocyte stem cells, even if it took 20 years from the proof of principle that human keratinocytes stem cells could be

efficiently transduced by means of recombinant retroviral vectors to the first trial in humans [5, 40]. The demonstration that autologous keratinocyte stem cells genetically corrected *ex vivo* can permanently repair the epidermis of a patient suffering from junctional epidermolysis bullosa brings hope to patients suffering from disabling hereditary skin diseases.

Autologous dermal fibroblasts can also be expanded in culture and used in combination with cultured autologous keratinocytes to treat small chronic wounds (e.g., leg ulcers) [41]; however, a technology tailored to a single patient is time consuming, with uncertain economic viability. Cell therapy for chronic wounds is therefore the field of cultured allogenic dermal fibroblasts, sometimes in combination with allogenic keratinocytes; several biotechnology companies worldwide propose allogenic fibroblast-based products [42, 43].

5.3 Future Challenges

5.3.1 Comprehending Engraftment

Identifying, isolating and cultivating *ad hoc* stem cells is surely difficult, but having stem cells survive transplantation, engraft and durably perform is even more challenging. Engraftment is the quintessence of stem cell behavior as it draws on all stem cell basic functions, i.e., homing, attachment, migration, proliferation, fate choice, renewal, differentiation and death. In a normal situation, these decisions are tightly controlled and influenced by the microenvironment (the niche). In therapy, the microenvironment may be diseased, damaged by the preconditioning treatment, or even completely missing as in third-degree burns or limbal deficiencies. Consequently, transplanted stem cells have to adapt to an environment that is far from ideal, if not hostile. Several observations support the notion that stem cell engraftment is not optimal. The necessity to transplant a large number of bone marrow stem cells per kg of body weight [44, 45], when a single hematopoietic stem cell is theoretically enough to reconstitute the marrow for a lifetime [46], is clearly an indication. Engraftment of fetal cells in the brain for neurodegenerative diseases is also largely uncontrolled [47, 48]. Similarly, engraftment of autologous keratinocyte stem cells is often unpredictable, ranging from excellent to poor [1, 3, 4], but, surprisingly, there is no report in the literature on the mechanism of engraftment of cultivated epidermal stem cells, even after 25 years of use worldwide [6, 37]. The reasons for it are many, including a lack of academic interests because of the difficulty in thoroughly studying stem cell engraftment in human for obvious ethical reasons, and because of the variability in surgical procedures. In addition, the industry has not anticipated that mastering engraftment will result in more efficient cell products. Consequently, transplantation guidelines are mainly based on clinical experience rather than scientific facts, and keratinocyte cell therapy has not significantly evolved since 1984 (<http://www.genzyme.com/business/biosurgery/burn/epicel>).

Comprehending engraftment is surely a challenge that can be tackled in large animal models recapitulating conditions observed in the human.

5.3.2 *Stem Cell Plasticity*

There are circumstances in which there are no available stem cells to initiate a culture, leading to the notion that stem cells of SSE may possibly substitute for each other. This has justified the transplantation of cultured stem/progenitor cells from the oral cavity onto the ocular surface to repair invalidating corneal deficiencies [7]. Even if patient sight improves, long-term phenotypic conversion of the oral epithelium in corneal epithelium remains to be demonstrated [49]. Indeed, epithelial plasticity is related to metaplasia, a situation in which an epithelium adopts the phenotype of another epithelium [50]. It is known that a human cornea can spontaneously undergo epidermal metaplasia, resulting in corneal opacity and impaired vision, and it is thus important to understand the cellular and molecular mechanisms that control epithelial plasticity because differentiation to an undesired lineage may be detrimental.

5.3.3 *Ex Vivo Gene Therapy*

Safety is an essential prerequisite for *ex vivo* gene therapy. Autologous diseased stem cells are transduced by means of recombinant viral vectors bearing an appropriate cDNA to repair the genetic deficit (for example, *Col7A1* for recessive dystrophic epidermolysis bullosa) and reverted to a normal phenotype; the engineered stem cells are then transplanted back onto the patient to improve or even cure the disease. In that approach, it is extremely difficult, if not impossible, to determine the insertion sites of the engineered virus in all infected stem cells, a serious concern for regulatory affairs that ultimately grant permission to transplant. The main concerns are (1) that the medicinal gene is not inserted in a region of the patient genome that could be deleterious (e.g., favoring aberrant expression of a proto-oncogene and cancer), (2) that the engineered stem cells are not cancerous, and (3) that the transplanted stem cells do not disseminate to vital organs of the body (e.g., the lungs, the brain, the liver, the spleen, or the kidneys). The design of self-inactivating (SIN) vectors represents a major improvement and reduces the risk of insertional mutagenesis and activation of a proto-oncogene. Similarly, the determination of the proviral insertion sites should permit to eliminate those stem cells at risk from the transduced population, but this remains a technological challenge. A promising strategy is to fully characterize a clonal population of phenotypically reverted stem cells obtained from the multiplication of a single transduced stem cell. Unambiguous demonstration of safe genomic insertion of the provirus and that the progeny of the transduced stem cell is not tumorigenic fulfill the most stringent safety requirements of

regulatory affairs. Obviously, a requirement for a successful clonal strategy is an efficient cell culture technology, as for keratinocyte stem cells. Our laboratory is currently developing this strategy for the treatment of recessive dystrophic epidermolysis bullosa that results from mutations in the gene encoding type VII collagen, a protein that participates in the formation of anchoring fibers.

5.3.4 Embryonic Stem Cells and iPS

The isolation of human embryonic stem cells (ES cells) [51], and more recently of adult human cells reprogrammed to ground state pluripotency, termed induced Pluripotent Stem Cells (iPS) [52], has generated a great amount of hype. Interestingly, iPS can also be obtained by reprogramming adult human keratinocytes [53]. Although robust keratinocyte differentiation can be obtained from human ES cells and iPS [54–56], potential clinical applications remain elusive, but iPS-derived keratinocytes may be of interest for disease modeling and drug testing.

In conclusion, keratinocyte stem cells are pioneers in the field of *ex vivo* autologous cell and gene therapy. Future research should aim at improving efficacy of the transplantation of autologous keratinocyte stem cells, at regenerating hair follicles and sweat glands, at exploring the plasticity of keratinocyte stem cells isolated from various stratified epithelia and at improving safety of *ex vivo* gene therapy. Finally, the capacity to robustly differentiate ES cells and iPS along the keratinocyte lineage opens interesting avenues to disease modeling.

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Chapter 6

Towards a Cell Therapy for Muscular Dystrophy: Technical and Ethical Issues

Giulio Cossu

Abstract Cell therapy is moving into clinical experimentation for a number of genetic diseases, including muscular dystrophy. Cell transplantation offers new hopes for so far incurable diseases but the road to success is still long and difficult, since major obstacles remain to be overcome. These include technical hurdles such as isolation, expansion, genetic correction, and storage of cells, validation and diffusion of the protocols. In addition, serious ethical issues such as patient and donor selection, and, related to these, the increasing costs of these therapies need to be solved before cell therapy may move into a standard therapeutic intervention.

Keywords Cell therapy • Muscular dystrophy • Patient selection • Rare diseases • Trial costs

6.1 Introduction

Muscular dystrophies are a family of diseases that primarily affect skeletal muscle; they are caused by mutations in a large number of genes, mainly encoding cytoskeletal and membrane proteins. They progressively compromise patient mobility and quality of life, and in the most severe cases lead to complete paralysis and premature death. Right now steroids represent the only palliative therapy; however several novel strategies are entering or are ready to enter clinical trials. These include drug, gene and cell therapies; the latter is the topic of this chapter, where recent advances will be outlined together with remaining technical and ethical problems to be solved.

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6.2 The Muscular Dystrophies

Despite their molecular and clinical heterogeneity, all muscular dystrophies cause primary wasting of skeletal muscle, and secondarily compromise cardiac and respiratory functions which, in the most severe Duchenne muscular dystrophy (DMD), are the leading causes of premature death [1]. In many cases, the mutation affects proteins that form a link between the cytoskeleton¹ and the basal lamina.² Absence of one protein often causes the disassembly of the whole multiprotein complex associated with dystrophin,³ leading to increased fragility of the sarcolemma,⁴ especially during intense contractile activity. This, in turn, results in increased calcium entry (although the molecular mechanisms have not been elucidated in detail) and focal or diffuse damage to the fiber [2]. Damaged or dead fibers can be repaired or replaced by satellite cells [3]. These cells, which were originally identified because of their location between the basal lamina and the membrane of the muscle fiber, are now considered the resident “stem-like” cells in skeletal muscle. They are responsible for muscle growth and regeneration in postnatal life [4]. However, dystrophic satellite cells share the same molecular defect and produce fibers that are also prone to degeneration. With time, the population of satellite cells is exhausted and the muscle tissue is progressively replaced by connective and adipose tissue.

Despite the almost complete identification of the responsible genes and a relatively well understood underlying pathogenesis, muscular dystrophies remain among the most difficult diseases to treat. This is mainly due to the fact that skeletal muscle is the most abundant tissue of the body and is composed of large multinucleated fibers, whose nuclei cannot divide. Consequently, any cell or gene replacement strategy must restore proper gene expression in hundreds of millions of postmitotic nuclei, which are embedded in a highly structured cytoplasm and surrounded by a thick basal lamina.

6.3 Strategies to Replace Affected Cells

The original demonstration by Partridge and his colleagues that myoblasts can be transplanted into mouse dystrophic muscle and give rise to dystrophin-expressing myofibers [5] led to several clinical trials in the early 1990s that demonstrated safety but absence of evident functional benefit in the injected muscles. Failure was mainly the result of poor migration and survival of donor myoblasts, worsened by

¹Cytoskeleton=a protein network that controls cell shape and its changes during locomotion or contraction

²Basal lamina=a proteic lamina that ensheaths each muscle fiber

³Dystrophin=a cytoskeletal protein, absent in Duchenne muscular dystrophy, that maintains muscle fiber integrity

⁴Sarcolemma=the plasma membrane of the muscle fiber

the immune response elicited [6]. Subsequent experimentation has been devoted to optimization of this technique, and a Phase I clinical trial has been completed [7]. Although encouraging results have been obtained by local administration, a widespread effect is still limited by the impossibility of delivering myoblasts systemically through the circulation.

In the last decade, several still ill-defined stem/progenitor cells have been isolated from different adult tissues, including bone marrow-derived multipotent adult progenitors (MAPS), blood- and muscle-derived CD133⁺ cells, muscle-derived stem cells (MDSC) and mesoangioblasts [8–11]. These cells have been partially characterized and used in animal transplantation experiments; the results have opened up new possibilities for cell therapy in muscular dystrophy. A few promising examples of stem cells are described below.

6.4 Bone Marrow-derived Stem Cells

Several studies have demonstrated that wild-type (wt) total bone marrow-derived cells or selected sub-populations (BM-SP) are incorporated into regenerating skeletal muscle fibers when transplanted into dystrophic mice [12–17]. However, in some cases transplanted cells failed to restore expression of the wt protein, suggesting that under standard conditions they have little therapeutic potency [18, 19]. In other cases, results have been more encouraging. For example, bone marrow mesenchymal stem cells, which show little myogenic differentiation, became highly myogenic when engineered to express raised levels of intracellular Notch⁶ protein, and produced dystrophin in many fibers when transplanted into mdx mice [20]. In the case of 133+ cells, a Phase I study was completed demonstrating safety of auto-transplantation in a small muscle of the hand of un-modified, DMD cells [21]. Clinical trials may also be planned with other types of mesodermal stem cells in the future, even though pre-clinical work is not completed for many of them.

6.5 Mesoangioblasts

So far, systemic delivery in dystrophic mice and dogs has been performed only with mesoangioblasts, which are vessel-associated progenitor cells [11]. Intra-arterial transplantation of donor mesoangioblasts ameliorated defective muscle structure and function in dystrophic mice [22] and dogs [23]. Recently, similar results were achieved with mesoangioblasts transplanted into mdx/utrophin⁷ null mice [24].

⁵CD133=an antigen present on the surface of certain stem cells

⁶Notch=a membrane protein regulating cell fate

⁷mdx/utrophin null=a mouse with very severe muscular dystrophy due to simultaneous absence of dystrophin and the similar protein utrophin

Moreover, isolation and characterization of the human counterparts of mouse mesoangioblasts from adult skeletal muscle revealed that these cells comprise a subpopulation of pericytes, cells associated with the endothelium that give rise to the vessel smooth muscle layer. Pericytes can be expanded *in vitro* and differentiate at elevated frequency into multinucleated muscle cells [25]. In addition to these results, pre-clinical work has been completed with extensive toxicology studies where normal dogs have been transplanted with a protocol similar to that planned for patients, but with a maximal dose of cells four times higher. The effect of cyclosporine A⁸ (CSA) alone, a matter of controversy that followed the publication of the dog study [26, 27], has been investigated and shown to decrease by 40% the force of contraction of dystrophic dogs; this indicates that the beneficial effect of donor mesoangioblasts occurred despite, and not because of CSA. The GMP⁹ production of donor mesoangioblasts is in progress at the time of writing. Therefore, an open-label, non-randomized, mono-center Phase I/II trial is planned for the spring of 2011 at the San Raffaele Hospital, Milan. The protocol is based on serial multi-district, intra-arterial transplantation of donor mesoangioblasts from HLA (human leukocyte antigen) identical siblings. In June 2009 a preliminary trial began, dedicated to the validation of outcome measures in 28 DMD patients, aged 5–11, six of them being eligible because of an HLA-matched, non-carrier¹⁰ sibling. Three of these six children will undergo cell transplantation and, in the absence of adverse events, will complete the study in the fall of 2011. These children will be treated with Tacrolimus and, should the transplantation result in a significant and persistent improvement of motility and clinical conditions, will continue immune suppression treatment indefinitely or at least until a clinical benefit will be clearly detectable. Of course, immune suppression, even at the low dose necessary for an HLA matched transplant, is accompanied by long-term complications, and it will be difficult to determine whether the transplantation-related benefit will outweigh the negative consequence of Tacrolimus treatment.

6.6 Future Autologous Cell Transplantation

In the future, cell therapy with autologous, genetically corrected cells will certainly represent an ideal strategy, as it will avoid potentially life-long immune suppression, required to prevent immune rejection of donor-derived cells. This may be achieved with several approaches that must, however, take into account the very large size of the

⁸Cyclosporine A=an immune suppressive drug

⁹GMP=Good Manufacturing Practice, a method to produce clinical grade cells, necessary for transplantation in patients

¹⁰Non carrier=a sister that does not carry the DMD mutation in one X chromosome and is therefore completely healthy

dystrophin gene, whose cDNA¹¹ is 14 kb¹² and does not accommodate into current integrating vectors¹³ [28]. These approaches essentially translate into *ex vivo* gene therapy strategies currently used for direct gene transfer or correction in the patient's muscle. Without going into much detail, these strategies can be summarized thus: (a) expression of a truncated but functional form of the protein (mini- and micro-dystrophin); (b) skipping of dystrophin gene exons containing a mutation [29] to restore the reading frame (through repeated administration of small oligonucleotides or integrating vectors expressing small nuclear RNA, both engineered to recognize the donor and acceptor sites of the exon[s] to be skipped, with only the latter being possible in cells); and (c) transfer of human artificial chromosomes or transposons¹⁴ encoding the whole dystrophin locus [30, 31], ideally the most desirable correction, but technically the least efficient in terms of transfer into patients' cells. In the more distant future, patients' cells, reprogrammed to an "embryonic" state, will be easier to transduce with vectors described in (c), but this will require a significant amount of work.

6.7 Patient Selection

At variance with drug-based clinical trials, cell and gene therapy protocols involve, at least initially, a small number of patients. This is due to both the risks associated with novel therapies and to their high cost. From an ethical perspective, as there are no papers on cell therapy, it is possible to refer to a recent paper discussing this issue for gene therapy [32]. Besides more general problems, such as the balance between potential risks and benefits, the initial selection becomes an issue when the number of eligible patients exceeds the number of those who will be actually selected. Once medical selection criteria (age, type of mutation, clinical condition, presence of suitable donors, etc.) are matched, it becomes problematic to choose one patient versus another. The truth is that, at least in the beginning, possible benefits may be outweighed by the risks associated with the novel procedure so that selection may not be necessarily an advantage. Indeed, cases have occurred where gene therapy led to patient death. These cases are quite rare, considering the high number of trials already carried out; moreover, other patients of the same trial benefited from it and are now alive and well while they would have not survived without the therapy. A more detailed discussion of this issue is reported elsewhere [33].

The situation could become more complicated, if a preliminary trial had shown lack of adverse events and significant efficacy. Then it would become morally

¹¹ cDNA = a fragment of DNA corresponding to the mature messenger RNA and able to produce a complete and functional protein

¹² kb = kilobase, a measure of the length of nucleic acids

¹³ Integrating vectors = viral vectors (usually RNA based) able to insert into the host cell genome

¹⁴ Transposons = segments of DNA able to move in the mammalian genome from one site to another

imperative to treat all eligible patients. However, money and logistics would become major hurdles. Beside the economical problems described below, it may become difficult to treat all eligible patients for practical problems. For example, GMP facilities are still few and usually cannot process cells from more than a very few patients at the same time. For diseases with thousands of patients, such as muscular dystrophy or cystic fibrosis, this may become a serious problem, considering the fact that with time the disease progresses to a stage when no therapy may bring any hope of efficacy. On the other hand, several different approaches are currently becoming available for these diseases and only a subset of patients may be eligible for one specific therapy. In the case of muscular dystrophy, for example, only patients with specific mutations can be treated with oligonucleotides or morpholinos, while only patients with an HLA-matched, non-affected sibling are eligible for donor-cell therapy. Obviously, only time will tell whether these will become significant problems and for which disease. All this does not apply to those very rare diseases such as the congenital immune deficiencies that until today represent the only full success of gene-corrected stem cell therapy.

6.8 Donor Selection

Donor selection has already posed several important ethical issues for organ as well as for bone marrow transplantation (BMT). In the first case, the irreplaceable nature of the transplanted organ makes the situation quite different from BMT, an appropriate example for future selection of donors in cell therapy [34]. In BMT, as in several other possible cases, donation implies a small but invasive procedure that may cause discomfort to donors, especially if they are children, the most likely case for genetic disorders. Children may feel obliged to donate against their will, especially if the donation should be repeated and under pressure from the parents. Common sense suggests that children, unless very young, may understand why they are requested to undergo a specific procedure and the potential benefit that this procedure may cause to their sibling. Overall, donor selection appears to pose far fewer relevant issues than patient selection and should not be a major issue in the future of cell therapy, especially for muscular dystrophy, where a small biopsy from skeletal muscle (or possibly other easily accessible sources) is a simple, standard, and almost painless procedure.

6.9 Costs

With the advent of molecular medicine, the hope of curing fatal diseases became a serious possibility, and in some cases a reality. However, the costs to the health systems rose dramatically, and this alone became a serious problem [35]. To give an example, a first trial of cell transplantation for Duchenne dystrophic patients is

going to cost, including pre-clinical work, toxicology, cell production, clinical costs and additional expenses, more than 2.5 million euros. Even assuming that certain costs will not be incurred again in subsequent trials, the costs per patient would remain extremely high. Moreover, while it has been relatively easy to raise money from funding agencies for a novel trial, it would become much more difficult to raise additional money for subsequent trials that do not present elements of novelty, in respect to the one previously funded. Patient Association would instead continue to fund such trials but this might not be enough and may create a condition of great stress at the idea of a potentially efficacious but economically unaffordable therapy. Several points need to be discussed here: (a) costs tend to be progressively reduced with increasing numbers of patients being treated and progressive savings on procedures and reagents; (b) a DMD patient will cost the national health system, from diagnosis to death, an enormous amount of money – for supportive therapy, continuous clinical testing, physiotherapy, surgery, daily assistance, etc. Overall, this money is probably more than what would be needed for efficacious therapy, but the cost would be diluted over several decades rather than being needed in one single step; and (c) some of the costs associated with cell and gene therapy trials are excessive and unnecessary, likely dictated more by the will of large Pharma to make very difficult, if not impossible, the trials by academics or small companies rather than by the need to guarantee patient safety.

6.10 Conclusions

This is a crucial period for research on muscular dystrophy as well as for many other genetic diseases. A decade or more of pre-clinical work has set the basis for safe and hopefully efficacious clinical translation, and this applies particularly to stem cells, which however raise a series of technical and ethical issues. Recently, the International Society for Stem Cell Research has published Guidelines for Clinical Translation of Stem Cell Research (<http://www.isscr.org/>). These Guidelines “provide a roadmap for the responsible development of safe and effective stem cell therapies for patients. They call for rigorous standards in the development of such therapies including stringent evaluation and oversight, a thorough informed consent process, and transparency in operations and reporting” [36].

These standards are met by research on muscular dystrophy and the time is probably ripe for starting clinical trials. Only time will tell the outcome, the answers provided and the new issues to be faced.

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Chapter 7

Towards Modeling and Therapy of Genetic Diseases Using Pluripotent Stem Cells

Petr Dvořák

Abstract Recent advances in basic and preclinical research on human embryonic stem (hES) cells and induced pluripotent stem (hiPS) cells have created the potential for a revolutionary change in medicine. Thanks to several technological developments, both of these cell types can now be derived, expanded, and differentiated under conditions that are compatible with use in cell replacement therapies. Moreover, hiPS cells can be generated from patients' somatic cells, providing a technically feasible means of overcoming immunological incompatibility between patient and donor cells. All of these achievements have already sparked great interest in the pharmaceutical industry. Along with the continuing efforts of stem cell researchers, this will undoubtedly bring about the future introduction of embryonic and induced pluripotent stem cell science into the clinic. Replacement of missing or damaged cells by healthy, functional cells derived in vitro from pluripotent stem cells is the most obvious clinical application. Eventually, ex vivo repair of genetic mutations in patient-derived somatic cells that are reprogrammed into pluripotent cells and then differentiated into the desired cell types will permit transplantation back into the patient without any risk of immune rejection. A less difficult task, and therefore a shorter-term goal, is to generate mutant pluripotent stem cell lines to facilitate studies of the pathophysiology of various human genetic diseases and for use in drug screening. Indeed, many researchers now see disease modelling and drug screening using mutant cell lines as the first and most important goal of stem cell research, and view cell replacement therapy based on hES or hiPS cells as an extremely challenging and distant goal. Here, the advantages and limitations of the current strategies and the most important achievements in these two streams of pluripotent stem cell research are reviewed.

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7.1 Introduction

Eleven years have passed since the derivation of embryonic stem cells from human blastocysts [1]. At that time, groundbreaking technologies had already been developed for directed differentiation of mouse embryonic stem (mES) cells into various functional cell types. This permitted not only the investigation of the therapeutic potential of mES cell-derived differentiated progeny in mouse transplantation experiments, but also efficient gene targeting to mimic the genetic bases and pathophysiological features of human diseases in mouse models (reviewed in [2]). As a result of this progress, the expectations for hES cell-based therapy to treat various human diseases, including those with a genetic basis, were initially enormous. Such hope on the part of patients is easily understandable, and helps to encourage and accelerate progress in hES cell research. However, a number of technical hurdles gradually cooled down this early optimism. Moreover, the embryonic origin of hES cells immediately triggered an ethical debate, resulting in extreme caution in the discussion of potential hES cell-based therapies. Although it is now apparent that transplantation of hES cell-derived cells and tissues as a routine means of treating disease is still far away, several achievements have provided reassurance that this is still a promising approach. For example, Geron, the US-owned biotech company, developed hES cell-derived oligodendrocytes that were able to improve locomotion in animal models with spinal cord injury. However, the path from such translational research to potential clinical applications is a long one. This work was first published in May 2005 and was subsequently strengthened by other convincing data [3, 4]. At the beginning of 2009, Geron finally received clearance from the US Food and Drug Administration to initiate a phase I clinical trial in patients with complete thoracic spinal cord injury. Geron expects that the clinical trial will be initiated in 2010, 5 years since their initial findings.

More recently, re-programming of human somatic cells into pluripotent stem cells represented a new milestone in efforts to bring stem cell research to the clinic [5]. hiPS cells resemble hES cells, but are derived from somatic cells, introducing an ethically acceptable approach towards custom-tailored cell therapy without immunological barriers. However, new challenges have been identified, mainly with respect to techniques for inducing pluripotency in differentiated cells and the unpredictable genomic and epigenomic alterations in reprogrammed cells [6, 7]. Although the latest achievements are promising, a more realistic short-term application of both pluripotent stem cell types in medicine is the creation of cellular models for human disease, rather than stem cell-based therapies.

It is also notable that stem cell therapy as a strategy for treating human disease is not new; several such methods have already been used to treat human genetic diseases for decades, including bone marrow and peripheral blood transplantation.

For example, this approach has been used to treat chronic myeloid leukemia, which is caused by a single molecular event, namely, BCR-ABL rearrangement. Taking advantage of previous developments, monogenic diseases may be the best targets for cell replacement therapy using hES cells, or patients' hiPS cells combined with ex vivo gene repair. These new options offered by human pluripotent stem cells, including genetic disease modeling, will shape the future of medicine.

7.2 Advantages and Limitations of Genetic Disease Modeling Using Pluripotent Stem Cells

An important question is whether we need human pluripotent stem cell-based genetic disease models when we already have a number of mutant animal models in the form of targeted or spontaneous mutant mouse strains. In addition, some models of human genetic diseases could be established in primary cells from patients or by gene targeting in transformed immortalized human cell lines. Not only do human pluripotent stem cells offer the advantage of human-specific genetic and epigenetic programs, but they can also self-renew indefinitely and differentiate into virtually any cell type. Thus, developmental processes may be recapitulated in human pluripotent stem cells. Moreover, the clinical symptoms for the same genetic lesion may differ between humans and animal models. Finally, human stem cell-based models permit the generation of differentiated cells that are not easily accessible from tissues or organs.

One potential limitation of cellular models of genetic diseases is the lack of cellular crosstalk that normally exists within complex tissues and organs. However, since genetic mutations usually manifest themselves in a cell-specific manner, this may not be as great a concern as it would appear. In addition, the clinical symptoms of even monogenic diseases vary between individual patients. Stem cell-based models might be developed from many individuals with different genetic backgrounds to get a more complex picture of disease pathophysiology at the cellular and molecular level (reviewed in [8]).

A serious, but often underestimated obstacle to genetic disease modeling with pluripotent stem cells, is the instability of cultured stem cells, which may lead to the clonal evolution of dominant mutant genotypes [9–13]. This complicates functional and molecular analyses and is a common problem for all cultured cell lines, regardless of their origin, and must be considered for each specific disease model. However, an advantage of embryo-derived cells over somatic cells is that they appear to have a lower mutation frequency, as demonstrated by loss of heterozygosity studies [14]. In hES cells, long-term culture predominantly results in amplification of chromosomes 12, 17, 20, and X [11–13, 15], likely due to association with gene clusters critical for adaptation of the cells to culture conditions. Although this issue has been discussed in the context of cell-replacement therapies, it could also hamper efforts towards ES cell-based genetic disease modeling. The genomic stability of hiPS cells is still being investigated, but it is

likely that a similar problem will be encountered, as in hES cells. In summary, there appear to be both advantages and obstacles to using human pluripotent stem cells for genetic disease modeling.

7.3 Strategies for Establishing Models of Human Genetic Diseases from Pluripotent Stem Cells

In some cases, a specific genetic change results in a particular clinical phenotype. However, genetic diseases or disorders can be monogenic, sex chromosome-linked, and/or polygenic, and result in complex phenotypes. In addition, some genetic diseases are linked to abnormalities of particular autosomes, such as missing or extra copies and chromosomal rearrangements caused by incorrectly repaired chromosome breaks. Moreover, rare genetic diseases also arise from mutations in non-chromosomal mitochondrial DNA. Importantly, even for the majority of seemingly simple monogenic genetic diseases, the clinical phenotype represents the manifestation of a long chain of causative factors and complex genetic interactions that might vary from individual to individual. Long-term mutagenesis programmes in the mouse are currently focusing on the development of clinically relevant mouse models of genetic diseases [16]. However, although the mouse is currently the model organism of choice for studying human genetic disease due to its fully sequenced genome and established genetic manipulation techniques, it differs from humans anatomically, physiologically, and developmentally. Equally problematic is the fact that many human genes do not have true orthologues in mice. Therefore, clear correlations between genotype and phenotype are unlikely even with the most sophisticated tools, such as knockout and transgenic mice and humanized mouse models.

The availability of technologies to derive and culture human pluripotent stem cells offers a new tool for studying human genetic diseases. Since hES and likely also hiPS cells can be induced to differentiate along the same paths taken during early development or in adult tissues and organs, virtually any stage of differentiation can be investigated. There are basically two strategies for establishing models of human genetic diseases from pluripotent stem cells (reviewed in [17]). The first one is to develop *de novo* models from existing, normal stem cell lines by mutagenesis. This could be accomplished either by homologous recombination (HR)-mediated targeting of a specific disease-related gene [18–20], or by gene-trap mutagenesis, which generates random loss-of-function mutations that can be identified through sequence tags and sequence-based screens [21]. The efficiency of these methods in hES and hiPS cells remains low, mainly due to the difficulty of selecting targeted clones, since these cells have low survival rates after dissociation and low cloning efficiency. However, recent advances, such as the use of engineered sequence-specific enzymes, can enhance the efficiency of HR-mediated mutation of disease-related genes [19, 20]. Moreover, the techniques can be made

safer by removing potentially problematic antibiotic resistance cassettes after positive selection of genetically modified clones [22].

The second strategy is the establishment of cellular models of human genetic diseases from disease carriers. For example, hES cell-based disease models could theoretically be generated by fusion of a healthy, enucleated oocyte with a somatic cell from a patient with a genetic disease, followed by *in vitro* development to the blastocyst stage and derivation of a hES cell line. This option has never been verified or perhaps even tested, due to ethical and technical obstacles. On the other hand, hES cells obtained from embryos following preimplantation genetic diagnosis represent an ethically acceptable and technically achievable disease model (reviewed in [23, 24]). Indeed, hES cell lines have successfully been derived from developmentally arrested embryos [25] or even single blastomeres [26]. However, the applications of such cell lines are limited, perhaps again due to technical obstacles. In contrast, hiPS cells offer the advantages of a robust derivation method and the near absence of ethical barriers associated with the donor cell source. Thus, genetic disease-specific iPS cell lines have rapidly taken center stage. In several proof-of-principle experiments, hiPS cell lines were derived from patients with monogenic and polygenic diseases and are expected to make significant contributions to the investigation of these diseases (reviewed in [27]). However, it should be noted that the most efficient tool to date for generating induced pluripotent stem cells involves the delivery of four or fewer reprogramming genes into patient's cells using viral vectors. Since viruses integrate randomly into the host genome, this can lead to dysregulation of endogenous genes or cause further mutations that interfere with disease-linked genetic lesions. However, it is likely that these technical hurdles will be resolved by a combination of methods: use of small chemical molecules to replace one or more reprogramming factor, enhancement of reprogramming efficiency [28–31], non-integrative gene delivery, and transient expression of reprogramming factors [32, 33].

An important issue is the banking and registering of pluripotent stem cell lines that might be used as models of human genetic diseases. Several international or national stem cell banks and registries interested in collecting and/or listing lines harboring genetic diseases with a high standard of input data have already been established. These include the European Human Embryonic Stem Cell Registry [34], UK Stem Cell Bank, the Spanish Stem Cell Bank, the US National Stem Cell Bank, the US-based NIH Human Pluripotent Stem Cell Registry and the Harvard Stem Cell Institute core facility for production of disease-specific hiPS cell lines, as well as the RIKEN BioResource Center Cell Bank that is based in Japan. For genetic disease modeling, a large collection of model cell lines for each disease will be necessary to understand the pathobiology of the disease and to develop new drugs that take into account the diverse genetic background of the human population. Such centralized activities not only have practical implications, but also have ethical and political meaning, stimulating international scientific collaboration, creating public transparency in pluripotent stem cell research, and stimulating expert debate about pluripotent stem cell-related regulatory issues.

7.4 Recent Advances in Modeling and Therapy of Human Genetic Diseases Using Embryonic and Induced Pluripotent Stem Cells

Human ES cells can be differentiated into multiple somatic cell types. Although the differentiation protocols developed for hES cells have not yet been reproduced in hiPS cells, it is reasonable to expect that their differentiation capacity will be comparable to that of hES cells. However, it is possible that hiPS cells retain some gene expression memory of the original cell type from which they were derived [35], suggesting that some differentiation pathways could be favored and thus easier to induce. *In vitro* differentiation of hES and hiPS cells carrying genetic changes that usually result in clinical phenotypes *in vivo* offers an unprecedented opportunity to study the molecular pathology of genetic, particularly congenital, diseases at earlier stages.

An advantage of hES cells is that they can be used to study the pathobiology of genetic diseases that show a high frequency of early lethality. For example, tissue-specific gene expression in hES cells with a spontaneous chromosomal abnormality (45, X0) suggested that early lethality of female embryos with monosomy X, the dominant hallmark of Turner's syndrome, could be caused by defects in placental development [36]. In addition, hES cell lines with a spontaneous, culture-induced gain of chromosome X in originally normal 46, XY cells [11], [our unpublished observations] may be useful for studying Klinefelter's syndrome, the most common genetic cause of male infertility (reviewed in [37]). The molecular mechanisms underlying the phenotype of Klinefelter's patients remain largely unclear, but it has been suggested that the defects result from impaired germ cell development. Thus, studies of the differentiation capacity of 47, XXY hES cells along with gene expression profiles might be extremely informative.

Another sex chromosome-linked genetic disorder, fragile X syndrome, is characterized by expanded CGG nucleotide repeats that result in epigenetic silencing of the fragile X mental retardation 1 (*FMRI*) gene. This syndrome has been also modeled using hES cells derived from genetically diagnosed embryos at preimplantation stages [38, 39]. Since epigenetic silencing occurs upon differentiation of mutant hES cells, it might be possible to prevent *FMRI* inactivation and rescue the abnormal phenotype [39]. Serious human genetic disorder with a completely different pathology, but that is also caused by expanded nucleotide repeats (CTG), is myotonic dystrophy type 1. Dominantly inherited myotonic dystrophy is the most common adult muscular dystrophy. Studies using hES cell lines carrying the myotonic dystrophy type 1 mutation showed that CTG repeats are highly unstable and increase in number and range of variability during prolonged cultivation [40]. This observation could extend our knowledge about the molecular pathology of this disease, since CTG repeats were found to be stable at later stages of embryonic and fetal development. Modest expansion of CAG nucleotide repeats is a typical feature of another incurable genetic disease, Huntington's disease, which manifests in progressive brain neurodegeneration. Several hES cell lines carrying the mutant

CAG repeat allele provide an attractive tool for analysis of neural differentiation in Huntington's disease that is limited only by the refinement and efficiency of differentiation protocols to obtain specific neuronal subtypes [41, 42].

The advantage of having multiple hES cell lines with specific disease-linked mutations is particularly apparent in the case of cystic fibrosis. Cystic fibrosis is a common lethal genetic disease caused by a mutation in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene, which is expressed in epithelial and blood cells. Clinical manifestations include pancreatic insufficiency, pulmonary disease, various metabolic disorders, and male infertility. Although more than 1,500 *CFTR* mutations have been described, only a few have been shown to have functional importance, suggesting that the availability of as many model cell lines as possible might be advantageous (reviewed in [43]). So far, hES cell lines carrying the most common *CFTR* mutation, deletion of phenylalanine at position 508, have been derived [44, 45]. In addition to the exciting potential for understanding the pathophysiology of cystic fibrosis, these cell lines may be exploited for drug screening.

A typical example of the failure of a human disease phenotype to be recapitulated in an animal model is Lesh-Nyhan disease [46]. Mutations in the X-linked hypoxanthine phosphoribosyl transferase (*HPRT*) gene cause accumulation of uric acid and clinical manifestations that include retarded motor development, dystonia and unconscious movements, and self-injury. Using homologous recombination in hES cells, a model for Lesch-Nyhan disease was created that exhibits a typical feature of the disease that was not manifested in animal models – increased production and accumulation of uric acid [47].

One important effort that has thus far been largely neglected is human pluripotent stem cell-based modeling of heritable forms of heart failure [48, 49]. Although numerous mouse models carrying mutations that cause cardiac diseases in humans have been generated, the physiological and genetic differences between mice and humans necessitate the creation of genetically modified hES and patient-derived mutant hiPS cells. This will provide a useful tool for studying the pathophysiological mechanisms of human cardiac diseases.

It is of special note that although many models of human genetic diseases using hES cells have been reported, they have not been significantly further exploited in drug screening or to understand the molecular and pathophysiological mechanisms underlying genetic diseases. One possible explanation for this is that too little time has elapsed since the first derivation of hES cells. However, recent rapid and fascinating achievements with mouse- and human-derived induced pluripotent stem cells suggest other technical reasons, namely, difficulties in derivation and maintenance of hES cell lines following preimplantation genetic diagnosis or with gene targeting in hES cells. Indeed, hiPS cells have already been successfully generated from dermal fibroblasts and epidermal keratinocytes of Fanconi anemia (FA) patients [50]. This autosomal or X-linked disorder is caused by specific mutations in any of 13 genes and is characterized by increased cellular sensitivity to DNA damaging agents and defects in the repair of damaged DNA. This results in progressive depletion of hematopoietic stem cells. Cells from FA patients can be

reprogrammed into pluripotent stem cells and differentiated into disease-free and genetically stable myeloid and erythroid progenitors [50]. This opens up the exciting possibility that such cells could be used for transplantation therapy in FA patients without danger of immunological rejection. Complementary cell therapy using a humanized disease mouse model and mouse-derived induced pluripotent stem cells has been achieved with sickle cell anemia (SCA). A knock-in mouse model of SCA, in which normal mouse globin genes were replaced with mutant human globin genes, developed typical SCA symptoms. The SCA-specific genetic defect of reprogrammed fibroblasts from mutant mice was repaired through homologous recombination, and corrected cells were differentiated into hematopoietic progenitors and transplanted back into irradiated donor mice. Remarkably, all SCA symptoms improved to the point where treated animals were comparable to healthy animals [51].

Similarly exciting outcomes of induced pluripotent stem cell research have been seen for Parkinson's disease (PD), whose etiology was recently shown to be influenced by genetic factors. Indeed, marked improvements in the behavior of rats with Parkinsonian symptoms were observed following therapy with neurons derived from reprogrammed fibroblasts [52]. It is also encouraging to mention the generation of hiPS cells using an improved method that allows excision of reprogramming factors from patients with PD and their subsequent differentiation into dopaminergic neurons [53], the generation of hiPS cells that differentiate into motor neurons from patients with slowly progressing forms of amyotrophic lateral sclerosis [54] and spinal muscular atrophy [55], and the generation of hiPS cells from patients with familial dysautonomia (FD) [56]. Regarding FD, patient-specific hiPS cells are not only capable of differentiating into autonomic and sensory neurons that are depleted in the disease, but have also been used to validate the candidate FD drug kinetin. Given these achievements and the feasibility of generating hiPS cell lines from patients with a variety of genetic diseases, including adenosine deaminase deficiency-related severe combined immunodeficiency, Schwachman-Bodian-Diamond syndrome, Gaucher disease type III, Duchenne and Becker muscular dystrophy, Huntington's disease, juvenile diabetes mellitus, and Down syndrome [57], it appears that with the possible exception of diseases with early embryonic lethality, future genetic disease modeling will focus largely on hiPS cells.

7.5 Future Challenges and Directions

The development of effective treatments for genetic diseases requires a better understanding of the pathophysiological mechanisms involved. This will undoubtedly be best addressed using patient-derived human pluripotent stem cells. However, the use of pluripotent stem cell lines for disease modeling and drug development is still in its infancy. One important barrier may be that the available collections of model cell lines that are relevant to particular diseases are still limited and do not provide enough information about the complexity and/or heterogeneity

of the diseases. It will be helpful to compare several cellular models and possibly identify patient-specific pathogenic pathways or modifier genes.

An even more significant barrier that is common to both disease modeling and stem cell-based regenerative therapy is the genomic instability of cultured pluripotent stem cells. Although there is no clear causal connection between chromosomal abnormalities and/or subtle genomic changes and tumorigenicity of human pluripotent stem cells, extreme caution must be used [15]. This issue is currently being discussed mainly in the context of hES cells, but it will almost certainly be an issue for hiPS cells as well. In addition to the fact that the genes used to produce hiPS cells are known oncogenes (*MYC* and *KLF4*) or are indirectly linked to tumorigenesis (*SOX2* and *OCT4*), undesirable effects from induced epigenetic changes may represent another challenge. However, several promising approaches towards generating safer hiPS cells have already been tested and have shown positive results (reviewed in [30]): (1) the development of efficient differentiation protocols, often combined with cell sorting, to obtain pure populations of donor cells for transplantation; (2) introduction of a suicide gene specifically designed to kill cells that escape the differentiation pathway and are potentially tumorigenic; and (3) selective killing of residual, self-renewing stem cells in cell transplants using chemical tools [58].

Another seemingly elementary but highly challenging task is the large-scale production and quality control of stem cell therapy products [59]. Strict guidelines and regulations will be necessary to ensure the efficiency of differentiation protocols, cost-effective technologies for automated good manufacturing practices (GMP conditions) for cell expansion, and the rapid implementation of specific therapeutic applications.

Challenges may also arise with diseases that affect several cell types. It is not yet clear if pluripotent stem cells should be differentiated into several cell types before transplantation or if transplanted progenitors might effectively differentiate, migrate, and integrate into damaged tissue regions in a hostile environment that is often full of harmful agents. Thus, a better understanding of the interactions between transplanted cells and the endogenous repair processes activated by concomitant treatment, transplantation, or even natural reactions of the organism to disease is necessary.

In summary, there are still many challenges, but by continuing to investigate and translate our insights into preclinical models of human genetic diseases, successful clinical application will ultimately be achieved.

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Chapter 8

Therapeutic Possibilities of Induced Pluripotent Stem Cells

Harold Ayetey

Abstract A fundamental goal of human cell therapy is to regenerate ailing organs affected by congenital and acquired disease processes. Pluripotent stem cells such as embryonic stem (ES) cells can be differentiated into progenitor and fully differentiated cell types of all adult organs such as the brain, pancreas and the heart and therefore represent a promising source of cells for use in cell therapy for a variety of diseases. Importantly, the recent discovery that terminally differentiated somatic cells can be reprogrammed into induced pluripotent stem (iPS) cells with many of the properties of ES cells including the potential to generate diverse adult cell types bypasses important ethical concerns surrounding the derivation and use of human ES cells. Here, the therapeutic promise and limitations of these pluripotent cell types are discussed with a focus on iPS cells and their possible use in regenerative medicine, disease modeling and the development of pharmacological agents.

Keywords Pluripotency • iPS cells • Disease modeling • Cell therapy • Drug screening

8.1 Pluripotent Stem Cells: Potential and Pitfalls

The goal of human cell therapy is to regenerate ailing organs such as the heart, brain and pancreas affected by degenerative disease processes. One approach is to develop a ready supply of suitably defined and safe transplantable “pluripotent”

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stem cell or progenitor cell derivatives. While the successful and established use of donor hematopoietic stem cells (HSC) to reconstitute ablated bone marrow demonstrates the promise of cell therapy, the restricted differentiation potential of blood stem cells render them unsuitable for cell therapy in other organ systems. Pluripotent stem cells such as embryonic stem cells and induced pluripotent stem cells have much broader potential and can differentiate into progenitor and fully differentiated cell types of all adult organs such as the brain, pancreas, and the heart. They therefore represent a promising source of cells for use in cell therapy for a variety of diseases ranging from Parkinson's disease to type I diabetes and heart failure.

Human embryonic stem (ES) cells are self-renewing cells of embryonic origin capable of differentiating into cells of the three embryonic germ layers – endoderm, mesoderm and ectoderm [1, 2]. They present a possible route to regenerate ailing human organs [3, 4]. However, this potential of ES cells for use in cell therapy has been limited by ethical concerns over their embryonic source and the problem of immunological rejection of non-autologous tissue. Although somatic nuclear transfer (SCNT), which results in the reprogramming of an adult cell nucleus housed in a nucleus-free egg into a pluripotent cell, was viewed as a promising way to solve the problem of immune rejection, its use in human cells has been limited by technical difficulties.

Thus, the discovery that fully differentiated adult cells can also be reprogrammed to acquire pluripotency [5–7] represents a major step forward in efforts to transfer the paradigm of HSC therapy in hemato-oncological diseases to other organ systems. These cells, termed iPS cells are derived over several weeks from adult cells, such as skin cells, by forced expression of known pluripotency genes. iPS cells possess many of the characteristics of ES cells [8–11]. They can be propagated indefinitely and can also differentiate into all three germ layers and in theory all tissues and organs of the mature adult. Further, they do not require embryos or oocytes for derivation and if used for autologous transplantation, are anticipated to avoid the problem of immune rejection. By circumventing these key issues, iPS cells bring potential applications such as direct cell therapy, disease modeling and drug screening, a step closer. Some genetic diseases might be treatable by a combination of stem cell transplantation and gene therapy. New strategies for correction of mutations, such as the use of Zinc Finger nucleases [12], artificial restriction enzymes capable of targeting and correcting disease causing genes in complex genomes, may be coupled to cell replacement strategies.

Many studies have shown that iPS and ES cells share many molecular properties, such as similarities in pluripotent gene-promoter methylation patterns and X-chromosome reactivation, which may be related to the two key characteristics of self-renewal and the potential for differentiation into all tissue types [9, 13–15]. However, a recent genome-wide study of gene expression profiles of mouse and human ES cells and iPS cells revealed gene expression patterns unique to iPS cells [16]. The authors suggest that the iPS cell signature gene expression differences may be due to differential promoter occupancy by the reprogramming factors. Reprogramming gene integration and over-expression is also likely to be a contributing factor to these differences since their excision results in a genome-wide expression profile more similar to ES cells. Further, iPS cells generated without detectable genomic integration [7] have greater similarity to human ES cells. This

dissimilarity between iPS cells at the molecular level could result in sufficient functional variation to interfere with the translational use of certain iPS cell lines.

The mechanisms underlying the reprogramming process remain a black box. It is still slow and inefficient and comprises potentially deleterious events. Indeed, it has recently been shown that abrogation of the p53 pathway, regarded as the “guardian of the genome,” improves reprogramming efficiency [17–20]. The use of viruses and other transgene delivery systems that result in genomic integration also remains a concern given the possibility of insertional mutagenesis, activation of oncogenic pathways during the reprogramming process and possible transgene reactivation. Reports of progress with efforts to develop non-viral, non-integrating and transgene-excisable reprogramming strategies including the use of small molecules [21, 22], proteins [23, 24], episomal vectors [7], and piggyBac transposition [25, 26], are encouraging. However, most of these have only been demonstrated in mouse cells and remain unsatisfactory in human systems. The risk of random genome integrations and reactivation of transgene expression hindering differentiation and driving tumor formation therefore still exists.

8.2 Therapeutic Promise

There is some proof of principle data on the potential of pluripotent stem cells for use in regenerative medicine. ES cells have been successfully differentiated into many clinically relevant cell types, including neurons, cardiomyocytes and hematopoietic precursor cells, amongst others [27]. Their therapeutic potential has also been shown in a number of animal models. Examples include the treatment of immunodeficient mice with hematopoietic precursors, the treatment of blind mice with ES-derived retinal cells, and the improvement of motor function in rat models of Parkinson’s disease (PD) [28–30].

However, the etiology of many degenerative disorders remains unknown. Therefore it is unclear how transplanted stem cells might achieve lasting organ regeneration and repair. For example, pre-existing disease processes, if non-cell autonomous, may eventually affect newly transplanted cells. Organ repair might be achieved either by direct engraftment or through the release of growth factors and endogenous repair signals to failing organ tissues without actual physical integration. In HSC transplantation for leukemia, cell replacement therapy is achieved through direct integration of HLA-matched progenitor cells after ablation of the recipient’s bone marrow. In contrast, mesenchymal cells may confer benefit following transplantation for heart failure by delivering factors to target tissues without engraftment [31]. Although much of the research done with ES cells has already been replicated with iPS cells with therapeutic potential in the latter, demonstrated in mouse models of PD [32] and hemophilia [33], there is little direct proof of therapeutic benefit in humans for either cell type. Proposed clinical trials of human ES cells in acute spinal cord injury [34, 35] and retinal disease have yet to begin.

8.2.1 *iPS Cells and Cell Therapy*

Effective tissue repair in humans therefore remains a huge challenge and has yet to be demonstrated. In neurodegenerative diseases such as PD and motor neuron disease, anatomically appropriate and physiologically efficacious engraftment is likely to be a pre-requisite for measurable therapeutic benefit. It is unclear whether functional engraftment will require fully differentiated neural cell types or neural progenitor cells dependent on niche signals. Also, while diseases such as motor neuron disease and idiopathic PD appear to have a genetic component, their etiology, which may be the result of interactions between the environment and their genetic make-up, remains unclear. Conditions such as these may involve epigenetic changes imposed by interaction with the environment. The epigenome is reset during the process of somatic cell reprogramming to pluripotency [8]. Thus, reprogramming of somatic cells with pathologies caused largely by epigenetic processes could result in the generation of disease-free and potentially curative cells after differentiation. Myelodysplastic syndrome is an example of such an epigenetic disorder that could be reversed during the reprogramming process and derived hematopoietic progenitors differentiated into normal blood cells for transplantation. Many conditions could eventually benefit from the advances being made in iPS biology today. A few examples are discussed below.

Cardiac regeneration. Severe heart failure is associated with damage to the myocardium that is irreversible with current medical therapies. The challenge in cardiac regenerative medicine is to establish therapeutic strategies that enhance the regeneration of normal cardiac muscle in the failing heart. This idea has received much recent attention. While bone marrow-derived cells have demonstrated limited, albeit controversial, benefit following cardiac transplantation in humans [36–39], stem cell-derived cardiomyocytes have to date only been tested in rodent models of heart failure [40, 41] and has yet to be demonstrated in human hearts. iPS cells are expected to bypass the problem of immune rejection, but they retain many of the properties that have limited the use of ES cells in the heart, including inefficient cardiac differentiation, poor survival following transplantation, and a tendency to form teratomas [42]. Cardiac progenitor cells that generate cardiomyocytes have been derived from both human ES and iPS cells and may represent a valuable way forward in efforts to achieve cardiac regeneration [43, 44]. Long-lasting functional repair will depend on stable and suitable electromechanical coupling of transplanted cells in host myocardium.

Diabetes. Type-1 diabetes is a commonly occurring childhood disorder and a cause of significant morbidity and mortality and healthcare costs. While traditional therapeutic approaches that utilize exogenous supplies of insulin remain effective, they require a lifetime of injections that carry a risk of lethal hypoglycemia. It is therefore of particular concern that the incidence in children under 5 years of age is predicted to double by 2020 [45]. Transplantation of a whole pancreas and/or islet cells has been performed as an alternative treatment, and although this may result in effective blood glucose control in the absence of exogenous insulin [46–48],

the requirement for immune suppression remains a drawback in these patients. iPS cell-derived islet cell transplantation may therefore be beneficial, although the differentiation of pluripotent stem cells into mature, fully differentiated and functional islet cells is particularly challenging and involves recapitulation of key developmental steps *in vitro*. Recent advances in the directed differentiation of ES and iPS cells to insulin-producing and glucagon responsive cells could represent an important step towards transplanted cell-mediated insulin therapy for diabetics [49–52]. However, beta cells may not be enough to confer lasting normoglycemia, which requires tight metabolic control involving other islet cell types. Thus, other islet cells, such as glucagon-producing cells, may need to be co-transplanted. Finally, to avoid disease recurrence, it may be essential to engineer diabetes-specific iPS cells that are resistant to autoimmune attack. However, this will only be possible with a better understanding of the pathophysiology of this complicated disease.

Ophthalmic diseases. Retinal degenerative diseases, such as age-related macular degeneration and retinitis pigmentosa, are the predominant causes of human blindness in the world but unfortunately remain difficult to treat. Knowledge of disease mechanisms remains rudimentary and to date no curative drugs are available. It has recently been reported that iPS cells can spontaneously differentiate into retinal pigment epithelium (RPE) cells that form a highly differentiated RPE monolayer similar to their fetal counterparts [53]. This follows earlier studies that showed that a similar cell type derived from ES cells can give rise to rhodopsin-positive material within grafted RPEs [54] and, more recently, iPS cell-derived multipotent retinal progenitor cells have been reported to differentiate into functional retinal cells *in vivo*, where they form neural circuits sufficient for vision and vision-induced behavior [29]. These findings add to evidence that ES and iPS-derived RPE might support visual function. The retina is an immunologically privileged anatomical site and is expected not to mount an immune response following transplantation of ES-derived cells. However, the huge uptake of laser eye surgery may become relevant in the future, given recent evidence that it abrogates immune privilege in the retina [55] and the likelihood that many prospective patients will have had this treatment.

iPS Cell-Gene Therapy:- A winning combination. The use of gene therapy approaches in combination with iPS cells could help restore defective cell function in degenerative conditions with a genetic cause. Indeed, Hanna and colleagues recently successfully generated iPS cells from a genetically modified mouse with a sickle cell anemia disease phenotype. They subsequently corrected the mutation by genetic recombination and differentiated the mutation-free iPS cells into hematopoietic cells which, on transplantation into the mutant mouse, gave rise to disease-free bone marrow cells [56]. Others have achieved similar results by correcting the defective gene before somatic cell reprogramming and differentiation [57]. These and other studies prove the principle of stem cell mediated gene therapy in a rodent model.

The candidate diseases discussed demonstrate the potential of cell therapy in clinical medicine. Numerous other clinical conditions are potentially amenable to cell therapies. It is hoped that the efforts to efficiently generate virus and transgene-free iPS cells and improvements to differentiation protocols may sufficiently minimize

the main obstacles to clinical application. It might be possible to decrease the risk of tumor formation by bringing residual or new tumorigenic cells under therapeutic control through the use of drug-inducible suicide genes for example. Regenerative medicine laboratories around the world will also need to submit to the challenging requirement of deriving and maintaining iPS cells under good manufacturing practice (GMP) conditions. Delivery strategies will need to be developed for various organ systems in collaboration with clinicians including physicians, surgeons and interventional radiologists. Finally, the cost of patient-specific therapy is likely to be prohibitive and will require creative biological, economic and regulatory solutions. As an alternative, the establishment of HLA-matched stem cell banks may make iPS cell therapy more affordable and accessible.

8.2.2 *iPS Cells and Disease Modeling*

Animal models have been instrumental in efforts to understand the biology of human disease. However, species differences result in many developmental biological, physiological and biochemical differences that can limit extrapolation of findings to humans. For example, the mouse which is widely used to model cardiac arrhythmias, has a heart rate of greater than 600 beats a minute, 10× that of humans [58]. Further, the genetic variability typical of humans adds a layer of complexity to human disease that cannot be modeled in animal models, particularly in inbred transgenic mice. There is therefore a pressing need for new systems for modeling and studying human disease.

iPS cells are promising tools for patient-specific disease modeling as they are genetically identical to the individuals they originate from. Differentiated tissue types obtained from disease-specific iPS cells carry genetic and, in some cases, pathological features of the disease [59, 60], and could in principle be used as *in vitro* human models of human disease. Importantly, iPS cells thus obtained will have the inherent ability to self-renew and differentiate into all tissue types, thus providing a tool and a limitless source of tissue for studying cellular pathology across organ systems. This clinical-scientific application of iPS cells is more achievable than direct cell replacement therapy in the short term. There are some limitations however, as disease-causing processes may reside in other tissue types. It has been shown, for example, that surrounding glial cells may be responsible for the neuronal death that leads to motor neuron disease [61–63]. This data, obtained from *in vitro* experiments with ES cell-derived neurons, demonstrate the concept of non-cell autonomous disease and highlight potential limitations of disease-specific stem cell models. iPS cell-derived patient-specific neuronal model of non-cell autonomous pathologies are therefore likely to be disease-free. In such cases, co-cultures with primary or iPS cell-derived disease-causing cells may be necessary to obtain a phenotype. However, a clear phenotype might not be observed even in diseases where the mutation and other disease-causing process are present in a particular cell type. There might be a requirement for

environmental triggers or other parameters, such as ageing for phenotypes to be unearthed. This is likely to be the case in stem cell models of Alzheimer's disease. It may be necessary to identify ways of mimicking environmental stresses in the laboratory.

Despite these limitations, iPS cells have already been derived from dermal fibroblasts of patients with a range of diseases with both Mendelian and complex inheritance patterns such as Huntington disease, Down syndrome and juvenile-onset, type 1 diabetes mellitus [51, 59]. Some of the neuropathology usually seen in the autosomal-recessive disease, spinal muscular atrophy, was shown to be present in nerve cells derived from iPS cells from patients with the condition [60], and a few studies have demonstrated a disease phenotype in differentiated cell types derived from patient-specific iPS cells [64–66]. Together these studies suggest that it is possible in principle to model human diseases with iPS cells *in vitro*.

There are also potential applications of iPS for discovering and testing the efficacy of novel drugs in high throughput systems using iPS cell-derived *in vitro* disease models such as those described above. It may be possible for new drugs for the treatment of neurodegenerative disorders, cardiac arrhythmias, diabetes and others to be evaluated *in vitro* in disease-carrying iPS-derived tissues obtained from patients with the respective diseases.

Long QT Syndrome; A candidate patient-specific disease model. Congenital Long QT syndrome (LQTS) is a genetically heterogeneous disorder caused by different genetic mutations in potassium or sodium channels that lead to a prolongation of action potential duration in cardiomyocytes.

Clinically, this manifests as a prolongation of the QT interval on the surface cardiac electrocardiogram and an increased risk of syncope and sudden death due to ventricular tachyarrhythmias [67]. Although drug (B-blocker) therapy is effective in minimizing this risk in some patients, many others do not respond to therapy and require invasive surgical procedures, such as placement of implantable cardioverter defibrillators (ICDs). Unfortunately, despite detailed molecular characterization of mutated ion channels, the mechanisms by which individual mutations lead to disease remains unclear. Of note, however, is the incomplete penetrance and variable expressivity of disease-causing mutations which points to the presence of symptom-modifying factors other than the primary mutation [67–70]. Identification of these factors, possibly genetic modifiers, could lead to improved risk stratification among mutation carriers and other potentially life-threatening arrhythmias.

Existing transgenic mouse models of long QT syndrome [71, 72] despite being an important tool for studying LQTS, do not always faithfully recapitulate the electrophysiology of the human myocyte. For example, the significantly higher heart rate of the mouse requires much shorter action potentials that are delivered by expression of repolarizing ion channel genes that differ from their major repolarizing channels in humans. These and other molecular and electrophysiological differences between mouse and human confers limitations on their use for modeling human arrhythmias and renders them unsuitable for the study of human genetic modifiers.

The development of patient-specific iPS cell-derived cardiomyocyte models of LQTS could therefore be an invaluable tool for studying the role of possible genetic modifiers in the pathogenesis of the disease as the cells will carry the exact genotype of the patients being studied as well as provide unlimited quantities of myocytes for molecular and electrophysiological characterization. Candidate genetic modifiers might be tested, for example, by studying the molecular and electrophysiological phenotype of iPS cell-derived cardiomyocytes in founder populations with identical disease-causing mutations. This will offer important advantages over the study of mixed populations with heterogeneous mutations in multiple genes.

Interestingly, a nitric oxide synthase adaptor protein (NOS1AP) genetic variant has been shown to increase the likelihood of a longer QT interval in mutation carriers in genome-wide association studies of a South African founder population [73]. This study also showed an increased risk of cardiac arrest and sudden death in LQTS patients carrying the NOS1AP gene variant. iPS-derived cardiomyocytes from patients with the relevant LQTS mutation and the NOS1AP gene variant will aid *in vitro* characterization of the molecular and electrophysiological mechanisms by which NOS1AP increases LQTS severity. In addition to aiding clinical risk stratification and the need ICDs for example, such an *in vitro* human long QT model could also help accelerate drug development by facilitating efficacy and safety testing. This could expedite delivery of new treatments.

8.2.3 Pharmaceutical Applications of iPS Cells

Human iPS cells may also be suitable for toxicology screening applications. Cardiotoxicity screens, for example, are an essential aspect of drug development. The HERG channel, which mediates an action potential repolarizing current is blocked by a wide number of compounds and drugs leading to a drug-induced form of long QT syndrome and the risk of sudden death. Indeed, drug-induced QT prolongation has led to the removal of several drugs from the US market and is now a standard pre-clinical assessment tool in drug development [74]. The poor proliferation capacity and scarcity of human cardiac tissue have limited the use of human cells for drug screening, as this necessitates repeated isolation of primary tissue. Canine primary cardiomyocytes are therefore frequently used as a pre-clinical model for cardiac safety pharmacological studies. However, their widespread use is expensive and raises ethical concerns. Heterologous cell lines expressing human cardiac ion channel proteins are available but do not recapitulate human cardiomyocyte ion channel function as they lack the complex interactions and control mechanisms present in human myocytes. These shortcomings may compromise the safety of drugs in clinical use and underlie drug withdrawals due to concerns about cardiotoxicity.

Another important cell-type drug toxicology testing is the hepatocyte. These cells, the major cell type in the liver, metabolize a large number of ingested compounds and drugs and can therefore be used to predict the metabolism or toxicity of

a new drug. Currently, primary human hepatocytes are the gold standard for assaying liver toxicity. However, like many primary cultures, primary hepatocytes proliferate poorly in culture and are therefore an expensive tool for drug screening.

Human iPS cell-derived cardiomyocytes and hepatocytes are thus expected to make a significant contribution to drug development as they can in principle recapitulate normal and abnormal human biology and could represent a scalable, reproducible and inexhaustible source of cells for high throughput toxicology screening. This will enable safety evaluation of large numbers of established and novel drug candidates, limiting the need for animal studies and potentially reducing the number of new drugs that fail in clinical trials.

8.2.4 Stem Cell Surveillance *in Vivo*

Clinical imaging is now an essential tool in modern medicine, indispensable for diagnosis, minimally invasive therapeutic procedures, and disease monitoring. If current HSC transplantation practices in hemato-oncological departments are anything to go by, it is highly likely that clinical imaging departments will be very much involved in pluripotent stem cell therapy in the future. Investigators will need to be able to track the location and behavior of transplanted cells in living subjects over time, both during pre-clinical studies and in the clinic. Transplanted cell engraftment has traditionally been studied with histological techniques but the development of sensitive and non-invasive imaging techniques capable of monitoring iPS and ES cells *in vitro* should expedite basic pre-clinical studies in preparation for clinical translation. Recently developed *in vitro* reporter gene imaging techniques allow detection of stem cell behavior *in vitro* by positron emission tomography (PET) [75], while superparamagnetic iron oxide (SPIO) labeling allows stem cells to be identified *in vitro* as hypointense signals in magnetic resonance (MR) images [76]. These and future advances in stem cell imaging will be essential for monitoring iPS cell therapies when they eventually arrive in the clinical arena.

8.3 Conclusion and Future Directions

The successful clinical application of hematopoietic stem cell biology for the treatment of hemato-oncological disorders such as leukemia and myeloma is evidence of what can be achieved through meticulous basic research and careful clinical translation within appropriate regulatory frameworks. The establishment of iPS cells as an inexhaustible and ethical source of non-immunogenic and pluripotent stem cells represents a big step towards the exciting possibility of universal stem cell therapy. The numerous scientific challenges may be overcome by collaboration between molecular and stem cell biologists, clinicians and gene therapy and bioengineering

experts. Justified concerns over safety, efficacy and cost will need addressing and should benefit from the decades of experience acquired in HSC therapy.

Nonetheless, it may take decades before stem cell therapies become commonly available in the clinic. In the meantime our ever-improving understanding of basic developmental biology will lead to further advances in this new and exciting field of regenerative medicine. It may soon be possible to generate cell and tissue types of interest without the need to first reprogramme somatic cells to pluripotency. Indeed, it has already been shown that functional neurons can be derived directly from fibroblasts by over-expression of neural lineage specific transcription factors [77]. However, since most terminally differentiated cells do not proliferate well, it may be difficult to expand cells generated in this way for clinical use. *In vivo* approaches may come to the fore in the coming years, perhaps building on the recent demonstration that the exocrine pancreas can be re-programmed into insulin-secreting beta-like cells that reduce hyperglycemia by *in vitro* re-expression of developmental regulators [78]. Whatever happens with iPS cells in the next decade or so, regenerative medicine, it appears, is here to stay.

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Chapter 9

Industrial Applications of Stem Cells

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Abstract Human embryonic stem cells (hESC) can be differentiated into all somatic cell types, expanded to unlimited numbers and subjected to genetic modification. These properties provide novel perspectives for drug development and biomedical applications. The introduction of disease-specific mutations into these cells and their subsequent *in vitro* differentiation can be used to study the effect of candidate disease genes on cellular processes and responses to pharmaceutical compounds, thus representing an ideal tool to study human diseases. However, a large number of diseases is based on multiple and mostly unknown cellular alterations and can, therefore, not be adequately modelled using a candidate gene approach. The derivation of disease-specific cells from patients' own tissues is an emerging new approach. One of the most promising routes in this regard is the generation of induced pluripotent stem cells (iPSC) by reprogramming cells derived, e.g., from patients' skin biopsies. In analogy to hESC, these iPSC can be differentiated into any cell type. Thus, the availability of both hESC and iPSC provides unprecedented opportunities to generate virtually unlimited numbers of disease-relevant tissue-specific cells *in vitro*. Key prerequisites for a broad application of hESC- and iPSC-based cellular disease models are industrial methods to generate large quantities of highly purified cells in standardized formats. In this chapter we review the state of the art in stem cell industrialization and discuss innovative perspectives and future applications in this field.

Keywords Pluripotent Stem Cells • Reprogramming • Disease Modelling • Drug Screening • Regenerative Medicine

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9.1 Introduction

The successful cloning of the sheep Dolly in 1997 [1] and the subsequent derivation of human embryonic stem (ES) cells in 1998 [2] brought forward the concept of therapeutic cloning, in which pluripotent ES cell lines tailored to the genetic makeup of specific individuals might provide a rich source of therapeutic cells [3]. Human ES cells, which are derived from the inner cell mass of blastocyst stage embryos, have the unique ability to self-renew indefinitely while they maintain their potential to give rise to all cell types in the human body. Induced pluripotent stem (iPS) cells share these salient characteristics of hES cells but are instead generated via reprogramming of somatic cells through the forced expression of key transcription factors [4]. The achievement of induced pluripotency holds a great potential for regenerative medicine since patient-specific iPS cells could provide useful platforms for disease-related research, drug development and, eventually, autologous cell and tissue replacement therapies.

9.2 Pluripotent Stem Cells – Their Sources and Characterization

Pluripotent stem cells have both the ability to differentiate into all types of cells in the body and to self-renew indefinitely; these properties harness pluripotent cells with an enormous potential for basic and disease-related research, regenerative medicine and drug discovery. In order to qualify as *pluripotent*, stem cells are required to meet the following criteria: (1) unlimited proliferation *in vitro* while maintaining their normal, diploid karyotype; (2) differentiation potential into cells of all three germ layers, viz, ectoderm, mesoderm and endoderm.

Several assays exist that can demonstrate the pluripotency of a cell. The expression of pluripotency marker proteins, like Nanog, Oct4, SSEA1, SSEA4, is an indicator for the pluripotent state of a cell *in vitro*.

The differentiation potential of a cell is analyzed by injecting cells into immunodeficient mice where they form teratomas, tumors containing tissues of all three germ layers. When injected into a blastocyst, pluripotent stem cells contribute to different germ layers of a chimeric embryo.

In another assay, referred to as *tetraploid complementation*, embryos are created that are entirely derived from ES cells. This assay is typically used to generate genetically modified mice for studying the consequences of certain mutations on embryonic development. The assay starts with the generation of a *tetraploid* cell by fusing the two cells of an embryo at the two-cell stage. The resulting tetraploid cell, which includes a duplicated set of chromosomes, will continue to divide, and all daughter cells will also be tetraploid. Due to the tetraploidy, these cells will not give

rise to an intact organism. In the *tetraploid complementation assay*, two tetraploid embryos at the morula or blastocyst stage are aggregated using the “sandwich technology” with normal diploid embryonic stem cells. The resulting blastocysts are selected and transferred to the uterus of a pseudopregnant female. The tetraploid cells will form the extra-embryonic tissues, whereas the ES cells form the embryo itself (which is then completely ES cell-derived). Obviously, such an assay is restricted to animal cells and cannot be used for the validation of human pluripotent stem cells.

The most well-known pluripotent stem cells are ES cells derived from the inner cell mass of a blastocyst [5]. In principle, these cells can be differentiated into more than 200 different types of cells [6]. In addition, parthenogenesis and somatic cell nuclear transfer (SCNT) represent alternative sources of blastocysts and embryonic stem cells. SCNT represents a technique, wherein the nucleus of an unfertilized egg is replaced by the nucleus of a somatic cell. Factors in the oocyte then ‘reprogram’ the somatic nucleus into an early embryonic, totipotent stage corresponding to that of a 1-cell-stage embryo.

Irrespective of the types of blastocysts used in experimental setups, the isolation and establishment of ES cell lines involve embryo destruction and – in case of SCNT – oocytes. Ever since the first generation of human ES cells, significant advances have been made with regard to derivation technologies and alternatives. One example is cell fusion, where a somatic cell is reprogrammed by fusion with a pluripotent cell, resulting in pluripotent, albeit tetraploid cells [7]. Despite their ability to self-renew indefinitely and to generate all somatic cell types, the application of ES cells for cell replacement and other cell-based therapies is restricted by the risk of immunological rejection. This important restriction and the need to resort to totipotent cells in order to generate pluripotent cells have been overcome with the advent of induced pluripotency.

9.3 Induced Pluripotent Stem Cells (iPS)

Cells within an organism are genetically almost identical, yet they form cell types as disparate as neurons, immune cells or pulsing cardiomyocytes. Novel techniques are able to convert cells from a terminally differentiated state into one in which they not only divide indefinitely but can, in theory, become any cell type found in adults. Advances in generating these so called ‘iPS cells’ from mice and humans have opened what could be a new era of pluripotent stem cell biology and are about to stimulate a paradigm shift away from the concept of the ES cells as the only source of pluripotent stem cells.

Back in the 1960s, John Gurdon transferred nuclei from differentiated somatic cells from the epithelium of the intestines of the African clawed frog (*Xenopus laevis*) into enucleated egg cells. He obtained normal tadpoles and thus demonstrated

that the nuclei retained their ability to originate totipotent cells [8]. Over thirty years later, in 1997, the generation of Dolly became possible by reprogramming the nucleus to pluripotency upon insertion into an enucleated oocyte [1]. Both approaches implied that there are factors, which can change the epigenetic status of somatic cells.

In 2006, Takahashi and Yamanaka succeeded in identifying a defined set of four factors, which, upon expression in fibroblasts, were able to convert these cells into a pluripotent, ES cell-like stage [4] (Fig. 9.1). Specifically, simultaneous expression of Oct4, Sox2, c-Myc and Klf4 was found to efficiently reprogram mouse embryonic fibroblasts (MEF) and mouse tail-tip fibroblasts to form colonies that showed a ES-like colony morphology, proliferated similar to ES cells and exhibited pluripotent differentiation into all three germ layers. Thus, with the ectopic expression of only a few transcription factors, embryonic, neo-natal, or adult somatic cells can be reprogrammed and give rise to pluripotent stem cells.

Several groups were able to reproduce the results by Yamanaka et al., using the same or alternative sets of reprogramming factors, e.g. Oct4, Sox2, Nanog and Lin28 [9]. It was shown that these iPS cells not only generated chimeric mice with high efficiency but also contributed to germ cell development [10]. After the successful generation of mouse iPS cells, human embryonic, neo-natal and adult fibroblasts were reprogrammed and the first human iPS cell lines were established [11].

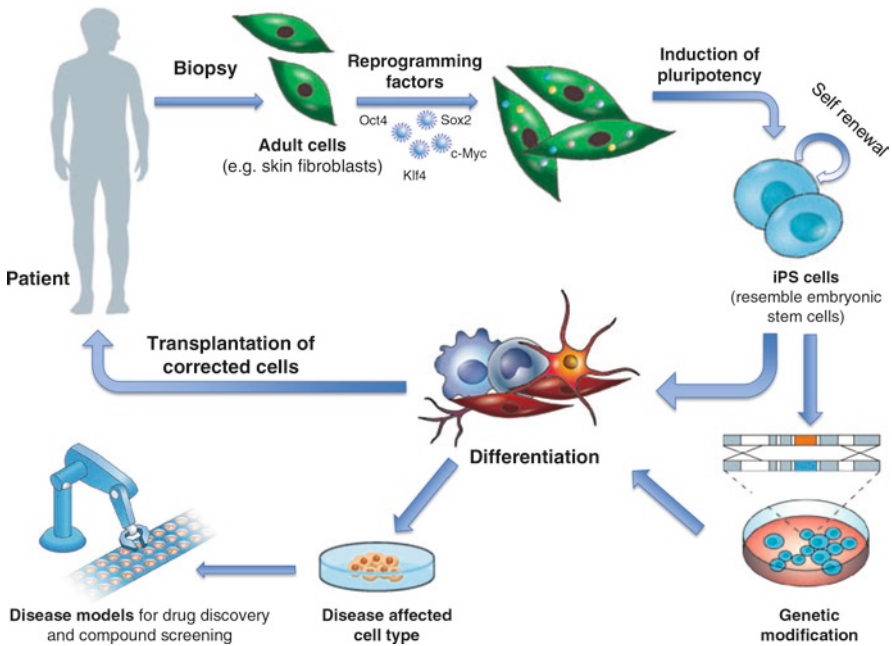


Fig. 9.1 Derivation and applications of iPS cells

Thus, with the advent of the iPS cell technology it has become possible to generate cell lines from a patient's own tissue, opening the way for the generation of *disease-affected* cells in unlimited numbers *in vitro*. Such patient-specific cells may be used for studying the molecular pathogenesis of diseases in cell culture models and to screen and develop therapeutic compounds directly in the context of the affected cell type. This approach is particularly valuable for tissues, which show a low regenerative potential and are thus not amenable to the isolation of expandable progenitor cells. Examples include cardiomyocytes, insulin-producing cells and neurons.

In the first iPS cell studies, retro- and lentiviruses were used to transduce the reprogramming factors into the somatic cells. While efficient, this approach has several drawbacks. For instance, random insertion of the virally transduced genes can result in frame shift mutations and activate or inactivate host genes. In addition, reprogramming factors such as c-myc are oncogenes. Despite the fact that retrovirally transduced transgenes typically undergo transcriptional silencing, insufficient silencing and/or reactivation may occur, thereby promoting neoplastic transformation and tumour formation.

To circumvent such problems, several alternative methods are currently under investigation, among them episomal or excisable systems such as the elimination of transgenes by a transposase system [12, 13] or the application of cell permeable proteins to deliver reprogramming proteins, instead of genes, into somatic cells to induce iPS cells [14–16]. In addition, it has become possible to reduce the number of reprogramming factors, which is particularly relevant when it comes to replacing oncogenic factors such as c-Myc. Several factors could be replaced by applying small molecules interfering in the regulatory pathways of gene expression. For example, reprogramming with only Oct4 and Sox2 was possible when cells were treated with the histone deacetylase inhibitor valproic acid (VPA) [17].

Reduction of reprogramming factors is particularly attractive when it comes to reprogramming cell types, which already show a high expression rate of one or more of the respective factors. It has, for instance, been shown that reprogramming of adult neural stem cells can be achieved by applying only Oct4, since Sox2, c-Myc and Klf4 were already expressed in the target cells [18]. However, such methods require further improvements, especially with respect to their efficiency. Advancements in this regard have recently been made by Singhal and co-workers, who identified factors that mediate reprogramming with a higher efficiency by establishing an assay to screen nuclear fractions from extracts of pluripotent mouse cells based on the reactivation of the reprogramming factor Oct4 [19]. Using proteomics, they identified components of the ATP-dependent BAF chromatin-remodelling complex, which significantly increase the reprogramming efficiency when used together with the four reprogramming factors Oct4, Sox2, Klf4 and c-Myc. The reprogrammed cells could transmit to the germline and exhibited pluripotency. The reprogramming process remained also highly efficient when c-Myc was not present but BAF components were overexpressed. It is believed that BAF complex components mediate this effect during reprogramming by facilitating enhanced Oct4 binding to target promoters. Thus, the use of chromatin-remodelling molecules for somatic cell reprogramming appears to represent an efficient method for improving the efficiency of iPS cell

generation. From a safety point of view, the induction of pluripotency with cell-permeable proteins and small molecules would represent the safest method, since no genetic modifications are involved during reprogramming.

9.4 Prospects and Challenges Associated with the Clinical Application of Induced Pluripotent Stem Cells

With regard to biomedical applications, both ES and iPS cells provide attractive though remote prospects for cell replacement therapies (Fig. 9.1). If produced safely, patient-specific iPS cells may eventually circumvent immunological rejection after cell engraftment. In the long term, it might not even be necessary to derive donor cells for each patient but to resort to iPS cell banks generated from a small number of carefully selected and broadly compatible donors. For example, there are calculations that 30 hiPSC lines could deliver a full match for 82.2% of the Japanese population [20]. In this regard, a translation from cord blood banks to iPS cell banks appears particularly attractive, since these neonatal cells do not show age-related mutations or alterations observed in adult donor cells.

To make iPS cell-based cell therapy work in patients with genetic diseases, the iPS cells would not only have to be derived from the same or a immunologically compatible patient, but also subjected to gene correction before re-introduction and transplantation. In 2007, Hanna and co-workers treated mice with sickle cell anemia using iPS cells generated from autologous skin [21]. Sickle cell anemia is caused by a defect in a single gene. In this study, the mutated gene that causes sickle cell anemia was replaced by its normal counterpart, using homologous recombination in the skin-derived iPS-cells. Subsequently, the gene-corrected iPS cells were differentiated to blood-forming stem cells and retransplanted into the donor mouse. This and similar recent studies [21–23] showcase the potential of cell reprogramming to provide autologous, gene-corrected donor cells for the treatment of a genetic disorder.

At present, there remain substantial challenges for the clinical application of somatic cells differentiated from patient-specific iPS cells. In addition to the risks associated with the cell reprogramming process discussed above, iPS and ES cell-derived preparations can lead to teratoma formation by residual pluripotent stem cells present in the donor cell suspension. As minor contaminations with pluripotent cells suffice to induce teratomas, it is of paramount importance that ES and iPS cell-derived donor cells are properly differentiated in the required somatic cell type and free of any undifferentiated pluripotent cells. Another drawback of iPS cells to be considered is the fact that the somatic cells used for reprogramming might have accumulated mutations across the patient's life span, which, too, might promote tumour formation.

Furthermore, there is still controversial as to whether iPS cells are molecularly and functionally equivalent to ES cells. Indeed, there is evidence that iPS cells differ from ES cells in both their biological properties, epigenetic status and gene-expression profiles [10, 24]. Recently, Stadtfeld and co-workers showed that the overall messenger RNA and microRNA expression patterns of genetically

identical mouse ES and iPS cells show pronounced differences in the expression of transcripts encoded in a cluster on chromosome 12 [25]. In consistence with the developmental role of this cluster, iPS cells with an aberrant expression of this gene cluster contributed only poorly to chimaeras and failed to support the development of entirely iPS cell-derived animals. Thus, the expression state of a single gene cluster may provide a criterium for the prospective identification of iPS cell clones that have the full development potential of ES cells.

Crucial challenges also exist with respect to controlled differentiation of iPS cells into defined somatic phenotypes, their targeted delivery to disease-affected host tissues and their functional integration in the transplant recipient. This is impressively illustrated in attempts to treat diseases such as amyotrophic lateral sclerosis (ALS), where motor neuron degeneration occurs both in the brain and throughout the spinal cord. Successful cell replacement would not only require long-term engraftment of iPS cell-derived motor neurons and establishment of correct axonal projections across long distances, but also sophisticated surgical approaches, which essentially permit donor cell delivery throughout the central nervous system.

Finally, any cell preparation scheduled for clinical application would have to be produced under industrial conditions in accordance with international standards of good manufacturing practice (GMP) and criteria of the International Organization of Standardization (ISO).

9.5 Prospects of iPS Cells for Cell-based Disease Modelling and Compound Development

The ability to derive disease-relevant cell types from patient-specific iPS cells provides a unique opportunity to study the cellular and molecular pathogenesis of human diseases under controlled conditions *in vitro*. This is relevant for several reasons. First, many diseases are human specific and can, therefore, only insufficiently be modelled in non-human systems such as transgenic mice. Second, access to primary human tissue for disease-related studies is very limited, particularly when it comes to organs with low regenerative potential such as heart or the nervous system. Third, even if human cells are used, classic transgenic approaches employing overexpression of a disease-relevant gene or its mutant versions do not reflect the intricate dosage relationships between the various proteins involved in the molecular pathogenesis. In contrast, patient-specific iPS cell-derived somatic cells should truly reflect the genotype and expression profile of disease-relevant somatic cells. Moreover the ability to generate these cells retrospectively enables a correlation of the patient history with the cellular phenotype. It is, therefore, not astonishing that the advent of transcription factor-based cell reprogramming has boosted interest in generating iPS cells and iPS cell derived somatic cells from patients suffering from a variety of diseases. Examples include amyotrophic lateral sclerosis (ALS), spinal muscular atrophy (SMA), Morbus Parkinson, Familial Dysautonomia (FD) and many others [26–29].

Indeed, some of these disease-specific cells have been shown to feature disease-specific pathological changes. One recent example is familial dysautonomia (FD), a peripheral neuropathy, which is caused by a point mutation in the IKBKAP8 gene and affects neurons of the peripheral nervous system. Studies on FD-specific iPS cells revealed tissue-specific splicing of the IKBKAP8 gene as well as defects in neurogenic differentiation and cell migration. Moreover, this and other studies demonstrated that such disease-specific iPS cell-derived somatic cells can be used to identify compounds, which ameliorate the pathological phenotype [27, 29].

Considering that the generation of iPS cells involves reprogramming of the epigenome, they could become particularly valuable for dissecting epigenetic and genetic effects. In a recent study, Urbach et al. demonstrated critical differences between embryonic and induced pluripotent stem cells with respect to their ability to model Fragile X syndrome, the most common form of inherited mental retardation [30]. This disease is associated with the expansion of a trinucleotide gene sequence (CGG) in the FMR1 gene on the X chromosome, which is associated with hypermethylation of the repeat region and its upstream promoter, thereby resulting in a loss of FMR1 expression. Urbach and colleagues showed that despite successful reprogramming of fibroblasts from Fragile X patients, the FMR1 gene remained inactive and continued to exhibit DNA methylation and histone modifications indicative of inactive heterochromatin. In marked contrast, ES cells derived from Fragile X embryos identified through preimplantation genetic diagnosis expressed FMR1. These data point to important epigenetic differences between human ES and iPS cells, which merit further investigation.

9.5.1 IPS Cell Applications in Other Species

Generation of pluripotent stem cells via somatic cell reprogramming might also bypass problems associated with the inability to derive ES cells from a variety of species. Here, replacement of ES cells by iPS cells might in the end enable to generate transgenic offspring with beneficial traits such as disease resistance in domestic or threatened animals. Freezing batches of iPS cells from endangered species may also help to preserve them.

9.6 The Potential of Pluripotent Stem Cells for Industrial Use in Compound Screening

The ability to generate virtually unlimited numbers of ES cells and patient-specific iPS cells makes them an ideal tool for testing pharmaceutical compounds in high throughput (HTS) and high content screenings (HCS). Applied e.g. to iPS cell-derived somatic cells such as neurons or heart muscle cells, this approach permits for the first time a direct assessment of the therapeutic effect of compound libraries on disease-affected human cells. The feasibility of using hES cells for HTS was

demonstrated by Desbordes et al. in 2008, who developed a high content assay suitable for screening a chemical compound library for candidates with a positive effect on hES cell self-renewal [31]. While pluripotent stem cells can generate any somatic cell type *in vitro*, this application is particularly interesting for cells of nonregenerative organs such as nervous system and heart, i.e. organs, from which it is difficult to derive primary cells in large numbers.

The application of ES and iPS cell-derived somatic cells in large-scale compound screening is further facilitated by advances in generating stable, self-renewing tissue-specific stem cells. For example, Koch et al. have recently established a population of stably proliferating human neural stem cells (NSCs) from human pluripotent stem cells. These cells remain stable across at least 100 passages while maintaining their ability to generate different types of neurons and glia. The fact that neural precursors, neurons and glia can be directly expanded from a stock of these NSCs without resorting to pluripotent cells contributes significantly to the standardization of cell preparation [32].

Recent studies provide first examples for the application of disease-specific iPS cells in the assessment of pharmaceutical compounds. In 2009, Swendsen and co-workers derived motor neurons from iPS cells of a patient suffering from spinal muscular atrophy (SMA), a progressive disease associated with impaired expression of the SMN (*survival motor neuron*) protein and subsequent degeneration of motor neurons. The authors could demonstrate that treating reprogrammed cells with compounds known to raise SMN levels could partially revert the pathological intracellular distribution of SMN protein in the affected cells [27].

Taken together, it is to be expected that the use of human cellular models will increase the predictability of screenings. For pharmaceutical companies this point is highly significant, as typically several million dollars have been spent before a new drug is entered into first clinical trials in humans. Human stem cell-based approaches provide an opportunity to screen compounds at an early stage of drug development in a human setting and thus to avoid late and costly drop-outs.

Stem cell-based screening approaches might also help elucidating the efficacy of drugs for patients from different genetic backgrounds. The reprogramming technology provides prospects to generate libraries of somatic cells from various genetic backgrounds, which could help dealing with the variability in individual responses to potential therapeutic agents – a major problem in effective drug development [33]. In that regard, stem cell-based screening approaches might also support the development of personalized medicine and tailor-made treatment plans.

9.7 Challenges on the Way to Industrial Applications of Stem Cells

To establish stem cell technologies in industrial processes, a close interaction between academic basic research and industry is necessary. Implementing industrial standards in early phases of research projects on pluripotent cell-derived disease models is a way to accelerate this process and to ensure compatibility of such cellular models for drug development. Considering the large numbers of cells eventually

required for screening applications or cell therapy, it becomes evident that novel and innovative technologies are required to produce large quantities of cells. To obtain meaningful results, cell culture and expansion requires automated, standardized and certified conditions. By definition, stem cells are a self-renewing cell type. However, the culture conditions, i.e., media components, supplements or surface characteristics, have a critical effect on the developmental potential of the cells.

In contrast to the two-dimensional (2D) cultivation of cells, three-dimensional (3D) culture techniques allow much higher cell densities. Within the last years, several improvements have been made in cell culture technologies in large-scale formats, for instance with stirred bioreactors. The application of such technologies for stem cell cultures is not trivial; many of the studies performed so far have been focused on the cultivation of cells in aggregates that include very heterogeneous populations of different cell lineages called embryoid bodies (EB) [34, 35]. This method is applied to obtain differentiated cell types in large numbers from pluripotent cells. The controlled differentiation of ES cells in spinner cultures has been achieved by genetic introduction of lineage-specific selection constructs and the adaptation of culture conditions [36, 37]. However, the cultivation of human pluripotent stem cells in bioreactors remains a major challenge. Stirring-induced shear forces influence aggregation of ES cells cultured in suspension and have a major effect on the differentiation potential of ES cells [38]. A promising approach appears to be the micro-carrier-mediated cultivation of hES cells in suspension, which has been shown to allow two to four times higher densities of cells than 2D colony culture [39, 40]. To circumvent batch-to-batch variations and to minimize contamination risks, further automation technologies must be implemented to standardize pluripotent stem cell cultures. A good example in this regard is the Cellhost™ system, which automates cell plating, media replacement, addition of growth factors and cell harvesting during stem cell culture and permits long-term sterility in antibiotic-free-cultures [41]. Taken together, the production of large numbers of stem cells and their derivatives under defined, reproducible and standardized conditions should enable their use in industrial, pharmaceutical and clinical applications.

9.8 Concluding Remarks

Recent advances in generating and differentiating pluripotent stem cells provide unprecedented prospects for disease modelling, compound development and cell therapy. Cell reprogramming and the possibility of deriving patient-specific cells will particularly promote further development of these applications towards personalized medicine. A key success factor in the *business ex academia* translation of these developments will be an early and close interaction of basic science, clinical medicine and industry. Such an interaction is not only required for convincing the pharma and health sector of the advantages of stem cell-based systems but for optimizing technology development for specific products and treatments. These points considered, human pluripotent stem cells and cell reprogramming may well ring in a new era in the translation of stem cell science *from bench to bedside*.

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Chapter 10

The Obstacles on the Road to Clinical Applications of Stem Cell-Based Therapies: What Has Been Done to Overcome These Obstacles and What Remains to Be Done?

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Abstract The advantages and problems in cell transplantation depend on the type of stem cells used. Blood stem cells have been used in clinical treatment for more than 40 years. Mesenchymal stem cells are already used in a large number of clinical trials. They are used in severe graft versus host reaction, and in forming bone, cartilage and muscle. Pluripotent stem cells, human embryonic stem cells (ESC) and induced pluripotent cells (hiPSC) have not yet been used in clinical trials, but the first trials in spinal cord injury have been planned. Safety issues are the concerns in stem cell transplantation. Risks of infection are known, and they can be tackled by careful testing of the donor and careful handling of the cells. A particular quality system, GMP, has been developed for this purpose. Immunogenicity is a problem in all situations in which patient's own cells cannot be used. For tackling immunogenicity, options are matching the tissue types of the donor and the recipient, immunosuppressive medication, possible modulation of the immune response, and the use of patient-specific stem cells induced from the recipient's somatic cells. Substances of animal origin in cultures may promote immune reactions. They can be avoided by using only xeno-free cultures. Tumorigenicity of cells differentiated from human embryonic and pluripotent stem cells is a risk if cells differentiated from such pluripotent cells are transplanted. Pluripotent cells need to be eliminated by using effective differentiation protocols and by removing the possible remaining pluripotent cells from the population aimed at transplantation.

Keywords Human embryonic stem cells • Induced pluripotent cells • Cell therapy • Tumorigenicity • Good manufacturing practice

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10.1 Introduction

Adult stem cells are already in clinical trials or use, while the use of pluripotent cells, human embryonic stem cells (hESC) and induced pluripotent cells (iPSC) in clinical trials and applications have not yet been initiated. Both of these cell types are very promising for cell transplantation because of their huge potential in differentiation to many cell types, and their capacity to form well-functioning different cell types [1]. Safety issues in addition to request of more defined and repeatable differentiation protocols have so far been the main obstacles in going further. Even though it may seem easier to use adult stem cells whenever possible, such cells are not without problems, either.

In 2008 the International Society of Stem Cell Research (ISSCR) [2] published guidelines for clinical translation of stem cells, followed by the National Institutes of Health (NIH, USA) in 2009 [3]. Safety, quality and ethical considerations are central issues in these two guidelines.

10.2 The Expected First Clinical Applications

Blood stem cells have already been used clinically for more than 40 years. There are many clinical trials going on using tissue-derived stem cells, particularly mesenchymal stem cells from bone marrow and adipose tissue. More than 80 clinical trials have been registered in the European Community (EudraCT) [4] and the NIH, USA [5]. Mesenchymal stem cells are studied, for instance, in bone, cartilage and muscle formation and in modifying the immune response. Few side effects have been reported, including tumorigenicity [6, 7]. Also, cells from skin and muscle are already in clinical trials.

Neural differentiation of embryonic stem cells has already been successful for 10 years, and the differentiation protocols have evolved to safer and more controllable direction, while more has been learned about the mechanisms regulating neural differentiation. There are many promising results when using hESC-derived neural cells in animal models. Hence, disorders of the nervous system are probably among the first ones to be treated using pluripotent cell derivatives. Disorders that can be treated by local transplantation are more likely than diffuse ones to be treated first.

There is good proof of principle in cell therapy of Parkinson's disease. Fetal midbrain cells have been used in the treatment of patients with Parkinson's disease [8]. Human ESC-derived neural cells improved symptoms in animal models of Parkinson's disease [9]. Also, spinal cord injury has been treated with some success in rats using hESC-derived oligodendrocytes [10]. Clinical trials have been planned, but not yet carried out.

Retinal pigment epithelium is a particular cell type that is formed spontaneously from hESC in culture, and retinal degeneration is a common cause of visual impairment in humans. Hence, retinal repair using hESC-derived pigment epithelial cells also seems to be a promising option [11].

The treatment of diabetes has been a candidate on the list of diseases to be treated, but it has proven more challenging. However, significant advances have been made in pancreatic islet cell differentiation, also making this disease close to treatment [12].

Cardiac repair is also being intensively studied in several ways, and sooner or later treatments will come [13]. With accumulating experience, other, more complicated diseases may also be tackled using pluripotent cell-derived differentiated cells.

10.3 Risks in Stem Cell Transplantation

10.3.1 *Infections and How to Avoid Them*

Microbial contamination of the cells can make them ineffective and cause severe infections in the recipient. The cell donor may be the source of contamination, hence the donors have to be tested for the possible known microbes that may complicate cell transplantation. Several viruses, such as immunodeficiency viruses (HIV), hepatitis B and C, cytomegalovirus, and HTLV I and II, human polyomavirus (JVC) can be tested using antibody measurements or by measuring the expression of the viral genome in the donor's blood. Virus particles can also be tested using electron microscopy [14], in which these extremely small particles can be visualized using very large magnifications. The cells themselves can and should be tested. Mycoplasma contamination is not uncommon in cell culture laboratories, and different types of molecular and microbiological tests exist for the large number of mycoplasmas.

Cells may also become contaminated through any component in their culture media. When serum is used as a culture medium constituent, the serum donor may be a source of contamination. Serum is still used as a constituent in many stem cell cultures, even though cultures with only constituents that are exactly known, have been adopted more and more. In addition to bearing a risk of infection, serum contains substances that may direct the differentiation of the cells to undesired directions. Small microbes called prions, which can cause Creutzfeldt-Jacob's disease that affects the central nervous system, can be mediated by bovine serum. Also, human serum may contain unknown microbes that may activate themselves in the recipient. The best method for avoiding such contamination is using a culture media that only contain synthetic human proteins. Feeder cells, which offer important nutrients and regulating factors for stem cells, have been largely used in culturing many types of stem cells, and they are still used for establishing pluripotent human embryonic stem cells and induced pluripotent cell lines. They may also be infected. Feeder-free cultures are more easily controlled because they have fewer different steps in the culture procedures. Hence, whenever possible, feeder-free cultures of stem cells are developed and used. It is always best to avoid any possible sources of contamination. Feeders are still often used because many cells grow better with feeder cells. Impurities and particles in the air of the laboratory may carry microbes,

and careless handling of the cultures, including non-sterile contact, may cause infections.

GMP and EU tissues and cells directive. There are established quality systems that help in avoiding contamination, such as Good Laboratory Practice (GLP) and Good Manufacturing Practice (GMP) [15]. The EU Tissues and Cells Directive (EUTC) [16] regulates the quality of all cells used in human therapy. It requires good manufacturing practice (GMP)-based production systems. In this directive and in the local laws written accordingly in the member countries, the quality of the procedures and cleanliness of the products have been regulated. There are additional requirements for human stem cell-derived cells. It offers a quality approach to manufacturing and enables the manufacturers to eliminate or minimize instances of contamination, mix-ups and errors. It protects the consumer from purchasing products that are not effective or even dangerous. GMP regulations address record-keeping, personnel qualification, sanitation, cleanliness, equipment verification, validation of the processes, and complaint handling.

10.3.2 Immunogenicity

If the recipient's own adult stem cells are used, they will not undergo immunological rejection. This is the big advantage of such autologous (patient's own) cells. There are many situations in which, however, allogenic (donated) cells are needed. The tissue injury may be so acute that there is no time to culture up large enough numbers of the patient's own cells, such as in the cases of spinal cord injury, stroke and cardiomyocyte repair in myocardial infarction. Cells differentiated from donated pluripotent cells are often more capable of repairing the injured tissue. iPS cells have infection and tumorigenicity risks similar to those of the hESC.

When allogenic cells are needed, rejection can be avoided by matching the donor's tissue type so that it resembles that of the recipient as much as possible. In blood stem cell transplantation, the optimal donors are found among the patient's close relatives. If no matching relative is found, the treating team can search for donors from the large international registries. If no matching donor can be found from them either, the situation may become difficult. It would be desirable to have donor registries with potential donors of different ethnic origins. In blood stem cell transplantation, umbilical cord blood banks are a huge possibility and help. Also, such blood banks containing blood from donors having as many different tissue types as possible would be highly desirable. There are only a few situations in which autologous cord blood stem cells can be used, but as donated stem cells they are very useful.

In organ transplantation, immunosuppressive medication is used. It can also be used in stem cell transplantation if necessary. Immunosuppressive medication has possible severe side effects, such as increased susceptibility to infections and tumors. These risks can only be taken if the cure for a severe disease or injury outweighs the side effects.

Human embryonic stem cells are as immunogenic as any other cell types [17]. If cells have been differentiated from hESC, complete matching may be difficult

because the numbers of hESC lines are limited. A combination of matching and immunosuppressive medication is probably the way to go forward. Modulating immune response by giving to the recipient mesenchymal stem cells [18] may be an alternative that still has to be explored. Banking and matching pluripotent cells (hESC and iPSC) would also be important [19].

A promising new alternative to avoid rejection in stem cell transplantation is the use of patient-specific induced pluripotent (iPS) cells [20, 21]. iPS cells can be established by inserting pluripotency genes to the recipient's somatic cells, such as skin or blood cells. These cells can then be differentiated to other cell types using similar methods that have been developed for embryonic stem cells.

From culture constituents containing animal-derived components, such as mouse feeder cells or fetal bovine serum, the cells can take in animal proteins, which in addition to possibly carrying microbes, are immunogenic and may promote the rejection of cells after transplantation [15]. Hence, culture systems free of any components with animal origin would help avoiding not only microbial contamination but also immune reactions in the recipient of the cell transplantation. Six human embryonic stem cell lines stated to be of clinical grade have so far been derived [14], but the derivation system was not animal-component free, as the culture medium contained animal proteins. Animal substance-free culture media, feeder cells and feeder cell-free cell culture matrices are now under extensive development and testing [15].

10.3.3 Tumorigenicity

10.3.3.1 Pluripotency-Related Tumors

A definition of pluripotent stem cells is that they can form any tissues in the body. This property is well demonstrated by their capacity to form tumors called teratomas when injected into immuno-incompetent mice. Teratoma formation is used as a test of pluripotency of human ESC and iPSC. Teratomas are benign tumors that consist of clumps of different tissues [22]. Teratomas as spontaneous human tumors are not very rare; so-called dermoid cysts occurring in human ovaries are teratomas. Getting a teratoma after cell transplantation would be a severe complication. Sometimes teratomas may also become malignant. Hence, in cell therapy and regenerative medicine it is extremely important to use only cell populations that do not contain any pluripotent cells. Tissue-derived stem cells may also give origin to tumors, as demonstrated by the case of a boy who received a transplant consisting of fetal brain stem cells [23].

10.3.3.2 Culture Adaptation

Mutations occur when cells divide, both *in vivo* and *in vitro*, in tissues, and in culture. In living organisms, there are several ways to eliminate mutated cells, but such control mechanisms do not function well in culture. If a mutation in cells dividing

in culture gives a growth advantage for the mutated cells, the proportion of such mutated cells starts to increase in the cultured population. These growth-promoting mutations are often also tumorigenic [24]. The more times the cells have divided in culture, the more likely the existence of mutated cells in the population. Cell generations in culture are called passages, and the number of passages are therefore directly related to the likelihood of mutations. This phenomenon is known as culture adaptation. Culture adaptation occurs not only in pluripotent stem cells, but also in adult mesenchymal stem cells that frequently undergo mutations in culture and transform to cancer cells [5, 6]. If such transformed mesenchymal stem cells have been expanded from the becoming recipient's own cells, the recipient has a high risk of cancer.

10.3.3.3 How to Detect Possible Tumor-Forming Cells, and How to Remove Them

Several molecular biology methods can be used to identify the different cell types in populations. Performing such tests is called characterization of the cells. A simple method to find out which proteins the cells express is immunocytochemistry. This method is based on an antibody made against a certain protein, and a colour reaction linked to the antibody. Expression of genes is routinely determined using a method called polymerase chain reaction (PCR), which can also be used in a quantitative manner. In PCR, the base order of the desired genes is multiplied as large numbers of copies, making it possible to detect. In practical microarray methods, large numbers of genes or proteins can be analyzed at a time. There are several systems to do it, and many of them are commercially available. New mutations can be identified by sequencing the base structures in the genes. Using such methodologies, it is possible to find cells that have undergone changes.

During directed differentiation of the cells, it is necessary to ensure that correct cells are obtained. The cells have to be characterized using molecular methods. Similarly, the presence of cancerous cells can be detected. Before any use of stem cells in cell transplantation or regenerative medicine, it is important to characterize the cells carefully to see that the differentiated cells are what they are aimed at, and that there are no pluripotent or tumor cells in the population.

A problem in the use of pluripotent cells is that all the known differentiation methods today result in miscellaneous cell populations in which the desired cell types are more or less enriched. Hence, it is important to select from the population the desired cells, for example, cardiomyocytes or neurons, and weed out the pluripotent cells. Cell sorting can be carried out using antibodies against their surface proteins, coupled with a fluorescing substance, a method called fluorescence-assisted cell sorting (FACS). A disadvantage of this method is that the process is relatively harsh to the cells. It can break down the filaments of neural cells, thus impairing the survival and function of the differentiated cells. Using an antibody that is linked to a magnetic bead, a somewhat milder process magnetic (sorting)

may be used. These kinds of methods are being further developed and improved to enable obtaining safe-for-use cell populations.

The introduction of so-called suicide genes to pluripotent cells is another strategy that is under development. Linking a gene that causes programmed cell death, thymidine kinase, to a pluripotency or cancer marker in the cells, is already under clinical studies in leukemia [25].

Because the likelihood of mutations increases division by division in culture, as low passage numbers (as few divisions) as possible should be used in treatment. Avoiding high passage requires a large number of cell lines. It is important to make new stem cell lines continuously.

Before use, it is still important to scan the population for the presence of mutated cells by an effective microarray-based method. An effective method would be to see if some pluripotency or cancer genes are over-expressed, or if some cancer protection genes are under-expressed is single nucleotide polymorphism (SNP) microarray [26].

10.4 Prospective

Adult stem cells, particularly blood stem cells, have been used in clinical treatment for more than 40 years. Mesenchymal stem cells are already used in a large number of clinical trials. Pluripotent stem cells, hESC and hiPSC are approaching the first clinical trials. Safety issues are the concerns in stem cell transplantation. Risks of infection are known, and they can be tackled by careful testing of the donor and careful handling of the cells. A particular quality system, GMP, has been developed for this purpose. For tackling immunogenicity, options are matching the tissue types of the donor and the recipient, immunosuppressive medication, possible modulation of the immune response, and the use of patient specific stem cells induced from the recipient's somatic cells. Avoiding substances in cultures that are of animal origin also helps to avoid immune rejection. Tumorigenicity of cultured pluripotent and mesenchymal stem cells is a risk factor that has to be eliminated as well as possible before cell transplantation treatment.

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Part II
Translating Stem Cell
Research Knowledge from Bench
to Bedside: Ethical Issues

Chapter 11

Translational Stem Cell Research and Animal Use: Examining Ethical Issues and Opportunities

Kate M. Millar

Abstract Recent developments in the field of stem cell research has generated notable ethical debate. This ethical discussion often emphasises *human-centred issues*, such as the ethical status of the embryo, with *animal-centred* issues being much less prominent. Further discussion of animal issues is needed given that stem cell research in its many forms has significant implications for the use of experimental animals and, more broadly, may impact on the changing nature of animal-human relationships. Three aspects of this new research phase raise prominent ethical issues relating to animal use: the creation of human-animal interspecies embryos; the use of *in vivo* animal models in translational stem cell research programmes; and the opportunity for embryonic stem cell tests to play a role as new alternatives to laboratory animal use. This chapter explores the increased focus on translational stem cell research and the implications for experimental animal use. When trying to set a context for ethical debate on this issue, applying an adapted ethical matrix for animal use in translational stem cell research may represent a useful tool, allowing users to explore not only individual ethical impacts, but also the ethical implications of long-term research trajectories.

Keywords Ethical matrix • Laboratory animals • 3Rs • Animal ethics • Alternatives

11.1 Introduction

Stem cell research has captivated societal imagination, not only in terms of the possibilities it presents for medical treatment in the future, but also for the ethical questions that are raised. A central question is about how the use of stem cell

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technologies may change the way in which we construct notions of health and aging during the twenty-first century.

The field of stem cell research has moved rapidly, from the first mouse embryonic stem cell (ESC) work in the 1970s and 1980s [1, 2], via further advances in the field of mouse genetics for which Martin Evans received a Nobel Prize (1997), through to the identification of human embryonic stem cells (hESC) by James Thomson in 1998 [3]. However, the last 10 years have witnessed the most dramatic developments. Since the publication of Thomson's landmark paper in 1998 [3], there has been an increase in the volume of research being conducted on human derived embryonic stem cells. In addition, further challenges and opportunities within the field have more recently been presented by the announcement of the derivation of induced pluripotent stem (iPS) cells by Yamanaka's group in Tokyo [4, 5].

These recent developments have generated notable ethical discussion – so much so that ethics is often mentioned in the same breath as stem cell research. This ethical discussion often emphasises what might be termed *human-centered issues*, such as the ethical status of the embryo, the implications of patenting cell lines (as discussed by Hermerén in Chap. 24 of this edition) and the wider long-term implications for the management of health care resources (as discussed by Hermerén in Chap. 31 of this edition). By contrast, discussion of *animal-centered issues* is much less prominent. In short, the implications for the use of experimental animals in both the *in vivo* and *in vitro* research does not often feature prominently on the typology of ethical issues raised.

This relative lack of discussion is problematic, given that stem cell research in its many forms has significant implications for the use of experimental animals and, more broadly, may impact on the changing nature of animal–human relationships. Innovative research in stem cell science has mainly focused on *in vitro* experiments and the use of mouse models [6]. However this area of research has recently entered a new phase. This chapter will highlight three aspects of this new research phase that raise prominent ethical issues relating to animal use, discussing two in more detail. These are the creation of human–animal interspecies embryos; the use of *in vitro* animal models in translational stem cell research programmes; and the opportunity for embryonic stem cell tests to play a role as new alternatives to laboratory animal use.

The first prominent issue of this new phase of stem cell research relates to the increase in production of animal models of disease and their use in pre-clinical trials. Increasing innovation in cell biology and the drive for treatment outcomes has resulted in a greater need for translational research and the development of more specific clinical animal models [7]. As full clinical trials are constructed as inevitable and expectations are raised, the use of non-human animal species other than that of mouse are deemed to be more likely. This shift, if it occurs, would challenge the current trend in animal experimentation governance that is increasingly urging scientists and clinicians to move away from the use of higher sentience animals.

The second notable aspect centers on the “alternatives to animal experimentation” agenda. The outcomes of stem cell biology research has presented new opportunities.

The role that embryonic stem cells could play in future research, particularly the use of hESC in toxicity screening, may herald new opportunities for animal alternatives that could change the face of *in vivo* experimentation. The debate is complex with many ethical questions specific to which experiments and what use. However, a number of aspects can and should be discussed in more detail. These first two aspects, use of *in vivo* models and alternatives, will be examined in further detail in Sect. 11.3 of this chapter.

The third, which relates to fundamental research involving the creation of human-animal interspecies embryos and chimeras, has been widely discussed over the last 10 years [8, 9] and thus will not be covered extensively here. The thrust of the ethical discussion has been human-centric, focusing on questions regarding what it means to be human, the nature of the infringement of species integrity and further implications for the eroding of human dignity [10, 11]. However, this debate is important for other biocentric reasons as it also impacts on the boundaries that are drawn between what is acceptable – or not – to do to animals. If it is acceptable to cross the human-animal species barrier for reasons of research innovation – in other words, if this boundary is not as ethically significant as has been previously argued – then this has implications not only for human dignity, but may also impact on the construction of ethically acceptable interactions with animals. Specifically and markedly, if this research is ethically permissible, what is deemed as an acceptable interaction with animals challenges the use of any species distinction, this further opens up the debate on whether treatment of non-human animals should focus on their similar interests or capacities. Since a deeper discussion of the ethical issues raised by human-animal interspecies embryo and chimera research is beyond the remit of this section, please refer to three chapters in this text, by Neville Cobbe and Valerie Wilson, Gisela Badura-Lotter and Marcus Düwell and, finally, Jochen Taupitz (Chaps. 15, 16, and 17, respectively); for other relevant work, see Baylis (2008), Robert and Baylis (2003), and Greene et al. (2005) [11–13].

The three aspects highlighted above emphasize how this research field is redefining boundaries, not only relating to human medicine. This area presents new opportunities for the growing “alternatives to animals” research community, yet also still presents ethical risks and challenges that require further reflection.

11.2 Animal Use in Research

Before discussing the specific issues of experimental animal use and stem cell research, it is important to explore the context in which this animal use debate is occurring.

The use of animals in biomedical research has been the subject of notable public and political discussion over the last few decades. In recent years it has been argued that there has been a significant shift in the ethical status of animals *per se* within society [14, 15]. It is claimed that we have become increasingly aware of animals’

welfare status and beyond, and this has resulted in a shift in the social ethic that applies to animals that leads to the point where ... *the legal minimum should be a life worth living where their integrity is respected and where animals' physical and mental needs are met* [16].

The global use of animals in experimentation is estimated to be between 100 and 200 million animals per year. It is the use of animals *in vivo*, vivisection experiments, that has stimulated the greatest public debate, particularly in Europe and North America. The debate is epitomized by the foreword of the recent UK Nuffield Council on Bioethics report that reviewed the ethics of animal experimentation [17], *The issues ... [raised by research involving animals] ... have been a subject of intense public debate over at least the past four hundred years. Feelings are strong on all sides of the issues, and in recent years reports of violent action against those conducting animal research in the UK have brought the matter to the forefront of public attention. Nearly all scientists would agree that the use of animals raises ethical issues. Assuming that an ideal situation would be an end to vivisection, the justification for research involving animals needs to be examined.*

When examining the use of animals in stem cell research, it is also important to highlight more general recent trends in animal experimentation. In some European countries quite detailed annual data is collected on the use of animals. This can include data sets on the number of experiments and the type of species used, through to the severity of the procedure conducted on the animal. Using the UK as an example, there are notable recent trends in animal use that are relevant in this context.

Firstly, in the UK the number of procedures conducted on animals is recorded each year. Detailed annual figures, going beyond pure numbers, have been recorded since the mid-1940s. During the period from the mid-1970s to 1990s there was significant reduction in the number of experiments using animals. This trend of reduction continued until 2000, when numbers began to increase; this raise is exemplified by the most recent figures, which show “*just under 3.7 million scientific procedures (conducted on animals) were started in 2008, a rise of 454,000 (14%) on 2007*” [18]. This increase in animal use partly results from a rising numbers of animals being used in breeding programmes, which are now included in animal experimentation data, but the increasing use of genetically modified (GM) animals also impacts on the figures. In 1995 only 8% of the animals used in the UK were GM. Over the following 10 years this number had increased six-fold, rising to 33% in 2005. In 2008, this figure had increased to 37%. GM animals now account for over a third of all animals used. It should be noted that of this number, the species used in 97% of these procedures are fish and predominately mice. This trend has been noted in other countries, including the USA.

A final notable aspect is the use of higher species in experimentation, with these species (including cats, dogs, and primates) currently accounting for less than 0.5% of overall procedures conducted in the UK. Interestingly, the combined figures for 2008 was lower than 2007. However, the 2008 figures show a fall in dog use and an increase in non-human primate use, an increase of 600 procedures using an additional 230 primates [18]. The use of higher mammals for *in vitro* work has been

a prominent point of debate across Europe. This issue, specifically with reference to primates, has received notable attention in the discussions surrounding the revision of the “protection of animals used for scientific purposes” EU Directive (COM[2008]543 final). These trends are important when we explore the implications of translational stem cell research.

Examining the way in which animals are used in experimentation, generally three types of classification are often referred to: (1) toxicity/safety testing, this use is often required by law; (2) applied research, including some pre-clinical experiments; and (3) basic research. Although this is a simplistic representation of a broad range of research activities, these categories at times can be useful as they intimate to the types of argument that are proposed as underlying justification for animal use for certain research purposes or priorities.

Having briefly outlined some of the key trends and debates within animal research, it is important to turn to a consideration of the ethical issues. The Nuffield report identifies two fundamental questions, “*First, does the scientific use of animals lead to valid, useful and relevant results in specific areas? Secondly, is it permissible for one species to cause pain, suffering and death to another to achieve aims that primarily benefit the former species?*” [17]. The justification for the use of animal experiments in translational stem cell research appeals to utilitarian arguments, answering the first question with arguments of “validity,” “need” and “least-harm/greatest good.” In other words, animal research is justified through the acquisition of knowledge; for example, in stem cell research this knowledge underpins the next phase of human trials. However, this utilitarian argument can “cut both ways,” as not all animal models provide “useful” data. If animal models are not ideal “causal analogue models” for human disease, then the ethical and scientific justification for animal use is weakened. This is an important aspect to consider when arguments are presented to conduct specific experiments with specific species as part of a translation pre-clinical strategy. A further prominent question for this research area is: Does translational stem cell research present new challenges that are not presented by other areas of the biomedical research, such as, for example, oncology or genetic research, and what are the opportunities to find alternatives? This question will be explored in the next section of this chapter.

11.3 Animal Use, 3Rs and Stem Cell Research

A central concept in the discussion of the ethics of animal experimentation is the “3Rs.” This concept, an important part of the application of utilitarian cost-benefit assessment that is embedded within a range of animal experimentation regulations, was originally famously proposed by Russell and Burch, published as “The Principles of Humane Experimental Technique” in 1959 [19]. This work was the first to set out “guiding principles” that all researchers should apply when conducting animal-based studies. This important text proposed that researchers should strive to Replace, Reduce and Refine animal use.

In specific terms, the 3Rs can be simply translated as the need to (1) replace living animals in scientific experiments with approaches that do not involve animals; (2) reduce the number of animals used to an absolute minimum that would be needed to obtain valid results; and (3) refinement requires the use of procedures that will have minimal adverse effect on the animal used. The application of refinement also prompts researchers to seek to review the species used and strive to use lower species whenever possible.

The 3Rs has been a central element of animal experimentation legislation across Europe, such as in the UK, requiring experimenters to consider the 3Rs before they proceed with their research. A number of academics have examined the nature of this concept, deliberating over whether it is a political, scientific or ethical concept [20]. It might be argued that in a pluralistic society, the concept is all of the above, but what is important in the discussion of new areas of research – for example, for translational stem cell research – is how the three components of replace, reduce, and refine are respected or infringed upon.

Refinement and Reduction Firstly, examining the principles of refinement and reduction as clinical opportunities emerge from stem cell biology, it is likely that an increasing number of pre-clinical animal models will be used [7]. One of the key questions when considering the impact that this may have on animal use is whether this new area of research will result in a new form or level of animal use in biomedical experimentation, i.e., a step change that is different from current trends. Do the drivers for stem cell therapies generate new imperatives to use animal models? More importantly, how might these changes challenge wider practice and priorities in animal experimentation?

When we explore our ethical responsibilities to laboratory animals, even when considering the diversity of ethical positions held, applying principles of refinement and replacement has played a notable role in assessment of acceptable experimental practice [15]. In terms of reduction and the impact of stem cell research on the numbers of animals used, due to the way in which data is collected within many jurisdictions, it is not possible to identify how many procedures or animals have been used within this research area. However, there has been notable ethical discussion around the need for pre-clinical trials. The International Society for Stem Cell Research (ISSCR) sets out a form of imperative for pre-clinical research: “*Recommendation 11: Sufficient preclinical studies in relevant animal models – whenever possible for the clinical condition and the tissue physiology to be studied – are necessary to make proposed stem cell-based clinical research ethical, unless approved, controlled, and conclusive humans studies are already available with the same cell source. Investigators should develop preclinical cell therapy protocols in small animal models, as well as in large animal models, when deemed necessary by independent peer review or regulatory review*” [7]. Many organizations would support the need for these studies, but this recommendation seems to give limited weight to the findings or views of other bodies that promote the value of alternatives in associated fields and the impact these can have on the overall number of animals used. For example, the National Research

Committee on Toxicity Testing and Assessment of Environmental Agents has highlighted the need to use culture cells in toxicity studies while pointing out the limitations of animal studies [21]. In a number of cases including stem cell research, striving to reduce the overall number of animals used or avoiding the use of higher animals may not only represent a strong 3Rs ethical position, but also a scientifically supported approach.

One significant challenge to the further application of refinement may be the claim that higher species are needed in some preclinical trials. The use of higher species in experimentation, particularly the use of non-human primates, has been a long-standing area of controversy and debate [22, 23]. The arguments against use draw on a number of ethical positions, from pathocentric concerns (for animal suffering), through speciesist challenges, to absolute rights-based positions [23]. The use of non-human primates has been widely discussed in the development of the new European Directive ("Protection of animals used for scientific purposes" EU Directive (COM[2008]543 final) and a number of countries are limiting their *in vivo* research in this area, often referring to "capacity arguments." It is still unclear how these positions and the implementation of the new EU legislation will play out in practice when new biomedical innovations, such as in the stem cell research area, are presented for pre-clinical assessment.

However, one point that may be worth noting in this discussion relates to refinement in experimentation. It might be argued that researchers working in translational stem cell research using hESCs have a particular responsibility to apply the principle of refinement, with regards to the need to reduce pain and suffering when conducting *in vivo* trials. The issue of pain perception and sentience has played an important role in the hESC ethical debate. It was the Warnock Committee (1982–1984) that first argued that it was ethically acceptable to use human embryos under 14 days of development as, among several important considerations, these embryos have not started to develop a spinal cord (primitive streak) and therefore have no sense or sensation of pain [24]. If this is indeed a persuasive argument, then this line of pathocentric argumentation should hold true for other areas and may therefore represent the need for a greater application of the principle of reduction in translational stem cell research when applied to sentient animals.

Replacement Of the two forms of replacement, an absolute replacement will not use any form of animal material, such as human studies or the use of computer modeling. Whereas relative replacements involve studies not conducted on live animals, they will involve the use of animal tissue *in vitro*, such as the use of cell cultures.

In an attempt to reduce the use of animals in toxicity testing that is conducted to protect the environment and human health, researchers are striving to identify robust *in vitro* models. For human toxicity studies, these models need to recreate the human system. At present, a number of mouse-based cell systems have been developed and assessed; however, these have been shown to be different from *in vivo* human systems. Hence there is growing interest in the use of hESC systems, since assays or the screening of potential hazards using these cells should be far better models. One area that appears to be showing notable progress is the use of

hESC in reproductive toxicity testing [25]. Krtolica et al. argue that hESCs can be used to examine the effects of toxins on cell differentiation (inner cell mass and trophoblast) and hence act as a valuable model for human embryo pre-implantation development [25].

The introduction of the new EU REACH regulations (Registration, Evaluation, Authorization and Restriction of Chemicals, 2007) will require greater toxicity testing of chemicals, and as a result, many animal protection groups are concerned that this will lead to much greater use of *in vivo* testing. Therefore the development of hESC systems may represent a much-needed opportunity to reduce the animal burden, and possibly providing more robust screening tests. The REACH agenda and the increasing desire across Europe to replace animal *in vivo* testing may even imply a research imperative in the *in vitro* stem cell field, with much more investment needed to ensure that tests can be developed and validated as quickly as possible in order to make a notable difference [26].

In addition to the REACH agenda, use of hESC cell lines, such as those using cardiomyocytes, may prove to be useful in drug screening and development [27]. Recent work has shown interesting opportunities in “cardiac safety pharmacology” with significant interest from the pharmaceutical industry [28]. The key question is whether these models can be adequately validated, but it is hoped that developments such as these will provide cost-effective and time-efficient screening, as well as eventually reducing large animal use and the overall number of *in vivo* studies carried out in drug development.

11.4 Applying Ethical Frameworks to Assist Decision-Making

As highlighted above, in a society that presents a plurality of values when considering the issues of animal use in experimentation, one of the most challenging aspects of any assessment is presented by the weighing of the ethical implications of animal experimentation against the ethical goods of potential societal and human benefit. This challenge is exemplified by reference to the cost-benefit analysis in UK legislation, where before granting a licence, every experiment must “weigh up” the costs to the animal against the benefits to society. This analysis is often done in isolation for individual experiments and does not include the collective impacts of a novel field of research.

There is no simple solution to the ethical challenges presented by these complex decisions; however, it can be helpful to include this cost-benefit analysis in a wider context by using decision-support tools. When considering these aspects and the weighing of ethical impacts, both positive and negative, for translational stem cell research, some areas initially appear much more justifiable than others; however, it is valuable to explore the ethical dimensions in more depth and this can be facilitated through the use of ethical frameworks.

A number of ethical frameworks has been developed, many based on a participatory approach applied to facilitate dialogue and ethical analysis; one such tool that

can support wide reflection and analysis is the tool proposed by Mepham [29], the Ethical Matrix (EM).

The EM, a conceptual tool, was originally proposed to help users map the ethical issues raised by the development and use of biotechnologies. Subsequently, the framework has been used by a number of organizations, from NGOs through to advisory committees and commercial companies. As well as analyzing the impacts of emerging biotechnologies, it can and has been applied to explore the ethical issues raised by bioscience research programmes that embed animal experimentation – for example, uses of the ethical matrix applied to animals include (1) GM animal biotechnology such as bST and GM fish [30, 31]; (2) to assess animal genomics research programmes [32]; and (3) companion animal reproduction [33].

Applying the EM requires the assessment of a proposed strategy; for example, this can be the use of an animal model in a pre-clinical stem cell research programme, in terms of respect (or lack of respect) for three specified ethical principles. These principles, wellbeing, autonomy and fairness, are *prima facie* and they are applied to the pre-defined set of interest groups. These ethical principles, often seen as foundational principles, are derived from the *prima facie* guiding principles originally proposed by the medical ethicists Beauchamp and Childress. For the case of animal experimentation (Table 11.1), the matrix consists of rows that indicate the ethically relevant interest groups. These are groups that may impact on or be impacted by a research programme, both positively and/or negatively. The interest groups defined here for animal use in experimentation are patients (consumers); experimental animal(s); clinicians/researchers; and industry and wider society. The three ethical principles are then applied to these interest groups to examine the impact for each “cell” of the matrix. Once all of the cells of the matrix (table) have been mapped, an ethical “weight” (significance) can then be assigned in order to determine an overall judgment.

As emphasized in previous work using the Matrix, “*It should be noted that this method is not prescriptive and therefore will not produce ‘an answer’, but the method can make the ethically relevant issues transparent and thus facilitate informed decision-making. The value of the approach is that, it makes explicit the evidence used to justify a position and encourages ethical reflection on the impacts for all ethically relevant interest groups. The method can also act as a starting point for ethical deliberation in public policy decision-making*” [33].

Table 11.1 Modified ethical matrix for translational stem cell research

Interest group	Respect for three ethical principles		
	Wellbeing	Autonomy	Justice
Patients (consumers)			
Experimental animal			
Clinicians/researchers			
Industry			
Society			

This adapted matrix shown in Table 11.1 could be applied to map the issues raised by the use of different animal models by mapping where the ethical principles for the groups are infringed upon or respected by animal use. It is noted that it is not within the remit of this chapter to conduct an analysis for a specific translational stem cell experiment or programme; however, reviewing the matrix's cells for this issue can help to identify the aspects that require broader considerations and may bring to light areas that will represent key ethical challenges and opportunities.

This modified matrix could be used by animal research committees or groups such as the ISSCR, allowing them to discuss the issues raised by research programmes as well as specific experiments. Alternatively, this tool can be used for “brainstorming” about research trajectories and futures. The method has previously been applied to allow communities of researchers to reflect on collective ethical responsibilities, by allowing them to hopefully look beyond the singular day-to-day decisions. This may help researchers look beyond the step-by-step slippery slope concerns presented by this type of research, and hence examine the wider context.

11.5 Future Challenges

As previously noted, stem cell research presents new challenges for the medical profession with prominent ethical questions raised by current and prospective research activities. Considering the potential role of animal models in the next phase of translation stem cell research, it is notable that the use of animals does not appear to have been framed as a significant ethical issue as part of the societal discourse that has emerged around this research area. It might be argued that the focus of the current stem cell ethical debate reduces the space for a sound ethical discussion of the issues raised by the potential increase in animal use in this translational research area.

Across the EU, with the challenges of the REACH agenda and the revision of the Directive that calls for increased harmonization of animal experiments, this new area of translational research area may both challenge and be challenged by shifting boundaries, and this will undoubtedly impact on the way animal experimentation is conducted and regulated.

Therefore of the three ethical aspects that have been identified in this chapter, the potential to replace *in vivo* models and to develop cell-based relative replacement alternatives presents a significant ethical opportunity. It appears that there is a major opportunity to drive forward the animal alternatives agenda with greater investment in this research area. At a time when we search for ways to minimize the use of animals at a global level, development of *in vitro* models could be a focal priority. It is important to bear in mind the drivers to use pre-clinical animal models, particularly higher species. However, if the work of organizations such as the National Research Committee on Toxicity Testing and Assessment of Environmental Agents has a pressing point, looking for alternatives may not only benefit animal-kind but human-kind, as we avoid using potentially ineffectual and expensive *in vivo* animal models.

Finally, when trying to set a context for ethical debate on this issue, applying an adapted ethical matrix for animal use in translational stem cell research may help place this work in an important broader animal wellbeing and societal setting, allowing users to explore not only individual ethical impacts, but also the ethical implications of long-term research trajectories.

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Chapter 12

Ethical Aspects of Stem Cell-Based Clinical Translation: Research, Innovation, and Delivering Unproven Interventions

Jeremy Sugarman and Douglas Sipp

Abstract The clinical translation of human pluripotent stem cells may take several different paths including clinical translational research, medical innovation, and the clinical use of untested or unproven interventions. Each of these translational pathways operates within particular ethical frameworks. These frameworks include distinct standards of practice and procedural mechanisms for addressing the ethical issues inherent to each pathway. As such, it is essential that these differences be recognized to best protect not only the well-being of patients, but also of the scientific enterprise as it seeks to understand the true value of stem-cell based interventions.

Keywords Clinical trials • Ethics • Research ethics • Stem cell therapies • Stem cell treatment

12.1 Introduction

Stem cell research has attracted much attention not only for its fundamental scientific interest, but also for its great therapeutic promise. With the exception of bone marrow and umbilical cord blood-derived hematopoietic stem cells in the treatment of disorders of the blood and immune systems, however, that promise currently remains largely unfulfilled. Efforts to bridge the results of basic stem cell research into clinical applications are now beginning to gain momentum, through a variety of translational pathways. These pathways include conventional clinical translational research, medical innovation, and the clinical use of unproven novel interventions.

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A number of universities, hospitals, and companies in the United States, Europe, Asia, and Latin America have received approval to conduct conventional clinical trials to test the safety and efficacy of stem cell interventions for a broad range of conditions. Others are also engaged in medical innovation, in which potential therapeutic value is assessed in small numbers of patients. And multiple companies, clinics, and individual clinicians now proffer stem cell-based untested interventions to patients, often attracting them to travel abroad to receive them. Each of these forms of clinical translation differs markedly in a number of respects. While other chapters in this volume examine the particular scientific aspects of stem cells in relation to the development of potentially therapeutic interventions, in this chapter we examine the ethical values inherent to clinical translation. Specifically, we describe each pathway in turn, outlining some of its most relevant aspects followed by a discussion of the relevant ethical frameworks associated with it.

12.2 Translational Research

We use the term “translational research” to refer to an experimental activity whose primary goal is the acquisition of knowledge, specifically in regard to the safety and/or efficacy of a clinical intervention. The standard translational research paradigm begins with experiments conducted at the laboratory bench, followed by experiments with non-human animals. Promising interventions are then tested in a staged series of experiments with human subjects. Phase I research typically involves a small number of subjects in which the primary concern involves testing for safety and dosing. If an intervention is found to be safe, Phase II research may be initiated to test for signs of efficacy among patients with a particular disease or condition. If the results are encouraging, Phase III research is conducted in a larger number of patients to assess the efficacy of the intervention in comparison to existing standard approaches, and to develop additional safety data in a larger set of subjects. Finally, if the intervention works well, and receives necessary regulatory approval for marketing, the longer-term effects of the intervention may be evaluated via post-marketing surveillance research.

The attributes of this classic pathway of clinical translation is that it is ideally suited to provide robust data about the safety and efficacy of interventions. In addition, when accompanied by multiple levels of oversight, it can help protect the rights and welfare of the participants. Such an approach is, however, time-consuming and expensive, which potentially precludes testing of some promising interventions.

Regardless, clinical trials involving several types of stem cells for many different indications are already under way or in planning around the world. In the United States, for example, Osiris Therapeutics has tested or is testing treatments using bone marrow-derived mesenchymal stem cells for graft-versus-host disease (GvHD; this trial was terminated in Phase III), Crohn’s disease, chronic obstructive pulmonary disease, Type I diabetes, and arthritis of the knee [Osiris]. The Geron

Corporation received approval to begin clinical trials of human embryonic stem cell (hESC)-derived oligodendrocytes in acute thoracic spinal cord injury, but several months later the trial was placed on clinical hold by the US Food and Drug Administration after newly-released results from animal trials indicated an unexpectedly high number of microscopic cysts developing in regenerative sites [Geron]. Neuralstem, also based in the US, has received permission to conduct a trial of spinal cord neural stem cells in the treatment of amyotrophic lateral sclerosis [Neuralstem]. ReNeuron, a company in the United Kingdom, has received approval to start a clinical trial for the use of fetal stem cells in the treatment of stroke [ReNeuron]. In addition, numerous trials are being conducted at academic hospitals in many countries, including a 1,200-subject study of the use of adult bone marrow cells in the treatment of cardiomyopathy [1].

Such research carries a range of practical and ethical considerations. While these have been discussed extensively elsewhere, there are a number of issues that are especially relevant to the clinical translation of stem cells, or their immediate derivatives, which warrant mention here.

First among these is the safety of pluripotent stem cells, such as human embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs). These cells, which exhibit differentiative capacity that enables them to give rise to any type of cell in the adult body, are also known to form teratomas when injected into mice. More importantly, the injection of differentiated cells derived from mouse ESCs in studies using animal models of various human diseases, including diabetes and neurodegenerative diseases, has been shown to carry a significant risk of tumorigenesis, presumably due to the inadvertent inclusion of undifferentiated (pluripotent) cells in the transplanted population [2, 3]. Such concerns were among the primary scientific obstacles to the approval of the first clinical trial using human ESC-derived cells, for the protocol developed by Geron to test the safety of ESC-derived oligodendrocytes in subacute thoracic spinal cord injury described above.

Early protocols for the generation of iPSCs used, among four transgenes, a known oncogene (*c-Myc*) as well as a retroviral vector for gene delivery, and carried a risk of genomic instability occasioned by the multiple gene insertions required to achieve the induction of pluripotency [4]. Each of these factors poses barriers to human use. While some of these concerns have been alleviated by recent methodological advances, legitimate concerns surrounding the long-term genomic and karyotypic stability of iPS cells and their derivatives remain [5].

A second, related aspect of stem cells (both pluripotent and multipotent) is the expectation that they will properly integrate, survive, and function indefinitely in the recipient's body. This expectation still remains to be proven, however. In addition, this "permanence" factor, while desirable if the intervention is successful, raises additional concerns about safety should there be detrimental effects from the integration of the cell-based intervention since, unlike the case for many drugs, it may not be possible to remove the entity causing harm.

In contrast to pluripotent stem cells, multipotent cells show reduced differentiative plasticity (i.e., they normally give rise to only a subset of related cell types), and no proclivity for teratoma formation. That is not, however, to say that

their use is risk-free. Transplants of allogeneic hematopoietic stem cells, both bone marrow-derived and those from umbilical cord blood, have resulted in graft-versus-host disease, as well as the delayed onset of malignancy such as leukemia [6, 7]. The real possibility of such late-onset complications from similar cell-based interventions and the putative lifelong biological activity of transplanted cells highlight the need for long-term evaluations of the safety of stem cells and their derivatives. Importantly, should truly curative applications be developed in the future, patients will presumably visit their physicians less frequently, which may make regular monitoring problematic.

The nature of the Phase III clinical trial itself entails certain tensions that, while perhaps not unique to stem cell research, are brought into sharper focus by the high degree of therapeutic promise currently attributed to the field. For example, there is substantial cost and expense involved in designing and carrying out a rigorous study capable of generating statistically significant and robust data. Some industry experts estimate that such a clinical trial of a cellular therapy is likely to cost from US \$100 to 200 million.

A number of patient groups have either driven clinical research through direct funding (such as the Juvenile Diabetes Research Fund, which provided \$160 million in internationally competitive basic and clinical research funding in 2008 [JDRF]), and through coordination and recruitment of patient-subjects to ongoing clinical trials. One notable example of this is the activity of an American group known as ALS Worldwide, which has worked with clinical researchers in Mexico (at the Tecnologico de Monterrey Hospital) to recruit amyotrophic lateral sclerosis patients from as far away as The Netherlands and New Zealand to a preliminary study of the safety and efficacy of autologous CD133⁺ stem cells in treatment of that disease [ALS Worldwide].

Activism on the part of patient groups will likely continue to be important to ensure the conduct of clinical research in neglected diseases. The vast majority of trials performed by industry inevitably tends to target disorders that would constitute a sufficiently large market to justify the research investment, and academic research in industrialized countries likewise tends to focus on ailments that are prevalent in those countries. Again, this “orphan disease” problem is not specific to stem cell clinical research, but is particularly salient given the high levels of visibility and expectations that surround the field.

In addition, many patients now look to stem cell research as a possible cure for their ailments, even though the research process is focused primarily on discovery. Such expectations themselves present a potentially confounding factor in the clinical translation of stem cell research. Pre-existing and deeply embedded therapeutic belief regarding these cells and their products among those who will be recruited to participate in research may exacerbate the likelihood that those enrolled will harbor a “therapeutic misconception” in which subjects erroneously believe that the primary goal of research is clinical care rather than science. This anticipation of efficacy may be associated with significant effects among trial subjects. In fact, a Stage III clinical trial of mesenchymal stem cells for the treatment of Crohn’s disease has to be redesigned after unexpectedly high placebo response rates [Osiris].

Ethical Frameworks Given the enormous promise of stem cell research and the risks associated with both pluripotent stem cell transplants (in animal studies) and multipotent stem cell transplants (in humans), scientists have begun to develop guidelines for translational research involving stem cells. The International Society for Stem Cell Research (ISSCR) issued its “Guidelines for the Clinical Translation of Stem Cells” in January 2008 [8]. These guidelines recommend minimum standards of practice for three aspects of translational stem cell research: cell processing and production, preclinical studies, and clinical studies. This third area addresses both clinical trials and medical innovation; additional discussion is devoted to aspects of social justice.

The guidelines call not only for the comprehensive characterization of the stem cells themselves, but also for clear assessments of manufacturing standards, pre-clinical data, and measures for mitigating risks such as tumorigenesis. Emphasis is placed on the need for extensive independent monitoring and peer review as well as careful attention to informed consent. Thus, in large part the guidelines bolster considerations and mechanisms now commonplace in clinical and translational research, specified to the context of stem cell research. For example, they seem to be largely consistent with recognized international documents such as the Declaration of Helsinki [9] as well as the International Council on Harmonization [10] and regulatory standards for the approval of biologic treatments. At a theoretical level, they also comport with well-recognized ethical principles for human subjects research, such as were articulated in the Belmont Report issued by the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research [11]. In broad terms, the Belmont Report argues that there are three primary ethical principles that are *prima facie* binding in research: respect for persons, beneficence, and justice. Respect for persons is manifested in the expectation of obtaining informed consent. Concern about the welfare of participants, including minimizing risks to them while maximizing scientific benefit, is subsumed under the principle of beneficence. Considerations regarding fair subject selection are a matter of justice. In now-familiar approaches in policy and ethics, attention is focused on these principles during prospective review of research as well as other procedures to help ensure that the rights and welfare of participants are protected.

Nevertheless, it is important to observe that the ISSCR guidelines not only recapitulate familiar mechanisms, but they also expand the range of relevant considerations to include the need for publishing research results as well as considerations related to social justice. Recommendations with respect to social justice include the need for frank reporting, public engagement, maximizing social good, and aspiring to make beneficial interventions available in resource-limited settings.

12.3 Medical Innovation

While the staged approach of translational research is scientifically appealing, some critically important advances in clinical practice eventuated from what may be termed “medical innovation.” Indeed, medical innovation is commonplace in

surgery [12], where it is a recognized pathway of clinical translation. In the case of surgery, “An innovation is a new or modified surgical procedure that differs from currently accepted local practice, the outcomes of which have not been described, and which may entail risk to the patient. Many innovations are used on an *ad hoc* basis as dictated by the clinical situation. Some innovations, however, may be developed in a more systematic fashion and may ultimately meet the criteria for human subjects research, although they do not meet the criteria at the time they are developed” [12]. Recent scholarship in the surgical literature distinguishes variations from medical innovation. Here, “A variation is defined as the minor modification of a surgical procedure that does not have the reasonable expectation of increasing the risk to the patient” [12].

An innovation paradigm is also familiar to those engaged in the transplantation of hematopoietic progenitor cells. Take, for example, the first transplantation of umbilical cord blood aimed at the treatment of a child with Fanconi anemia [13]. In this case, the patient was eligible for an allogeneic bone marrow transplantation but a suitable bone marrow donor could not be identified. However, the patient’s mother was pregnant at the time that transplantation was being considered and it was possible that the newborn would be a good match for transplantation. Based on *in vitro* work as well as studies in an animal model, the clinicians raised the possibility of using the newborn’s umbilical cord blood for transplantation. With parental consent, the innovative transplantation was performed successfully, sparking the field of cord blood transplantation. Of note, a series of patients with malignant and non-malignant conditions received innovative experimental cord blood transplants before other efforts began to be considered under more formalized research proposals [14, 15].

Given the clear need to evaluate promising therapeutic interventions for safety and efficacy, especially when there may be other available interventions, the extent to which a medically innovative case series may proceed before the transition to a controlled clinical trial is made unclear. Nevertheless, absent concrete recommendations, there would appear to be a very real risk of the unintended routinization of treatments which have not been adequately tested for safety and efficacy. Such concerns have been raised in other fields, such as the use of maternal fetal surgery for spina bifida and the use of live donor liver grafts for transplantation [16, 17]. Despite good evidence of efficacy for some hematopoietic cell-based interventions, the need for evaluating safety and efficacy under a research paradigm is underscored by the example of autologous bone marrow transplantation for the treatment of breast cancer. Despite “success” described in numerous case series, the risky, expensive, and arduous procedure was not found to be useful after being subjected to randomized experimentation [18].

Ethical Frameworks In its *Guidelines for the Clinical Translation of Stem Cells*, the ISSCR notes that, “Responsible clinician-scientists may have an interest in providing medically innovative care to a few patients using stem cells or their derivatives prior to proceeding to a formal clinical trial” [8]. The guidelines go on to explain the conditions under which such innovative treatment, which puts

potential therapeutic benefit ahead of generalizable knowledge, should be permissible, including a stated scientific rationale, the potential therapeutic value in comparison with existing treatments, full characterization of the cell types used, a detailed description of the treatment protocol, and a plan for follow-up to assess both efficacy and the incidence of adverse events, if any. The plans for the innovative treatment should be approved in advance, subjected to quality assurance and ethical oversight, and conducted with appropriate protections for patients [8].

Lindvall and Hyun, who co-chaired the Task Force that prepared the *Guidelines* subsequently suggested that “The ethics of medical innovation is the ethics of patient care, not research. The research ethics paradigm views innovative treatment as a departure from standard treatment and overlooks clinical situations in which the currently accepted treatments are ineffective or burdensome” [19]. However, it is not clear that it follows that innovative treatment must therefore fall under the ethical rubric of patient care; the dichotomy may be false. While most variations in practice would be considered within the ambit of responsible clinical practice, medical innovation seems to share aspects of both practice and research, calling for a more precise delineation of the ethical values at stake [20]. Responsible medical practice privileges the use of evidence-based approaches to diagnosis and treatment. Responsible medical innovation requires an in-depth knowledge of both the disease and the proposed intervention, as was true in the case of those engaged in the first transplants of cord blood. While the concerns of the patient were paramount, the desire to try out a promising idea in a very controlled setting and to report the results cannot be ignored. As such, it seems unjustifiable to conclude that the only relevant considerations here are the ethical considerations that inform clinical practice.

While developing a moral theory of innovation is beyond the scope of this chapter, it seems that such a theory would include, but not be limited to, the basic ethical principles of clinical practice, perhaps capturing the fiduciary nature of such relationships as well as a means of identifying and managing competing scientific interests. In addition, the types of interventions that might responsibly be used under an innovation paradigm would seem to differ only slightly from available interventions about which there exists substantial knowledge and experience. That is, there should be minimal incremental risk involved.

As conceptual approaches to innovation are developed, and experience using the proposed procedural frameworks for the responsible innovation proffered for surgery and stem cell transplantation is garnered, there is a tangible need to ensure that the innovation pathway is not misconstrued in such a way that it redounds in harm to current and future patients. Innovation should not be viewed as a shortcut to be taken simply because the research ethics paradigm may involve burdensome processes of review and oversight. In addition, it should not be employed simply because the testing and evaluation of interventions under a research ethics paradigm may be expensive, or so that those engaged can profit financially by labeling the intervention as innovative instead of experimental.

12.4 Clinical Use of Unproven Interventions

Although the clinical use of unproven interventions may occur in many clinical settings, the acceptability of such practices seems to differ greatly across the globe. In addition, there are different mechanisms and standards for licensure and use in different jurisdictions. Thus, what constitutes adequate testing or proof is itself a complex issue. Nevertheless, the clinical use of untested or unproven interventions under an established and reasonable rubric of assessment, especially those that carry risk and/or preclude the delivery of alternative treatments that are known to be safe and effective, is morally problematic.

The clinical use of unapproved or unproven interventions can be exacerbated by medical tourism (also known as medical travel or global healthcare), which involves international travel by patients to receive routine medical, cosmetic, or dental procedures. Legitimate medical tourism is understandably driven by lower costs or shorter wait times available in overseas hospitals offering similar (or superior) standards of care. Estimates of current market size range from 65,000 to 750,000 patients per year [21, 22], and this is projected to grow, indicating the increasing acceptance of the concept of medical travel.

With respect to stem cells, the growing acceptance of medical tourism may spur patients to travel not because of cost or convenience, but by the availability of procedures that are unapproved for use in the patient's country of residence. This practice of regulatory arbitrage has been seen previously with procedures such as LASIK (laser-assisted *in situ* keratomileusis), which was available outside the United States for years prior to its approval in that country.

In fact, a global industry centered on travel by patients to receive stem cell injections unavailable in their own countries has arisen and continues to grow across a very broad geographical range, with companies and individuals operating in both developing and advanced economies. This phenomenon, commonly referred to as "stem cell tourism" (although this is something of a misnomer, as many patients are seriously ill and presumably not traveling for the purposes of sightseeing or shopping), has given rise to widespread controversy for the nature of claims being made in regard to conditions being treated and cell types being used, and for the tendency of such companies to rely on patient testimonials and viral marketing techniques in lieu of peer-reviewed publications and rigorous experimental data.

A review of online advertisements by such offshore stem cell clinics reveals a great variety of claims regarding stem cell types, including hematopoietic (derived from bone marrow, peripheral blood, or umbilical cord blood), adipose, neural, mesenchymal, fetal, and embryonic, as well as numerous claims regarding the use of animal cells. In nearly all cases, detailed information on the cells being used is not publicly available, making it impossible to determine whether such claims reflect the true nature of the cellular product(s) being injected. The range of therapeutic claims is even broader, extending from cosmetic and quality-of-life applications, such as facial wrinkles and erectile dysfunction, to chronic or life-threatening

conditions including spinal cord injury, congestive heart failure, HIV/AIDS, Parkinson disease, and amyotrophic lateral sclerosis [23]. One survey of the medical literature of claims made by 19 such clinics revealed that their claims of efficacy consistently exceeded the evidence available from published studies [24].

In addition to exploiting notions of the comparative slowness of the drug approval systems in Western countries, providers of stem cell-based interventions also capitalize on widely held conceptions of how stem cells might work in the treatment of medical conditions. Claims typically include that stem cells home to damaged sites in the body and instinctively “know” what to do [Medra], which can take the form of differentiating into the needed cell type(s) or secreting unnamed cytokines or paracrine factors [Beike]. Some cite early-phase clinical trials conducted internally or by other institutions, but the claims generally exaggerate benefit and understate risks [24].

Some physicians involved in stem cell tourism have also advanced the argument that it is unethical not to provide a treatment to a patient who has no other options, or that stem cell clinical research has been stymied by the medical establishment and the global pharmaceutical industry because of the damage a one-time, lifelong cure would do to their business models [International Cellular Medicine Society]. Such arguments are lent a degree of superficial plausibility by recent scandals in the testing and marketing of drug products, and reports of the dysfunctionality of regulatory agencies such as the US FDA.

Ethical Frameworks Across the globe, there is a wide range of local standards of medical practice, which are often embedded in local laws and policies. Analyzing the range of goals of medical practice turns out to be quite complex [25], but despite the lack of clear international consensus on what constitutes ethically appropriate practice and how to justify it, the interests of the patient are a consistent priority. Thus, it seems fair to claim that a core defining ethical goal of medical practice is beneficence. That is, there is an obligation for clinicians to help their patients while minimizing potential harms related to medical interventions. As such, clinicians have a fiduciary obligation to their patients, which places the patient’s best interests above their own.

Accordingly, it would seem to be unethical for clinicians to prescribe or administer untested or unproven interventions to patients unless there is both no other reasonably available alternative treatment, and there is good reason to believe that such interventions will provide meaningful benefit without undue harm. This calls into question many of the reported practices seen in stem cell tourism for which there seems to be an insufficient evidence base for the intervention, and where there is a strong possibility that the interventions may be harmful.

Of course, it is conceivable that a clinician may have discovered, through serendipity or innovation, a particularly effective intervention. While it would obviously be consistent with a fiduciary obligation to provide such an intervention, it is also arguably important for the clinician to take measures to ensure that the interventions are indeed safe and efficacious (including over time) and to share this information with colleagues through peer-reviewed mechanisms.

12.5 Concluding Comments

Given the different pathways of clinical translation, it is important for those engaged in the process to understand precisely where their particular endeavor fits, since each is characterized by a different set of ethical frameworks that have unique procedural mechanisms associated with them. Conventional clinical translational research prioritizes the ethical frameworks applied to research that find application in international guidance documents and in oversight that includes independent review of proposed interventions. Medical innovation falls somewhere between the paradigm of medical research and clinical practice. Although new mechanisms of oversight have been suggested, there is little accumulated experience regarding their implementation and efficacy. Clinical practice is in large part subject to professional norms and an array of local forms of oversight, such as licensing boards. In each of these domains, there are standards of responsible practice that are critical to ensure that translation best protect the rights and welfare of patients and society. We suggest that clinicians who bypass such accepted paradigms in the provision of novel, unproven interventions based on stem cells or their derivatives, may be operating in an ethically problematic manner.

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Chapter 13

Translational Stem Cell Research in Pediatrics: Ethical Issues

Michael Fuchs

Abstract The ethics of medical research constitutes one part within the field of medical ethics and bioethics where a far reaching consensus could be found at least as far as the principles and necessary procedures are concerned. Much more moral conflict is, however, prevalent in other parts of bioethics. The consensus achieved in the ethics of medical research was built upon the concept of informed consent, which is deemed a central aspect in the ethics of research on human beings. The focus on informed consent, however, causes problems in cases where research subjects are unable to give consent for whatever reason. Minors make up for one of the groups where the capability to give free and informed consent is, at least, problematic.

The lack of informed consent in the case of minors creates a dilemma in that paediatric research is needed for good paediatric practice and for the benefit of future minor individuals.

Against this background, alternative concepts such as assumed consent (McCormick), educational benefit or minimal risk have been developed.

With reference to the clinical translation of stem cells, detailed ethical and regulatory guidelines have been discussed. Nevertheless, no special recommendations for the clinical translation of stem cells to minors are in place at the current moment. The article examines the helpfulness of the distinct alternative concepts.

Keywords Gene therapy • Instrumentalization • Minimal risk • Pediatric research • Therapeutic attempt

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13.1 Introduction

Along with many of his contemporaries in the late eighteenth century, Edward Jenner made the experience that milkmaids who had contracted cowpox, did not, or only very rarely, come down with real pox [1]. In order to develop a reliable procedure for vaccination, based on this realization, the doctor vaccinated a healthy 8-year-old boy with cowpox, which he had extracted from a cowpox pustule. After his article about the successful immunization had been rejected by the Royal Society, he conducted many more trials, including one on his own son, who was 11 months old. Jenner was well aware of the risks connected to his trials, discussed these risks in his scientific papers, and searched for strategies of risk minimization. For him, the fact that many of his test subjects were, in fact, children, did not, however, serve as a motive for particular ethical reflection [2]. Over the past 120 years only, the reference point of the ethical reflection concerning this matter has shifted. Due to some major court rulings, the development of medical law as well as the emergence of biomedical ethics, “informed consent” gained the status of a general demand of both the medical treatment and the medical research on the human being [3–5].

13.2 The Requirement of Informed Consent in Research on Humans

In view of the ethical debate over the past 100 years, the question concerning research on minors presents itself in the following way: On the one hand, it became apparent in the debate among lawyers, ethicists and physicians that the informed consent of the person concerned is the constitutive prerequisite for any kind of research on the human being. Informed consent is based on the principle of self-determination, which applies to all human beings as bearers of basic rights. The principle of self-determination and its operationalization in the concept of informed consent constitute the core of the development of bioethics in the twentieth century. By now it globally represents a central ethical claim regarding research on the human being and can be considered a core area of a comprehensive consensus within the fields of bioethics and research ethics.

The focus on informed consent, however, causes problems in cases where research subjects are incapable of giving consent. Minors make up one of the groups where the capability of giving free and informed consent is, at the very least, problematic. The legal orders assume that consent, that could, legally, serve as justification for research, cannot be provided by minors. Going beyond state laws, this insight is also being in the ethical codices.

As a consequence of this standardization, research projects could be conducted on minors only in cases where a realistic therapeutic purpose was given in the narrowest sense. Drug trials conducted by means of the regular procedure, in which

testing for toxicity constitutes the starting point of the research on the human being, were not allowed to be carried out on minors. All research projects including minors in the trials posited special standards for the persons involved and caused substantial additional costs in the commercial field of research.

13.3 Pediatrics and Its Demand for Scrutinized Procedures and Products

Many physicians therefore complained that numerous efficient drugs had never been clinically tested on minors and could thus be prescribed to minors only if the doctor prescribing the drug did this within the scope of his individual responsibility and freedom of therapy for single-patient use. Does this mean that the special protection granted to children is being reversed into the opposite, meaning into the situation that within the group of children deemed to be extraordinarily vulnerable those children who are particularly vulnerable due to disease have to take riskier medications than sick adults do? Over the past few years, legislation in both the USA and in the EU has tried to change this situation. With the Food and Drug Administration Modernization Act (Pub. L. No. 105-115, § 111 Stat. 2296) of 1997, as well as the Best Pharmaceuticals for Children Act (Pub. L. No. 107-109, 115 Stat. 1408) of 2002, drug companies were granted an extension of patent for 6 months if a drug was also being tested on minors. The Food and Drug Administration (FDA) has the authority to require pharmaceutical companies to test specific products in children. The European model combines this American solution, which uses incentives, with specific obligations. As is the case with the FDA, the EMEA as the European pharmaceutical agency is thus given the task to provide hints and to request specific surveys. This aims at establishing an intensive exchange about the demand and respective plans for development among industry, academic science and admission agencies [6]; this also includes the exchange of data concerning negative findings. The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) also aims in this direction and stresses the morally justified demand for according studies [7]. What has not changed, meanwhile, is the ethical difficulty of justifying such trials.

13.4 Research Without Informed Consent

The lack of informed consent in the case of minors creates a problem, if not a dilemma, in that pediatric research is needed for good pediatric practice and for the benefit of minors in the future. Against this background, alternative concepts, such as assumed consent, educational benefit, or minimal risk have been developed.

How is it possible to argue for the validity of such a concept without generally questioning the informed consent?

The concept of minimal risk and minimal burden has established itself in many legal systems as the most important legal instrument apart from the consent of the parents and representatives. Research can be conducted without legal consent only if a certain threshold of burden and the potential hazard is not crossed. Different practices for the classification can be offered to refute the obvious objection that the term “minimal” could not be defined unambiguously. From the sphere of daily life, for instance, burdens and risks can be drawn that we regularly and, in a sense, naturally expect of minors, too.

In their article on risk-benefit assessment in pediatric research, Sumeeta Varma and David Wedler present data showing that the risks children undergo from activities of daily life are really high and much greater than the risks they face from routine examinations and tests [8]. In the explanatory report of the Convention for the Protection of Human Rights and Dignity of the Human Being with Regard to the Application of Biology and Medicine of the Council of Europe, examples are given as to what can be considered research intervention with minimal risk. “While Article 16. ii restricts research in general by establishing a criterion of risk/benefit proportionality, Article 17 lays down a more stringent requirement for research without direct benefit to persons incapable of giving consent, namely only minimal risk and minimal burden for the individual concerned. Indeed, it is only in respecting these conditions that such research may be carried out without constituting an instrumentalisation of these persons contrary to their dignity. For example, taking a single blood sample from a child would generally only present a minimal risk, and might therefore be regarded as acceptable” [9]. Even if forming analogies is not easy and certainly cannot be done without entailing subjective estimates, the problem of comparison and demarcation does not necessarily have to be regarded as invincible. The question does, however, have to be posed as to whether the concept of minimal risk is convincing. The explanations provided by the Council of Europe constitute an *argumentum ad hominem*. In a strictly logical consideration, someone can be used as a means only even if the integrity of the person concerned is touched upon only slightly.

Paul Ramsey initiated the debate on the question concerning experimental interventions with children with his statement that persons unable to give their consent are principally and, without any restrictions, out-of-bounds for non-beneficial research. By neglecting this taboo, a child would be used as a means only and not also as an end. Ramsey’s famous formula reads as follows: “A subject can be wronged without being harmed” [10]. Does the attempt to set thresholds for significant damage come to nothing herewith? Richard McCormick, who has become Ramsey’s opponent in the by now classical debate, pointed towards Ramsey’s own feelings of discomfort as regards the puristic position that this non-therapeutic research on minors has to be strictly banned, which makes children, as he states, “therapeutic orphans” [11]. He does not content himself with defining a threshold of the permissible risk. Rather, he takes the analysis of those assumptions and imputations as a starting point that enables parents to justify therapeutic interventions on their child. The assumption that a child *would* give his or her consent to such an

intervention resulted, he argues, from the conviction that the child *should* give consent. He then infers that there are other things that the child ought to choose, simply as a human being. The participation in research protocol that is of use to others and with which, under low costs, the common welfare would be served, could be considered a similar thing. Even though the child was not yet a moral subject and, as such, bore responsibilities, the child was, however, no unrelated entity as Ramsey was treating him or her [11]. As social human beings, “it is reasonable to expect that they will want certain goods for others and contribute to these goods if there is no discernible risk, discomfort, or inconvenience” [11]. As regards determining grounds of justification of non-therapeutical research with minors, Dan Brock, too, begins by comparing research to therapy: “Unlike medical therapy, in which professional traditions and norms require the physician to promote the well being of his or her patient, the fundamental aim of research is the advancement of generalizable knowledge” [12]. Other than McCormick, he nonetheless does not wish to allege consent but aims at demonstrating other forms of benefit apart from the medical-therapeutical one. In a first step, he does, however, point towards the important role that family members can generally assume for those who are unable to give their consent: “[W]ithout specific reasons that disqualify the family member from acting as surrogate for an incompetent individual, the presumption for the family as surrogate will hold” [12]. In the case of children, there are even special reasons for letting parents decide as surrogates. As regards research with a potential therapeutic benefit, “virtually any significant degree of potential therapeutic benefit from research participation, in comparison with standard therapy, could justify exposure of the child to minimal risks [...] unless parental permission is refused” [12]. Brock further explains that participating in a medical research project entails a communitarian-educational benefit for the participating child as well: “Thus, when parents teach their children that they should be willing to participate in medical research designed to benefit others, this is a small, though not inconsequential, respect in which they are helped to become moral beings, with a concern for the well-being of others besides themselves and their close relations and friends” [12]. Finally, there is a justificatory benefit that others can have due to research-generated knowledge. Brock does, however, consider this benefit as decisive only if the risk is tolerable and if the parents’ consent is at hand.

In various contexts, the concept of a special solidarity among children has been brought up independent of this debate about an individual benefit not related to health and a benefit for others. This concept sometimes attempted to designate the benefit of the group as a third category between the benefit of others and individual benefit [13]. At times, this happens simply by stating “that it is in the interest of children to evaluate medicinal products with scientifically proven methods, if possible by pediatric placebo-controlled trials, which should only be justified when their design, enrolment and conduct ensure that they really address the best interest of the children-participants with the view to their health and a concern of their dignity” [14]. Nevertheless, it may be asked if children as children constitute a group in a morally relevant sense that could result in the requirement of specific solidarity between the members of that group. Since the relevant groups cannot

really be considered as a community in the communitarian sense, a communitarian justification is difficult to find. Therefore, the concept of group benefit is too vague and ethically not sufficiently plausible to be able to carry by itself the load of justification for research with minors. It can therefore not be assumed that, by means of this concept, presumed consent could be insinuated, or rather, that the concept of the child's well being could, as a pediatric benchmark, include the engagement for the well being of other children.

The concept of minimal risk is not clear, either; it can, however, be expatiated and concretized and is of considerable significance as regards the weighting function. Where the minimal risk is transcended, only the individual benefit can be of an exculpatory nature. This comes into consideration first and foremost as medical benefit and, more specifically, as a therapeutic benefit or one that has a therapeutic impact. When focusing on the therapeutic goal, the danger of therapeutic misconception has to be borne in mind.

13.5 Features of Stem Cell Therapy

With reference to the clinical translation of stem cells, detailed ethical and regulatory guidelines have been discussed. The International Society for Stem Cell Research developed and adopted "Guidelines for the Clinical Translation of Stem Cells" in December 2008 [15]. They address quality and safety standards for cell processing and manufacture and preclinical studies. Stem cell-based clinical research and clinical applications will be subjected to independent review, for the scientific as well as for the ethical aspects, no matter whether the source of the stem cells is the adult body, the embryo or the fetus. Only under very strict conditions should clinician-scientists be allowed to provide unproven stem cell-based interventions to, at most, a very small number of patients outside the context of a formal clinical trial. Nevertheless, high expectations in the scientific community and the therapeutic promise of stem cells regarding regeneration of implantable tissues have led to huge expectations of people suffering from severe, and up until now, incurable afflictions. Apart from researchers relying on clinical studies and working within the established safeguards of good clinical practice, there are many providers offering stem cell applications as a remedy for a wide range of ailments, without clinical data to prove the soundness of these treatments. While some of those providers clearly violate international standards of good clinical practice, others work in regulatory gray areas, even in Europe. A survey analyzing Web-contents of stem cell clinics reveals that clinics all over the world offer treatments using different sources (adult stem cells, fetal cells, cord blood stem cells and embryonic stem cells) with different goals (therapy, health improvement and cosmetics) and various indications (ranging from neurologic diseases to allergies) without clinical evidence [16]. This diversity of procedures and indications also leads to regulatory problems, as these procedures do not fall under one and the same law [17, 18].

From an international perspective, the regulatory requirements and procedures are not standardized.

Although it may seem obvious from an ethical perspective that therapeutic procedures that are not validated by clinical data should not be allowed, there are also good reasons to allow the application of unproven procedures under certain conditions. In the tradition of medical ethics and medical law, those procedures have been named therapeutic experimentation or therapeutic attempt. The Helsinki Declaration makes such attempts the subject of regulation under the title of combinations of research and care as well as of treatments where proven interventions do not exist: “31. The physician may combine medical research with medical care only to the extent that the research is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects. [...] 35. In the treatment of a patient, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician’s judgment it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available.”

While those therapeutic attempts may be justified under certain conditions, it is important to take a very careful look at individual cases. One of the specific features that has to be considered is the age of the patient and his or her capability to assent or consent.

Within the entire sphere of stem cell transplantation, there are good reasons for conducting therapeutic trials or therapeutic attempts on minors and entering unknown territory specifically in the field of pediatrics. Often, a large number of cells would be necessary for a successful transplantation in adult patients, occasionally a larger number than the number of suitable donor cells available. Nevertheless, no special recommendations for the clinical translation of stem cells to minors are in place at the current moment.

There are, however, other spheres of innovative experimental therapies, which can serve as a field of experience and of reflection on these experiences. This applies to the field of somatic gene therapy, which frequently makes use of approaches based on stem cells.

Research into gene therapy and its application raises questions about how to deal with the patients’ or participants’ inability to consent. These questions acquire special relevance because the intervention at the earliest possible stage is closely linked to the paradigm of gene therapy as a causal therapy, and also because of particularly great problems constituted by risks and uncertainties.

Indeed, in the field of gene therapy, in particular as regards hereditary diseases, trials have proven to be especially efficient and helpful when conducted on minors. Immunodeficiency diseases accompanied by malfunctions in the hematopoietic

system have become subject to particular attention. The first clinically tested gene therapeutic protocols with hematopoietic stem cells that were virally induced *ex vivo*, were developed for genes whose genetic products have a strong significance for the physiological functioning of different cell types of the hematopoietic system. A gene that belongs to these genes is the autosomal localized gene of the adenosine-deaminase, whose mutation leads to an impairment of the purine-metabolism and the clinical disease of ADA-deficiency (ADA-SCID), a severe primary immunodeficiency syndrome with metabolic impairment of the red blood cells and the liver cells, which occurs as early as in infancy and which affects both the T-lymphocytes as well as the B-lymphocytes [19–22]. A severe immunodeficiency syndrome occurring in infancy, which is triggered by mutation of the gene located on the γ_c -subunit of several cytokine-receptors and which is characterized by an impairment of the differentiation of T-lymphocytes and natural killer cells as well as by the absence of these cells in the blood (γ_c -SCID, SCID-X1, “bubble boy disease”) [23, 24], has also proven to be accessible for a gene therapy approach. After the insertion of applicable functional genes into the patients’ hematopoietic stem cells, a clinical cure in the majority of the treated patients was being reported, both in the case of ADA-deficiency and in the case of γ_c -SCID. It holds for both diseases that there is particular hope for children to significantly improve their condition and to possibly even achieve a cure by experimental genetic therapy.

However, as regards patients reaching adulthood, oftentimes so many, or severe additional diseases have to be taken into account that the efficiency of the gene therapeutic approach can be expected only rarely. From a research strategic standpoint, it therefore seems viable to conduct trials on minors. This also applies to other diseases causing similarly severe secondary diseases. This research strategy can be justified within the scope of bioethical reflection.

The ethical perspective did, however, result in streams of argumentation for at least two strategies. “The classical position, formulated in the early 1970s in the United States, was that clinical trials should be completed in adults first before children are exposed to the potential harms of such trials. The revisionist position of the late 1980s and 1990s is that participants in clinical trials are carefully monitored and that they often are the first people in a society to have access to new and possibly effective treatments. Therefore no class of individuals, whether women or members of ethnic minorities or children, should be excluded from the potential benefits of timely participation in clinical trials” [25]. The revisionist position that children should not be excluded really reflects the fact that proof of clinical efficacy of gene therapy could be obtained in studies that were conducted in pediatric clinics. A very high number of patients successfully treated with experimental gene therapy were children. Although many of these children continue to benefit from gene therapy, five of the patients with X-SCID developed acute T-cell leukemias and one of these patients died. In the meantime, researchers have enhanced the viral vectors that are used for the gene transfer. Nevertheless, oncogenic side effects due to so-called insertional mutagenesis can still occur at high risk. There is no benefit that could justify these risks besides the direct individual benefit for the health of the child suffering from a serious disease. Many of the sick children

with X-SCID would not have reached adulthood, nor even adolescence, without experimental gene therapy. This is the justification that can be given retrospectively, *ex-post*.

Since the first-time application of gene therapy protocols is characterized by high risk and uncertainty of the outcome, a group of authors [26] – making use of the example of Wiskott Aldrich Syndrome – argues that human experimentation that has the advancement of knowledge as its primary goal cannot provide an ethically adequate framework for such an application. Rather, they call for the establishment of a controlled individual therapeutic attempt as a conceptual as well as procedural framework. Such healing attempts are to benefit those who can profit most from it and for whom an alternative to traditional therapeutic instruments seems to be the most imperative. At the same time, and as is the case with clinical trials, too, a high level of good scientific and clinical practice as well as a control by means of a review system of competent ethics committees, need to be guaranteed. It is debatable whether such an instrument can be put into practice or whether it would be at risk of creating new gray areas. The diligence as regards the determination of risks and benefit and the restriction to an individual benefit seems to be imperative independent of the chosen conceptual framework in cases of risky procedures with minors if an instrumentalization of children is to be eliminated. The requirements for scientific and ethical controls conducted by independent bodies thus have to be taken into account at any rate.

Even without any experiences regarding insertional mutagenesis, a large number of risks as well as substantial uncertainty concerning the consequences seem to persist for other areas of translational stem cell research. The requirements that were being discussed and reflected upon concerning gene therapeutic stem cell transplantation, can hence serve as a benchmark. Only where the new practice has proven to be the medically more successful practice over a larger range of individual trials can the traditional rules for clinical surveys be adhered to. Purely toxicological experiments with children are being excluded in these cases as well.

13.6 Strategies of Ethically Justifying Translational Stem Cell Research on Minors

If bioethics' traditional principles are being attributed to the superordinate principle of human dignity, as is, for instance, in the case of the European Convention on Human Rights and Biomedicine of the European Council, avoiding instrumentalization of any kind is the authoritative imperative for any research on the human being. Since an application of stem cell research in the field of pediatrics by no means falls under the threshold of a slight burden and a marginal risk only, the only justification strategy coming into question is the therapeutic benefit; this holds true only for cases in which the therapeutic benefit entails a probability of exceeding the risk and proving *ex-post* to be the better option for the person concerned than is the case with any

other available alternative. In the sphere of experimental therapy, another danger does, however, arise herewith; this option has been discussed as therapeutic misconception for several decades now and is of particular significance here. Therapeutic misconception (TM) is the label for a mistaken belief that decisions about one's treatment as a research subject would be made being based solely on one's individual condition and needs [27]. Such a misunderstanding seems to be a phenomenon of quantitative relevance for the entire sphere of research on the human being. "Taken as a whole, the existing research indicates that TM is an extremely prevalent phenomenon among patient/participants" [27], p. 637]. Appelbaum and Lidz see a high significance from an ethical-qualitative viewpoint as well since, with a correct evaluation of the research by the person concerned, the validity of the consent is put into question: "To the extent that patients decide to enter research projects in the belief that they will receive personal care, we believe that it cannot be said that they appreciate the implications of their decision, and the validity of their consent is in question" [27], p. 640].

In fact, for the field of pediatrics, the validity of the consent is questionable in any case; a therapeutic misconception would, however, also jeopardize the assent, the representative consent and the right assessment of the benefit. Early clinical application of stem cell research thus has to act within the narrow boundaries defined by the necessity of avoiding both instrumentalization and therapeutic misconception, which can be assured only through procedural elements. Since the physician/researcher himself or herself is at risk succumbing to therapeutic misconception, there is an indispensable need for control and its accompanying reflection by experts outside of the teams involved as well as independent persons, some of whom have some experience in ethical judgment formation [28].

It is furthermore important to realize that there is no fixed hierarchy of principles regarding content in this field. The ethical debate on this lays open an uncertainty concerning decisive fundamental concepts of a normative judgment: What should serve as a starting point – rights and responsibilities, or interests and goods? At the same time, an uncertainty manifests itself as regards the appropriate weighting of the principles that are relevant here.

Quasi in the sense of a provisional moral, several panels have tried to account for the various principles and to illustrate criteria of justifiable research that goes beyond Ramsey's blanket prohibition. Both the National Commission for the Protection of Biomedical and Behavioral Research in the United States and the Council of Europe seem not to base the demand for making children objects of research benefitting others only in cases where this research cannot be conducted on other people onto the concept of a group benefit; rather, they seem to advocate a lack of alternatives independent of the above and thus a necessity in view of the high ranking of the intended goals. In fact, the concept of a group benefit seems hardly suitable here. The function of the minimal risk and the minimal burden as a threshold therefore assumes a decisive role for the question regarding translational stem cell research. If this threshold is surpassed, only an independent judgment regarding a positive weighing of individual benefit and individual risk is authoritative, and only the therapeutic benefit can be of relevance here.

13.7 Conclusion

The ethics of medical research constitutes one part within the field of medical ethics and bioethics where a far-reaching consensus could be found, at least as far as the principles and necessary procedures are concerned. Much more moral conflict is, however, prevalent in other areas of bioethics. The consensus achieved in the ethics of medical research was built upon the concept of informed consent, which is deemed a central aspect in the ethics of research on human beings. The difficulties of morally judging research on the human being can be attributed to the problem of determining the relation of the principle of no harm and the principle of self-determination.

There are three possibilities to define the relation: The self-determination can be superordinated to the principle of avoiding harm; it can be considered equal and applied as an instrument for the enforcement of damage limitation. The discussion of the last 150 years has suspended the precedence of avoiding harm in medical ethics in favor of a weighing of the self-determination that is becoming stronger and stronger. In the dealings with children, in particular, aspects of care still have to be respected. This opens up a principal justification for research with a potential benefit. For research that is solely benefitting others, high standards have to be defined. At any rate, the child, no matter what his or her age, has to have the right to veto based on good information; this right to veto persists at all times throughout the course of the study. What is to be further kept in mind is the need to have the consent of the parents as well as the need not to exceed the threshold of minimal risk and minimal burden.

For research with potential benefit, each self-misunderstanding and each deception concerning the true character of the study or the trial has to be avoided. Individual healing attempts, in particular, require control instruments in order not to become scopes for the development for research that is, in effect, benefitting others. The danger of such self-deception is particularly high in the spheres onto which patients put high hopes and in which research sees important steps for the establishment of novel therapies. Both of these apply to stem cell research and its transfer to the clinic.

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Chapter 14

Experimental Stem Cell-Based Therapy in Pediatrics: A Fictional Case Study

Kristina Hug and Anders Castor

Abstract This chapter analyzes the complex process of decision making when applying highly experimental stem cell-based therapy to children. We focus particularly on the special case of life-threatening conditions and with no available alternative treatment, when the experimental therapy is completely untested, or only tried on a few adults. The reader finds himself/herself in the shoes of a surgeon who is torn by a dilemma: Does he have morally justifiable reasons to treat a 4-year old child with an experimental therapy, and if no, why? The reader considers, together with the surgeon, the issues that have to be raised in the process of decision making. When are experimental stem cell-based therapies in pediatrics justified? What rules governing pharmaceutical research on children should be applied in the case of stem cell-based therapies? How to weigh the risks and benefits in the case of this particular child when the stakes are high, and a balance has to be found between life and unknown risks of unknown certitude and magnitude. What if the known risks and burdens of treatment may almost amount to torture in the perception of a 4-year-old patient? The child is not capable of making an informed choice on whether to accept this treatment or not. Which values should be given priority in such a case? The surgeon searches for the answer in different ethical theories, and considers arguments drawn from consequentialist, human rights, dignitarian and other deontological theories. The surgeon also questions whether free and informed consent in the context of experimental stem cell-based therapies is possible, when the seriousness of the child's condition may influence the parental decision making regarding the experimental treatment of the child, and when the risk of therapeutic misconception is high.

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Keywords Ethical theories • Experimental stem cell-based therapy • Informed consent • Pediatric research • Risk-benefit ratio

14.1 Introduction

A severely injured 4-year-old boy was admitted to a university hospital several weeks ago. He was rescued from a fire, and was at initial assessment found to have deep burns covering at least 80% of his body. The situation was evaluated as very critical, with overwhelming risks of death within days or weeks due to shock or ensuing infections, and in the unlikely situation of survival a high degree of morbidity would be expected. The boy was admitted to the pediatric intensive care unit, and resuscitation with intravenous fluids and analgesia was promptly started. The development of the burns made it clear within a few days that for the most part they were full-thickness, making the prognosis dismal.

After a few days, necrotic burned tissues had to be removed and skin was mostly excised down to the muscular fascia. As there was not enough healthy skin to harvest autologous epidermis for conventional split-thickness grafts, wounds were transiently covered with cryopreserved allograft and bioengineered synthetic dressings. A small biopsy was taken and sent to the cell culture laboratory for production of cultured epidermal autografts (CEA). Two weeks later, the wounds could be entirely covered with these standard cell culture products.

One week later, the young boy is again in the operating room for redressing of the wounds. The plastic surgeon carefully opens the dressings and removes the small gauzes that are in contact with the CEA. However, what he discovers is totally unexpected and extremely serious: the take of the CEA was almost null. Everything needs to be done again. New CEA must be prepared, and meanwhile the wounds must be covered. Unfortunately, another obese adult burned patient has just been treated in the morning, and there is no more stock of allograft or synthetic dressing in the hospital. The attending physician knows that if he cannot cover the wounds, the prognosis will be extremely poor.

Of course, he can try to make standard dressing waiting for the arrival of new material within few days. But he is also aware of a highly experimental research protocol, approved by a Research Ethics Committee, currently ongoing in his hospital. According to this protocol, patients suffering from extremely large and severe burns are treated with novel experimental stem cell-based therapy, in the form of immediately available full-thickness skin with all appendages (hair, sweat glands and so on). As this is an allograft, the patient needs to be immunosuppressed as long as this new skin will remain on the patient but the application of this in the acute setting is believed to reduce the mortality, and possibly give a much better end result (if the patient survives). The protocol is designed for adults, not for children. Four adult patients have been treated so far, of whom two have died, one very shortly after commencement of treatment, and one after prolonged suffering due to complicated side effects of the experimental treatment. Two patients are alive 2 and 6 months after experimental treatment has begun, and it seems that the transplanted grafts are healing well and forming new skin.

The surgeon on duty is torn by a dilemma. He could give the boy standard care, which by all chances will not save his life. Alternatively, he could try the experimental stem cell-based treatment. However, the protocol is not approved for children, and depends on an informed consent, due to involved unknown risks and known risks of unknown magnitude. The latter may include severe and chronic pain, malignant transformation of the graft, uncontrolled, cosmetically unacceptable overgrowth of new skin, heat intolerance due to possible lack of sweating, intolerance of sun exposure or chronic blistering. Risks may also include infections, which are common in the care of all burns, but carries additional risks in this setting due to possible infectious agents within the stem cell graft, and due to the required immune-suppression for preventing graft rejection. It may happen that the boy will live somewhat longer, but will die suffering from great pain. It may also happen that only some of these risks will materialize, their magnitude will not be significant and the experimental therapy will significantly prolong or even save the boy's life, even with a cosmetically acceptable end-result. The very limited experience from the treatment protocol on adults so far is not very positive. The therapy has not yet been tried on children, and the known and unknown risks might be quite different in very young patients. Children are not included in the ethical approval of the experimental protocol. Should the surgeon not treat the boy suffering from exactly the same injury as the adult patient next door, just because he is a child?

How should the surgeon make his decision? Now the 4-year-old boy is intubated, but even if he were conscious he would not be in a position to give informed consent, due to his age. The parents are still in a state of shock, and have had great difficulties in absorbing all the information and the hectic activity around their child. They are grieving the probable loss of their son, and his severe disfigurement, and might want that everything be done to save his life. But due to the possible severe side effects and suffering, an attempt to save the boy's life might not be in his best interest. The surgeon needs to act quickly. Should he propose to the boy's parents the possibility of experimental treatment on compassionate grounds?

Experimental stem cell-based therapy (ESCT) is a wide-ranging term. There are many different interventions that could fall under this term, from already widely applied, but still considered experimental therapies, such as using cord blood cells in the treatment of leukemia, to completely novel first-in-human experimental interventions, such as stem cell-based treatment of very severe burn victims. Thus, a first distinction can be made concerning how much the therapy in question has already been tested on human subjects. The scope of ESCT may also range from treatment of life-threatening diseases without alternative therapies to cosmetic therapies, thus involving a different risk-benefit ratio. Therefore, a second distinction can be made concerning the possible benefit that can potentially be gained by applying an ESCT. A third distinction can be made concerning the type of cells used in such therapy. There are different kinds of stem cells, among them multipotent stem cells (e.g., hematopoietic stem cells), which can differentiate into several other types of cells, and pluripotent stem cells (e.g., embryonic stem cells), which are capable of differentiating into all cell types of an organism, including the germline. Consequently, there are experimental therapies based on different types of

stem cells. For example, hematopoietic stem cells from bone marrow have been used for decades to treat leukemia. However, pre-clinical research is still conducted to investigate practicability and safety of many ESCTs. A fourth distinction can be made between experimental approaches towards diseases with effective (but maybe suboptimal) treatment alternatives (e.g., diabetes type 1) and conditions without known and effective alternative treatment options (e.g., severe burns). A fifth distinction can be made between ESCTs involving stem cells that are placed within a limited space in a non-vital tissue (e.g., β cells to parts of the pancreas of a diabetes patient) and ESCTs involving stem cells that would be spread throughout the patient's body (like hematopoietic stem cells in the blood system of the patient, or very large areas of the skin). The former cells can be removed if the treatment goes wrong, whereas the latter cells cannot.

Bearing in mind these distinctions, it is important to be explicit about what exactly is meant by "ESCT." This chapter focuses on ethical issues in the use of such therapies in pediatrics for treatment of disorders *without effective alternative therapies*. For the sake of argumentation, we have chosen an example of ESCT of burn injuries where more than 80% of the body surface is damaged. This is an injury that may occur in both adults and children. Burns of 10% or greater area of the total body surface in children is a potentially life threatening condition. When more than 80% of the body surface is affected by burns, the victim can be treated by cultured epidermal autografts (CEA). But in our particular case this is not possible due to unsuccessfully cultured CEA, thus providing the situation where no effective alternative therapy is available, at least until new CEA are cultured in about two weeks, if the boy survives until then. In this chapter, we discuss the ethical issues of using ESCT *in this particular case*.

Partly, the answers to the surgeon's question will depend on the concept of suffering and the probability and magnitude of risks involved. Partly, the answers will depend on the chosen normative point of reference.

14.2 Experimental Stem Cell-Based Therapies in Pediatrics – Are They Justified?

The first question that some may naturally pose when considering ESCTs in pediatrics is whether we really need to do the initial experimentation of such therapies on children. In the example of our vignette, the fictional experimental protocol is designed and approved for adults only. As Michael Fuchs discusses extensively in this book, children deserve special protection as research subjects, because they do not have the legal capacity to consent to participation in research. In trials involving children, one must balance the vulnerability that arises from their incompetence to protect their interests, while promoting the potential benefits of being involved in research [1]. Therefore is it not enough if we obtain the results of ESCT in adults having the capacity to consent and then

use the knowledge obtained in treating children? The answer to this question depends on several factors.

Research on minors is normally approved only if all of the following three conditions are fulfilled:

- (a) There is no other way to gain the information necessary to answer the research question;
- (b) The risk of harm is in proportion to the expected benefits, and procedures exist for reporting harm and for stopping a clinical trial if the safety of subjects is threatened;
- (c) Parental consent that respects the child's presumed interests is granted and children are informed and involved – to the extent possible – in the decision [2] and do not object to participation in research.

We will further explore whether these three conditions may be fulfilled if ESCTs are applied to minors.

14.2.1 Must Children Be Involved to Answer the Research Question?

It is well documented that children are not small adults [3–7], with regard to their developing physiology [3, 5, 7], the partly different diseases afflicting them [7, 8], difference in uptake, distribution and susceptibility to various drugs [8], and clinical needs [4]. Although we have a responsibility to protect children, we also have an obligation to ensure that they receive the best treatment [9, 10]. Therefore, there is now a wide consensus that medications should be tested in children before their introduction into practice in order to obtain safe and effective medical care in pediatrics and to protect children from untested, potentially harmful practices [2, 7, 9–14].

ESCTs might have similarities and dissimilarities with drug research. Are the biological differences between adults and children requiring testing of such therapies in children in addition to such testing in adults? Or can there be cases where there is no doubt about the superiority of such therapy in adults but clinical equipoise still exists when the same treatment is applied to children? These questions still remain to be answered when clinical testing of ESCTs begins.

Provided the necessity of studying a certain ESCT on minors is evident, the next question to be answered is whether we should test such interventions on children *only after* they have been tested on adults. This question becomes even more critical when we take into consideration the irreversibility of some stem cell-based interventions. The answer to this question depends on several factors.

The first factor is whether the disease or condition, for the treatment of which the intervention is tested, may occur in both adults and children or only in children. In the first case, the usual approach to design a study that involves children is to conduct preliminary studies in animals, adults, and older children (e.g., teenagers and schoolchildren) before young children (e.g., pre-school children and toddlers)

are involved [11, 12, 15]¹. The common strategy is to wait with enrollment into clinical research until the child reaches the age when he/she can provide his/her own consent (such as Parkinson's).

In the second case, when the disease or disorder occurs only in children, it is not possible to test the experimental therapy on adults, and the testing therefore needs to be done on children. So far, ESCTs are not modeled for treatment of diseases affecting only children. If the only factor we consider is the aim of the therapy (i.e., to treat adults or to treat adults and children), it follows that such experimental therapies should indeed be tried on children only after they have been tested on adults.

However, there is another factor influencing the answer to this question – the availability of effective alternative therapies. A distinction needs to be made between diseases or conditions for which there are effective alternative treatments and diseases or conditions for the treatment of which there are no such alternatives. In the case of diabetes, for example, where alternative treatment with insulin exists, the accepted practice would be to wait until the child grows up, becomes legally competent to provide his/her own informed consent and can decide for himself/herself if he/she wants to participate in research on stem cell-based therapy for diabetes. Such a choice, however, does not exist in the case of a fatal burn injury, as in our example, where there are no effective alternative therapies and death is unavoidable. In this case the risk-benefit balance is between certain death and potential side effects and/or ineffectiveness of the experimental therapy. In such cases, even though the condition exists in adults, the risk of *not* applying the experimental therapy on children whose death is inevitable may outweigh the benefit of their protection from the risks of the experimental therapy. This issue brings us to our next question concerning the risk-benefit ratio.

14.2.2 The Difficult Balance Between Risk of Harm and Expected Benefit

If there is a medical and ethically justified need to test certain ESCTs on children, the second question to be answered is whether the risk of harm is in proportion to the expected benefits to the patient. The difficulties of assessment of risk and benefit in stem cell-based interventions in general are extensively discussed in this

¹Children cannot be considered as a single homogenous population when it comes to studying medications? [16]. The International Conference on Harmonization (ICH) Guideline [17] distinguishes at least four subgroups: neonates including preterm and term from birth to 28 days of life; infants from 1 to 23 months of age; children from 2 to 11 years of age; and young people from 12 to 18 years of age [17]. Children are a very heterogeneous group, from newborns to adolescents with great developmental differences as well as physiological, pathophysiological and psychical differences within the group [5]. Each of these subgroups has its own characteristics, which may require separate trials [8]. Whether the same also holds true in the context of testing experimental stem cell-base therapies on children would depend on the kind of therapy applied.

book by James Anderson and Jonathan Kimmelman. As Michael Fuchs also discusses in his chapter, the concept of minimal risk in pediatric clinical trials is not entirely clear, and it may be even less clear in the context of ESCTs, since such therapies may include risks that are difficult to estimate. The question therefore arises what should be considered as “acceptable risks” for a pediatric population. The researchers and the Research Ethics Committee have to determine whether the level of risk in the research project is ethically acceptable. But both children and parents also need to determine what risks are acceptable to them. These two issues need to be considered separately.

Evaluation of risks acceptable to a particular child is very problematic. No one other than the patient himself/herself is in a position to decide if the burden of a painful or debilitating chronic disease is of such a degree that the risk of death or a seriously increased amount of suffering caused by adverse effects of the treatment in pursuit of cure is worth taking. But when the patient is a small child, it is impossible to know whether the child would prefer to risk non-existence in pursuit of the cure from the painful chronic disease. The parents may have an opinion (which should be carefully listened to and respected), but it is clearly only a surrogate answer to the question.

For evaluation of the magnitude and risk of harm acceptable in a particular research project, most guidelines distinguish four categories of risk: negligible, minimal, minor increment above minimal and major [1]. Minimal risk is defined as a probability of harm or discomfort not greater than that ordinarily encountered in daily life or during the performance of routine physical or psychological examination [10]. This definition can be debated. Minimal risk should be related to the alternatives available. Besides, a single car trip across town during rush hour poses approximately a 1 in 100,000 chance of death in a child, but if a research study poses a risk of death of 1 in 100,000, is it no more dangerous than an ordinary activity of normal life [10]? The following levels of risk are considered to be in balance with the benefit:

- Minimal risk with benefit for an individual;
- Minor increase over minimal risk, with benefit to the individual, and with the benefit to risk balance as being at least as favorable as that of the available alternatives;
- Greater than minor over increased risk with benefit for the individual that is especially favorable in relation to the alternative available approaches for the individual's condition [10].

Analogous distinctions can be made on the group level, but “group” raises further problems, the discussion of which is out of the scope of this chapter. Which rules should apply to ESCTs in children depends on the seriousness of the disease and the availability of alternative therapies. For example, one study found that parents of chronically and terminally ill children stated that they were prepared to take greater risks in treatment in the hope of a cure [18]. Similarly, parents of healthy children considering participation in vaccine research believed that children should only take part in research where the medical benefits outweigh any potential risk [19]. It has therefore been argued that the definition of “minimal risk” should be different for a healthy child than a child with an illness (and even more so with a

terminal illness with no effective treatment alternatives). What is usual for a child in the midst of an aggressive treatment protocol for malignancy is far different than what is usual for an otherwise healthy child undergoing a tonsillectomy [3]. Therefore, risks can only be determined based on the severity of the disease, the age of the child, the risks and benefits of alternative treatments [10], including the risk of non-intervention, and the expected benefit of treatment. For example, Phase I trials in pediatric oncology are considered ethically acceptable when no effective curative treatment is available, and provided the inclusion criteria and risks are clearly defined even if the probability of a benefit in terms of disease control is usually very low, but greater than zero [20].

In a research setting, it can be argued that not only the expected benefit of the experimental intervention should be taken into consideration, but also the effect of the research setting itself. Some researchers have argued that patients enrolled in trials have improved outcomes as compared to similar patients treated outside of trials [21, 22].

Suppose we accept that it is unethical not to test experimental therapies as a last remedy, provided the requirements for free and informed consent are met. A question we should then answer is how much damage and suffering we should be prepared to accept in order to save a child's life. Would our answers depend on what kind of risk we are considering? For example, would we prefer a known risk over an unknown risk, or vice-versa? The information available about risks may play an important role in decision making. To take a recent example, some have preferred to take a known higher (although still small) risk to die from swine flu rather than an unknown, considerably smaller (but unclear how small) risk to develop a neurological complication called Guillain-Barré syndrome from vaccination against the disease.

As children are a protected research population because of their vulnerability, stemming from their lack of capacity to decide for themselves, a question arises whether in the case of ESCT of a lethal burn victim, this protection of children does not result in "overprotection" with the harm this can entail. Is it justified to treat an adult suffering from severe burn injuries with an experimental therapy, but not a child suffering from exactly the same injuries in the next room, only because he/she is a child? In a different context, several authors have already raised the question regarding "overprotection" of vulnerable populations (see, for example, articles by D. Thomas [23] or R. Rhodes [24]).

The surgeon in our example has to decide quickly. How should he weigh the risks and benefits in the case of his young patient? In order to make the risk-benefit balance, he must know which values should be given priority in this particular case. He thinks hard.

14.2.2.1 Consequentialist Arguments

The surgeon first considers the consequences of different ways of acting in the case of his 4-year-old patient. If he looks at the problem from a consequentialist point of view, the maximization of utility and the minimization of disutility is all that counts [25].

If the surgeon grounds his decisions on consequentialist principles, he should not count the risks and benefits to the boy alone, but rather the sums of risks and benefits to the boy, the parents, future pediatric burn victims, the society, etc. Even if experimental therapy proves to be non-efficient and even risky to his patient, the knowledge obtained from testing this therapy would be beneficial to society – in the form of negative results, if they are published. The risks related to ESCT (such as tumorigenicity or misdifferentiation of transplanted stem cells) may be important if the boy continues to live. It can also be argued, however, that these risks, weighed against the benefit of a saved life and the benefit to society, would count as a lesser disutility than the risk in non-treatment (if this equals certain death). Accepting the values of classical utilitarianism, one may view a decision not to intervene as the one promoting disutility, and the decision to intervene as promoting utility. If he bases his choices on the values endorsed by preference utilitarianism, the surgeon might say that his choice would depend not only on the preferences of the patient, but also on the preferences of society, especially since the society constitutes a larger number of persons than the boy, the parents and the clinicians, although the preferences of the society might be weaker than those of the patient.

From a consequentialist point of view, higher risks could be acceptable in experimental potentially life-saving therapy than in similar conventional therapy, since administration of experimental therapy may result in greater benefit (to the patient and society) than administration of conventional therapy.

If the choice needs to be made between an adult and a child, and if the surgeon bases his decision on the values endorsed by the consequentialist way of thinking, he may consider that it is not only imperative to try to save the child's life with the help of an experimental therapy, but it is actually more beneficial to save the life of a child than the life of an adult. A child has a longer life expectancy, a better healing capacity and probably a higher chance to benefit from administered treatment, especially if the adult is and suffers from other diseases. This might, however, be counterweighed by the fact that long-term consequences will add up to a total greater amount of suffering for a surviving child with a long life expectancy than an elderly adult with a shorter life expectancy. Furthermore, a child's life is usually valued more than an adult's in many societies. Children ought to be saved first from a sinking boat.

Let us suppose that the surgeon realizes that if he sticks to the consequentialist way of thinking, he will face an unsurmountable problem of balancing suffering against death or weighing the patient's strong preferences against many, but weaker, preferences of the society. He next starts thinking in terms of rights – what rights does his 4-year-old patient have?

14.2.2.2 Arguments Based on Human Rights

The surgeon realizes that looking at his dilemma from the viewpoint of human rights, the key is not positive or negative consequences. Focus is on respect for individual human rights [25]. On the one hand, human rights' theory would require

the protection of the right to life of the relevant rights' holder (the child) at the same time as it would require the protection of the child's human right not to be treated inhumanely. With patients in serious condition, a potential conflict between saving life and very burdensome treatment is not rare.

The human rights theory also requires free and informed consent by relevant rights-holders [25]. Therefore, from the human rights perspective, both intervention and non-intervention in the case of experimental treatment would be acceptable as long as there is free and informed consent. Under some jurisdictions (e.g., Sweden) a teenager between 15 and 18 years of age may give his/her informed consent provided he/she understands what participation in research means in his/her case. If the child cannot provide consent/assent, then it may be difficult to justify an intervention from the human rights perspective, which would consider the child as the starting point. The issue of informed consent is discussed in greater detail in the next section of this chapter.

If the surgeon's decision making is based on human rights arguments, he would not approve of making a difference between an adult and a child in the case of ESCT because of the need to protect the child's right to life provided the requirement for free and informed consent has been met.

The surgeon again realizes that if he looks for an answer to his dilemma only in human rights arguments, he would face the problem of who has the right to decide about the balance between suffering and death. Do the parents have the right to make this decision for the child? If the surgeon considers only the relevant provisions of the legislation in his country regarding this matter, his decision might not seem so difficult; by law, until a certain age, it is the parents who give the informed consent on behalf of the child (provided that the child, who is capable of understanding the problem on a certain level, does not object). However, if the surgeon considers that it is the child who has the human right not to be subject to inhumane treatment (as burdensome treatment may become) regardless of the consent of the parents, the decision is no longer simple.

Still searching for the answer to his dilemma, the surgeon next considers his question from the point of view of human dignity rather than human rights. How would his acts affect the human dignity of his 4-year-old patient?

14.2.2.3 Dignitarian Arguments

The dignitarian perspective condemns any practice, process, or product that it judges to compromise human dignity [25]. Although it is notoriously unclear what constitutes the positive content of "human dignity," the negative content of this concept is sufficiently clear for most practical purposes. One interpretation of human dignity originates in Kant's idea that there is a difference between something that can have a price and something that has no price, such as human life. According to this interpretation, slavery, torture, eugenics, stigmatization and discrimination constitute the infringement of human dignity. The surgeon vividly remembers two of the four adult patients who suffered greatly from the experimental

treatment before they died. He imagines the child a few months from now, with skin stem cell treatment irreversibly underway, with gross disfigurement due to graft overgrowth, and in severe pain. He shudders at the thought, and feels that this would certainly compromise the child's dignity, not least because it did not ask for, or gave its consent to, the procedures ending up in this unfortunate state. But severe side effects are but one side of the coin, with a saved life on the other. Can he risk the dignity of the child (in the sense that very severe side effects can be likened to inhumane, torture-like treatment) in pursuit of saving its life? However, it may also happen that only some of these risks will materialize, their magnitude will not be significant and the experimental therapy will significantly prolong or even save the boy's life, even with a cosmetically acceptable end-result. The very limited experience from the treatment protocol on adults so far is not providing solid guidance, and, as the therapy has not yet been tried on children, the known and unknown risks might be quite different in very young patients. Thus, the choice a gamble. This thought makes the surgeon very uneasy.

Could a patient's consent justify a possibly painful, almost torture-like treatment? This can be disputed. From a dignitarian point of view, it would severely reduce the dignity of a person to freely accept torture, for example, or to make oneself the slave of another. And if this is not morally acceptable, who is the wrongdoer – the torturer or the consentor? Or is it the act itself that would be morally questionable? However, in our particular case, the one whose human dignity might be threatened by the experimental treatment is not of a maturity that allows informed consent.

It then occurs to the surgeon that the concept of human dignity can also be applied to the source of cells used in the experimental skin grafts. Some people hold the view that human life should be protected and respected from the point of conception onwards [25]. The surgeon knows that human embryonic stem cells have been used as the source of the cultured skin stem cells, and he knows that there exist objections to using human embryonic stem cells in stem cell-based therapy. He himself does not share this view, but he feels that this is something that he needs to discuss with the boy's parents.

Still unable to find a solution to his dilemma, the surgeon considers the duties that he owes to this child as a fellow human being and as his patient.

14.2.2.4 Other Deontological Arguments

According to the deontological approach, morality is concerned with duties and principles that require moral agents to behave in specific ways regardless of the consequences [26]. This approach narrows the scope of ethics and leaves agents with a fairly limited number of duties – it accepts that everyone occupies a different place in the world and has moral duties particular to that place [26]. In the case of a conflict of duties, one duty has a greater claim on moral agents than other duties [26]. Therefore, the duty of a parent to the child and the duty of the clinician to the patient would certainly override their duties (if any) to the society.

Exactly what duties does the surgeon owe to the child as a fellow human being and as his patient? The European Convention on Human Rights and Fundamental Freedoms protects everyone's right to life and prohibits torture and inhuman or degrading treatment [27]. Therefore, the boy in front of him has a basic right to have his life saved, if it is possible, but also to be protected from inhumane and painful treatment. Which duty weighs more—the one to save the life of the child, or the one to protect the child from inhumane and painful treatment? If doctors and parents perceive the duties differently (e.g., doctors do not deliver too-painful treatment in the face of a bad treatment prognosis, whereas parents want everything that is possible done in order to save their child) – whose duties are to be counted?

The surgeon realizes that there is no ready answer to this question. The child's and/or the parents' wish, especially in life-and-death situations, often guides decision making. However, the decision making ought to be based on what is right for the child, but there should be no black-and-white thinking in this case. Although the parents' role in decision-making is very important, the opinion of physicians should also be taken into consideration; otherwise, their role is reduced to that of a technician, who is just fulfilling the client's wish. In important issues such as these, the market economy dictum "the customer is always right" might not always be justifiable.

The surgeon remembers that the positions sustained by human rights theorists and deontologists are reflected in, for example, the UN Convention on the Rights of the Child, which requires, among other things, devotion to the best interests of the child as well as respect for the views of the child [28], and the Declaration of Helsinki, which states that the well being of the individual research subject must take precedence over all other interests [29]. The human rights position is also reflected in the European Convention on Human Rights and Fundamental Freedoms (ECHR), which protects everyone's right to life and prohibits torture and inhumane or degrading treatment [27], or the European Convention on human rights and biomedicine [30], which states that research on persons who are not able to provide their informed consent (thus including children) is ethical only if the research subjects concerned do not object to participation in the research.

The surgeon finds himself thinking that on several occasions he has considered whether the consent of the boy's parents can be free and informed. This thought brings him to his next question: Is free and informed consent of the parents of his young patient really possible in the given situation?

14.2.3 Free and Informed Consent in the Context of Experimental Stem Cell-Based Therapies

The surgeon remembers reading in the literature about two important concerns regarding parental consent in serious cases like his:

1. The influence of the seriousness of the child's condition on parental decision making regarding the child's participation in a research project, and

2. The risk of therapeutic misconception when the decision about participation in research has to be done on behalf of a child.

Both concerns are very interrelated. Therapeutic misconception may be caused by the fact that the parents, affected by the seriousness of their child's condition, would not base their decision on all available information, but rather would focus on the facts indicating that the proposed experimental therapy can be effective in their child's case. However, therapeutic misconception may not necessarily be caused by the aforementioned conduct. The surgeon quickly considers these concerns one after the other.

He remembers that other studies have reported that the seriousness of the child's condition and the urgency surrounding trial entry are important influences on the parents' sense of vulnerability, and the success of communication [31–34]. For example, some parents of children whose lives are endangered struggle even to understand or recall that research participation is voluntary [34]. Other parents report that occasionally they doubt their own decisions [35]. By contrast, parents of children with less serious, chronic illnesses report more comfort in making decisions about trial entry [35] and in judging what is in the best interests of the child because they have experience with the disease [36]. These examples confirm the findings of one study that the parental decision making regarding enrollment of their sick child in a clinical trial is dependent on the child's pathology [31].

The surgeon also remembers reading several studies that have shown that for parents, the decision-making about participation in a research project is likely to feel more serious and possibly overwhelming when this decision is made on behalf of their child, rather than to serve their own rights and interests [36, 37]. Parents in one study felt personally and directly accountable for their child's outcome in a trial and thought that giving consent for a child would be much more difficult than deciding to take part in a trial themselves [18]. Empirical research confirms that the responsibility to act in the best interests of one's child is keenly felt. For example, one third of parents in one study reported that while they might accept certain research risks for themselves, they were much less certain about accepting the same risks for their baby [38]. Parents find themselves in a situation facing the incompatibility of wanting to do what is best for the child, but not knowing what the best course of action is [36]. Another study has reported that what may seem to the researcher to be a misunderstanding of trial rationale may be the parents' constructing the situation in a way that is acceptable to their need to protect their child [36]. To the clinician, research can be considered low risk when it involves no greater risk than conventional treatment, but to the parent everything is high risk because they have a child [36]. Other studies have also reported that it is unlikely that parents will always approach decisions about trials in the rational and mechanistic process of weighing all the available information before reaching a decision [39, 40], and that confidence in the treating physician is very often part of the informed consent process [31]. Empirical research has shown that parents of chronically and terminally ill children are prepared to take greater risks in treatment in the hope of a cure [18], and parents' decisions about Phase 1 cancer trials, which can bring side

effects and offer little chance of long-term medical benefit, may turn upon a fear of “giving up” on their child and the need to “leave no stone unturned” [41, 42].

In his thoughts, the surgeon quickly summarizes the situation: According to the law, he needs to obtain the informed consent of the boy’s parents before he starts either the experimental or the compassionate treatment. However, it is not at all certain that the parents will be able to give *free and informed* consent. In that case, can their objection to whichever form of treatment be overridden? By whom?

The surgeon thinks as follows: If, based on the theoretical knowledge and the results of preclinical experiments, he assumes – though without certainty – that experimental therapy has a rather big chance of being effective – say, 50% – and that risks are rather small, and if without the intervention the child will certainly die, what grounds does he have not to treat the child? If parents do not give their consent – for example, they think that accepting unknown risks may make the child suffer more – does the surgeon have the right (or the duty) to administer the experimental therapy against their will or take temporary custody of the child? If he does not do this, what are his reasons? Do they depend on his considerations for the child, the parents, or the fear of legal consequences?

14.3 Epilogue

The surgeon realizes that he is facing a real dilemma. The normative points of reference that came to his mind do not seem to provide him with a direct answer to his question and are to some degree conflicting. However, considering the values endorsed by different ethical theories enables him to see the full spectrum of ethical problems present in his dilemma. With a sigh, the surgeon leaves the operating theatre to talk with the parents...

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Part III
Creation of Human-Animal Entities for
Translational Stem Cell Research:
Scientific, Ethical and Regulatory
Challenges

Chapter 15

Creation of Human–Animal Entities for Translational Stem Cell Research: Scientific Explanation of Issues That Are Often Confused

Neville Cobbe and Valerie Wilson

Abstract In keeping with the Nuremberg Code and the Declaration of Helsinki, the novel use of certain stem cells in patient treatments is likely to require prior testing in animals in order to minimize risks. When human cells are combined with those of other animals in a living creature, this generates something referred to as a chimera. However, a chimera is only one example of the various possible types of interspecies entities that have been used in biological research. How exactly might these different entities be used in translating basic stem cell research towards clinical therapies? Could the mixing of human and nonhuman materials threaten human identity and, if so, how might this happen? This chapter explores such questions in the light of current biological understanding.

Keywords Chimera • Interspecies hybrid • Cybrid • Transgene • Human identity

15.1 Introduction

In a dramatic scene, the hero in David Lynch's 1980 film *The Elephant Man* exclaims: "*I am not an animal! I am a human being! I... am... a man!*" This appeal for respect illustrates how distinctions based on our shared humanity are deeply engrained, notwithstanding recognition that we are a particular species of animal. It is therefore little wonder that various entities containing human and nonhuman components may lead to controversy [1–4]. In this chapter, we will attempt to guide readers in distinguishing between different interspecies combinations and discussing the scientific rationale for their particular use in research. This is followed by an exploration of how humans might be distinguished from other species, providing a

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backdrop for some of the ethical concerns and legal issues arising from such research, which will be discussed further in ensuing chapters.

15.2 Classifying Interspecies Entities

Whereas many different sorts of cross-species mixtures may now be envisaged, some overlapping usage of nomenclature has unfortunately led to confusion in public discourse [5]. This is sometimes apparent in the juxtaposition of a wide variety of different entities, ranging from those that might otherwise seem relatively innocuous or inconsequential to more problematic human–nonhuman mixtures [6]. In this chapter, we will attempt to firstly distinguish between two broad categories, designated simply as various subcellular mixtures (as shown in Table 15.1) and “chimeras,” of which the latter appear to be of greater interest in translational stem cell research.

The name chimera (Greek: Χίμαιρα) originally designated a mythological fire-breathing monster that was part lion, part goat and part snake. This term has been appropriated by biologists to describe various different entities [7–11] and has therefore been used rather broadly to describe any “single biological entity that is composed of a mixing of materials from two or more different organisms” [6]. For example, the descriptions ‘chimeric’ and ‘hybrid’ may be used interchangeably by molecular biologists when referring to an engineered protein or DNA construct [7, 8], whilst all eukaryotic cells have been described as chimeric in discussing their possible evolutionary origins via endosymbiosis [10].

However, in the context of developmental biology (or applied research with stem cells), a chimera is typically understood to be an organism containing a mixture of genetically distinct populations of whole cells¹ ultimately originating from different embryos [11, 12]. In such creatures, the cells from different sources are mixed but the separate cells are generally distinguishable, with the rare exception of tissues such as skeletal muscle in which cell fusion is part of normal development [13]. These entities are thereby distinguished from hybrids and various other mixtures, which generally involve mingling of subcellular components (such as either organelles or nuclear genes from different sources) within an individual cell.

¹The terms “chimera” and “mosaic” are both used to describe organisms that have more than one genetically distinct population of cells, so it may be necessary to further distinguish between these. The key point is that the genetically differing cell types in a chimera originate from two or more different zygotes (in which the embryos may be members of the same or different species). By contrast, the genetically distinct cells in a mosaic all originate from the same zygote, typically acquiring different genotypes as a result of either somatic mutation or recombination events. However, the term “mosaic” is also applied to phenotypically distinct populations of cells arising from random inactivation of X chromosomes carrying different alleles in female mammals. In either case, a mosaic would only contain cells from the same species, rather than combinations of cells from different species (as in interspecies chimeras).

Table 15.1 Subcellular combinations

Type	Definition	How is it made?	Example of purpose	Example
“true” hybrids	Fusion of gametes from different species to form a hybrid zygote (the product of fertilization)	Either through the mixing of gametes <i>in vitro</i> or through rarer mating events between species	To test the ability of human sperm to penetrate eggs	Zona-free hamster-test for sperm
Somatic cell hybrids	Tissue culture cell containing chromosomes of more than one strain or species	Often through fusing two cell types together (<i>in vitro</i>) and selecting for those cells containing DNA from both sources. NB: The term “somatic” arguably may be misleading since one of the cells could be an embryonic stem cell	To look for the properties of one cell that alter the properties of the other, or to find which genes lie close to one another on a chromosome	Human–mouse hybrid tissue culture cells used to map genes on human chromosomes. So far, this has only been done in tissue culture

(continued)

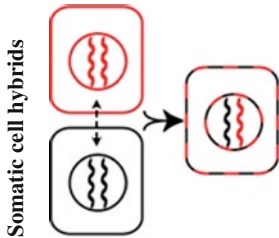
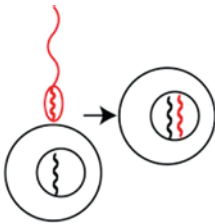


Table 15.1 (continued)

Type	Definition	How is it made?	Example of purpose	Example
Cytoplasmic hybrids (cybrids)	Cells made from the cytoplasm of one cell and the nucleus of another. Cybrids can be derived from different species or different strains of the same species, in which case they would be respectively termed “interspecies” or “inter-strain” cybrids	Created through injection of the nucleus of one cell into the cytoplasm of another cell from which the nucleus has been previously removed. This process is referred to as “nuclear transfer” and may involve somatic cells or the earliest cells from an embryo (referred to as blastomeres)	To make cloned animals or to make embryonic stem cells which are clones of the donor individual	“Dolly” the sheep and other cloned post-natal animals, or <i>in vitro</i> research with embryos created by nuclear transfer.
Transgenic animals	Individual containing DNA introduced from outside, so that all of its cells contain the genetic modification	Can be created by injecting DNA from one species into an early embryo, or by transfecting embryonic stem cells and then creating a chimera (see below). The resulting animals can be bred.	To study the effect of a gene modification, to produce proteins of interest for biotechnology	Transgenic mice e.g., mouse models of human genetic disorders and Down Syndrome

15.2.1 *Interspecies Hybrids*

Conventional hybrids result from fertilization between gametes from different species, such as the interspecies progeny resulting from sexual reproduction [7]. Typical examples include the mule, which is the offspring of a male donkey and a female horse, or a “liger” resulting from a rare hybrid cross between a male lion (*Panthera leo*) and a tigress (*Panthera tigris*). In these examples, the species are sufficiently closely related for viable offspring to be produced, though the progeny are generally infertile. However, non-viable hybrids could also be produced between more distantly related species by combining their gametes *in vitro*. Such “true hybrids” will not be discussed in any significant further detail, as they do not seem to have much use in translational stem cell research. Instead, the main interest in creating such entities with human material appears limited to previous tests concerning the fertilization potential of human sperm [14], which has seemingly been largely obviated by the use of intracytoplasmic sperm injection in fertility treatment [15]. Nevertheless, it may be worth noting in passing that human sperm is reportedly able to penetrate gibbon eggs [16] and previous attempts to generate chimp–human hybrids have been recorded [17], though all such attempts were seemingly unsuccessful.

In addition to hybrids generated by mixing gametes from different species, the term “somatic cell hybrid” has been applied to describe the fusion *in vitro* of two unrelated cell types. One of the best-known applications of somatic cell hybrids has been the production of monoclonal antibodies against known antigens by hybridoma cells [18], created by artificially fusing B cells from an immunized mouse with murine myeloma cells (with the capacity to proliferate and survive indefinitely). However, fusion of somatic cells from different species has also been used in other areas of research. For example, Sendai virus-mediated fusion of human and mouse cells has been used to assist mapping of human genes to particular chromosomes, thanks to the gradual elimination of human chromosomes over successive mitotic divisions and the ability to readily distinguish between the individual chromosomes of the respective species [19]. In addition, the formation of somatic cell hybrids *in vivo* has been used in attempts to explain how tumors progress, since human glioma cells injected into hamsters were able to fuse with hamster cells, and the resulting interspecies hybrid cells that metastasized were found more widely than non-fused cells [20].

15.2.2 *Cybrids and Transgenic Animals*

Other interspecies entities can be generated by means of nuclear transfer experiments that use oocytes and nuclear donor cells from two different species, which are consequently described as interspecies nuclear transfers [21]. Although the resulting entities have been referred to by various different names [22–24], a possible

consensus has emerged in which the term “cybrid” (a portmanteau of “cytoplasmic hybrid”) may be used to describe an embryo created by interspecies nuclear transfer, in which cells contain nuclear DNA and mitochondrial DNA from different species [25, 26]. Such experiments were initially performed by combining the cell nuclei from an endangered species with oocytes from a closely related species, where the latter was more common and could also act as a surrogate [22]. Increased interest in creating interspecies cybrids by combining human cells with the enucleated eggs of other species [21, 27] was prompted by revelations regarding the number of women’s eggs that had been used in attempts to clone human embryos [28]. Contrary to dubious suggestions that stem cells derived from interspecies cybrids would be immunologically compatible with the somatic cell donor [23, 29, 30], cells derived from cybrid embryos were latterly proposed simply for laboratory studies [31], partly due to concerns that components of divergent species may not be tolerated by a human patient’s immune system [32].

However, it had also been doubtful whether interspecies cybrid embryos would survive particularly long due to various incompatibilities between either the reprogramming process or mitochondrial proteins of more divergent species [21, 33–35]. Indeed, it was subsequently reported that embryos fail to grow beyond 16 cells if generated by inserting human nuclei into the eggs of more distantly related species such as cows, mice or rabbits [36]. This outcome in particular is unsurprising if one examines the branch lengths of a phylogenetic tree based on available sequence data for all mitochondrial proteins from these and other selected species (Fig. 15.1). The only pair of species illustrated here for which interspecies nuclear transfer has reportedly yielded viable offspring are the domestic dog and a particular species of wolf [37], and it is clear from the short branch lengths separating these species that their mitochondrial protein sequences are almost identical. By contrast, far greater differences are apparent in the long branch lengths separating human mitochondrial proteins from those of mice, rabbits, cows or pigs. On the other hand, using the eggs of more closely related nonhuman animals would seem difficult to justify if this might require non-consenting members of an intelligent and endangered ape species [35]. Meanwhile, the proposed use of nuclear transfer to generate patient-matched stem cells has been robustly out-competed by stunningly rapid research progress with induced pluripotent stem (iPS) cells [38, 39]. So although the mismatch between human nuclei and the oocytes of less closely related species may remain a subject of curiosity [26, 33], it seems increasingly unlikely that interspecies cybrids would play a significant role in translational stem cell research.

Lastly, transgenic animals [40, 41] may be considered to be a form of subcellular interspecies mixture, as they contain a mix of DNA from different species within the nuclei of their cells. Such creatures are not routinely considered to be “hybrids,” as the transgene from another species typically constitutes just one or a few genes (and therefore a minuscule proportion of an organism’s genome). Indeed, transgenic animals carrying no more than a few human genes might therefore be considered an utter contrast to interspecies cybrids (in which most of an entity’s genetic diversity is contributed by a human nuclear genome). However, novel strategies have also been developed to allow larger segments of the mouse genome to be replaced with

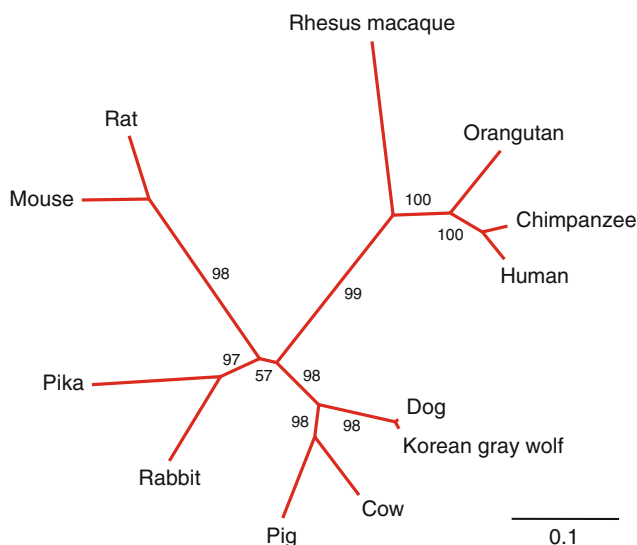


Fig. 15.1 Phylogenetic tree based on proteins encoded by mitochondria in selected organisms for which complete mitochondrial sequence data are available. The tree was constructed following concatenation of ungapped positions in alignments of all protein sequences. Distances shown are based on the Whelan and Goldman substitution matrix (modeling rate heterogeneity among sites according to a gamma distribution), with confidence scores for internal branches indicated by % bootstrap values. Similarly short distances to those observed between the dog and Korean gray wolf mitochondrial proteins are also observed when comparing cytochrome *b* sequences between various other pairs of species in which interspecies nuclear transfer has led to pregnancies (data not shown).

the equivalent human region [42, 43]. Moreover, a strain of mice with a nearly complete copy of human chromosome 21 has been generated [44], providing the most complete animal model for Down syndrome currently available. Nevertheless, even this feat is evidently insufficient to confer significant “humanlike” traits, as about a third of brain cells in these mice appear to lack the extra chromosome (which also constitutes a relatively small proportion of the total human genome). Whereas the insertion of DNA into other species by transgenesis is usually limited to a relatively minor proportion of the genome, more significant proportions of cells in organisms can be derived from different sources in the case of chimeras.

15.2.3 *Different Categories of Chimeras*

In contrast to the monster of Greek legend, a biological chimera may not necessarily combine elements from different species (Table 15.2). For example, bone marrow transplant recipients may be described as chimeras, as the cells in their blood originate from another individual and would usually differ genetically from

Table 15.2 Examples of chimeras

Type	Definition	How is it made?	Example of purpose	Example
Blastocyst injection or aggregation chimera	A chimera containing a mixture of cells throughout its body	Mixing either (1) blastomeres from different embryos of the same species or (2) pluripotent stem cells injected into a preimplantation embryo	Research to study the effects of the cell mixture and as a route to the production of transgenic mice	Mixing mutant cells (which carry a gene altered by mutation) with wild type ones (the “normal” form of the cell) to study whether wild type cells can rescue the phenotype (observable traits) of the mutant cells.
Transplant recipient	Individual that has received cells or organ(s) from another individual, either the same or another species	Transplantation of cells or organ(s) from one individual to another	Used in: (1) medicine for replacement of damaged organs or tissues; (2) clinical research to test the properties of cells including human embryonic stem cells; and (3) for testing of stem cell lines	(1) Human bone marrow or pig heart valve transplant into human; (2) to test whether stem cells can alleviate symptoms of Parkinson’s disease in mice; (3) to determine whether embryonic stem cells can differentiate into different cell types <i>in vivo</i> , as demonstrated through the creation of human embryonic stem cell derived tumors in mice.
Interspecies chimera	Chimera containing a mixture of cells from two species	Mixing either (1) blastomeres from embryos of two different species or (2) pluripotent stem cells from one species injected into the preimplantation embryo of another	Research to study differences in organism development; some researchers have suggested that injecting human embryonic stem cells into a developing animal embryo would enable testing for pluripotency	Sheep/goat and chick/quail chimeras

cells in most other parts of the body (unless the donor is an identical twin). Interestingly, human–human chimeras can also occur naturally on rare occasions, following the amalgamation of two different zygotes to form a single embryo [45, 46]. In the context of laboratory research with mice, preimplantation chimeras between mutant and wild-type embryos have been extensively used to discover when and where a gene is required in development [12], whilst transplantation chimeras have also been used to establish the functional behavior of injected cells based on their ability to reverse a specific pathology [47].

However, chimeras can also be created by combining the cells of different species. Such interspecies chimeras can include grafts of animal cells or tissue into postnatal humans (more commonly referred to as xenotransplants) that have been proposed as a solution to a shortage of donated human organs available for transplantation [48]. For example, pig heart valves have been used in human heart surgery for more than 30 years, with the advantage over mechanical replacements that complications due to thrombosis are generally reduced [49]. Although pig valves are chemically treated prior to human use, the use of xenografting has otherwise been largely beset by complications related to immune rejection [50–52] and concerns related to potentially increased transmission of viruses from other species to humans [53, 54]. Consequently, the use of other species to provide organs for transplantation has become an increasingly remote prospect, possibly accounting for the decline in associated regulation [55].

On the other hand, chimeras generated by grafting human cells into postnatal animals have been used extensively in research to explore the ability of human cells to differentiate and contribute to complex tissues. This includes the injection of human embryonic stem cells into immunodeficient mice to form a teratoma [56], which has become a standard assay for pluripotency. Furthermore, the transplantation of human tumor cells into immunocompromised mice [57], either under the skin or into the organ type in which the tumor originated, has become a widely used model for human tumor progression [58]. However, questions have been raised as to whether such assays may underestimate the frequency of human tumor-initiating cells, if both soluble and membrane-bound factors from one species are unable to engage with the corresponding receptors in another species [59]. For example, undifferentiated murine embryonic stem cells were found to rarely form tumors following injection into the brains of rats [60]. By contrast, when the same murine cell line was implanted into mouse brains (either as undifferentiated embryonic stem cells or following *in vitro* predifferentiation to neural progenitors), most of the mice exhibited large and visible tumors at the site of implantation upon examination a few weeks later [60]. Similarly, unselected hematopoietic precursors differentiated from monkey embryonic stem cells hardly ever formed tumors when transplanted into immunodeficient mice or fetal sheep liver, yet they formed tumors within a few months when transplanted back into the same monkey species [61].

Consistent findings have subsequently been reported with human embryonic stem cells following injection into human fetal tissues engrafted in immunodeficient mice, resulting in more aggressive growth of tumors than observed with the injection of such cells into mouse tissues alone [62]. Therefore, the phenomenon

of host-dependent tumorigenesis raises grave uncertainties and safety concerns regarding the therapeutic use of human pluripotent stem cells and their derivatives in patients (whether derived from embryonic stem cells or iPS cells). Nevertheless, the ability to study tumor progression in human tissue within a mouse [62] could also point towards additional uses of chimeras as a means for evaluating the behavior of cells intended either for study or therapeutic use. Rather than engrafting tissue from a human fetus into mature mice, one could instead generate chimeric mice by combining stem cells from different species at earlier stages of development. Such work could conceivably yield mice in which particular organs predominantly consist of human cells, potentially providing improved animal models for cancer research – but also raising additional sources of controversy...

15.3 Developmental Human–Nonhuman Chimeras and Associated Issues

The injection of human embryonic stem cells into mouse embryos to create interspecies chimeras has proven to be a source of divided opinion amongst biologists. Whereas some have argued that such experiments may be required to test the potential of existing human cell lines, others have questioned their necessity and feared that the resulting public disquiet might prompt further opposition to research involving human embryos [1]. However, the relevant issues are not necessarily limited to debate about the moral status of human preimplantation embryos, as the injection of human pluripotent stem cells into an embryo of another species may be subject to different regulatory constraints and could therefore raise novel questions. For example, research in the UK with various nonhuman species is governed by the Animals (Scientific Procedures) Act 1986, which defines any living mammal (other than humans) as a “protected animal” subject to regulation only after it has developed half-way through the normal period of gestation [63]. This markedly contrasts with the regulation of research with human embryos, where this is restricted to ensure that development does not progress beyond 14 days or the appearance of the primitive streak [64]. Consequently, a chimeric animal with a significant proportion of human tissue may be allowed to develop to considerably later stages than would be legally permissible with human embryos used in research.

Whilst the creation of mature chimeras through xenografting has been plagued by confounding issues of immune rejection [50–52], chimeras created at the earliest stages of development can be more readily generated due to the absence of a mature immune system in embryos. The use of embryonic and fetal chimeras between quails and chickens has been used for many decades to study neural development, since the cells of each species could be clearly distinguished [65]. This facilitated a series of dramatic experiments in which sections of presumptive brain tissue from embryonic quails were transplanted into

chicken embryos. The resulting chickens crowed and bobbed their heads in a manner typical of quails, demonstrating that the transplanted parts of the brain directed the corresponding quail behaviors [66, 67]. As it was also possible to create viable chimeras between distinct mammal species [68–71] and complex behaviors could be transferred across species, some interspecies chimeras could pose challenging quandaries if an experimental subject containing predominantly human brain tissue might exhibit greater potential for higher cognitive capacities.

Evidently aware of such concerns, the National Academy of Sciences in the USA recommended that research in which human embryonic stem cells are introduced into nonhuman primate embryos should not be permitted for the time being [72]. Similarly, a multidisciplinary working group concluded that it would be unacceptable to graft human neural cells into closely related species at an early developmental stage if the human cells would potentially constitute a large proportion of the host animal's brain [73]. However, as Jason Scott Robert has pointed out [74], there may be an important tension when some studies that might be more questionable scientifically could also be those that are less likely to pose significant ethical concerns and *vice versa*. For example, although human embryonic stem cells engrafted into mouse blastocysts could proliferate and differentiate within the embryos when cultured *in vitro*, the vast majority of the resulting mouse/human chimeric embryos failed to retain derivatives of the slower-dividing human cells after implantation in the uterus of foster mice [75].

Nevertheless, others had shown that human embryonic stem cells could rapidly produce neurons following transplantation next to partially differentiated tissue in early chicken embryos [76], and could also generate functional human neurons following injection into the ventricles of developing mouse brains [77]. Separate proposals have already been considered to create mice in which the neurons of their brains would be almost completely human in origin, using a particular mutant strain in which most or all of the developing neurons die so these might be functionally replaced by transplanted human cells [78]. Whilst the significantly different structure and size of the mouse brain would be expected to prevent human cells from contributing traits that might reflect human consciousness in this instance, the possibility of using an analogous approach with human pluripotent stem cells and mutant embryos of other mammals cannot yet be precluded. The potential outcomes of transplanting substantial numbers of human pluripotent stem cells into the developing embryos of larger mammals are far less certain at present. Notably, any relevant issues relating to such applications could similarly apply to human stem cells from other sources [79], not just those derived from embryos.

In addition to issues raised by the potential conferral of human mental traits, there is the possibility of human pluripotent stem cells contributing to the germline of some chimeras, particularly if such cells are incorporated sufficiently early in development. Perhaps due in part to the inefficiency of complete gametogenesis from sources such as embryonic stem cells *in vitro* [80], other species had previously

been used as surrogates in attempts to make human gametes *in vivo* [81, 82]. Despite seeming an unlikely prospect at present, more widespread use of such chimeras involving primates [83], rather than mice, has raised the hypothetical spectre of a human fetus trapped in the uterus of another species if the resulting creatures are allowed to interbreed, prompting recommendations that any such prospects should be prohibited [72, 78]. Concern has also been expressed about the prospect of unregulated reproductive cloning in some jurisdictions [84] by an alternative route involving human iPS cells and application of tetraploid complementation, as demonstrated in mice [85–87]. As it happens, it seems that tetraploid cells would not contribute almost exclusively to extraembryonic tissues in primates [88–90] as in mice [91], so it is more doubtful that tetraploid complementation would facilitate human reproductive cloning. Nevertheless, this does not rule out the use of an older reconstituted blastocyst approach [92], whereby greater contributions to development by human cells might be observed. So, if it is possible to imagine interspecies chimeras with predominantly human brains, babies, or bodies, should such creatures not be treated as equivalent human beings? Before attempting to answer this question fully, it may be helpful to explore why humans might be considered as special in the first place.

15.4 Distinguishing Humans from Other Animals

How are humans unique? At least at the DNA sequence level, no sharp dividing line between humans and all other species is readily apparent. In particular, the human genome differs from that of chimpanzees by only a few percent [93] and only a tiny number of genes of unknown function have been suggested to be specific to humans [94, 95]. As the protein sequences encoded by so many human and chimpanzee genes appear identical, most outwardly observable differences have been thought to result from differential regulation of gene expression [96, 97]. One particular noncoding region with a relatively high concentration of substitutions in the human lineage has already been implicated in hominid evolution, since it can also drive gene expression in developing limbs [98]. This has prompted speculation that the human sequence may have facilitated specializations of the hand that facilitate tool use and modifications of the foot associated with bipedalism [99], though its actual role in limb development remains to be determined.

Despite the lack of difference at the genome sequence level, various attempts have been made throughout history to distinguish humans on the basis of intellectual capacities for reason and conscience. However, this distinction becomes increasingly problematic in the absence of a radical discontinuity between the mental attributes of humans and those of related species, or when observable differences appear to be primarily quantitative and graded in nature. As Charles Darwin commented, “the difference in mind between man and the higher animals, great as it is, certainly is one of degree and not of kind” [100]. Even the state of being conscious of oneself may not be a feature that is strictly unique to humans.

For example, self-recognition in a mirror has now been experimentally demonstrated in a number of species, including chimpanzees [101], dolphins [102] and an elephant [103]. As with humans, it appears from a host of psychological experiments that chimpanzees understand the actions of others in terms of underlying goals or intentions, rather than simply in terms of surface behaviors [104]. Meanwhile, dolphins are known to display highly complex social behaviors otherwise associated with primates [105] and have the highest degrees of encephalization of all animals apart from humans [106, 107]. Further evidence that complex cognition is not restricted to humans comes from studies of food caching behavior and tool use in large-brained crows [108], which are also deemed to be capable of solving complex physical problems by reasoning both causally and analogically [109]. As for other mental abilities associated with humans, some young chimpanzees have similarly been shown to have a remarkable capability for numerical recollection. Strikingly, one particular 7-year-old chimp has been described as performing better than university students at a memory game when using the same apparatus and testing procedure [110].

Nevertheless, the average human brain at birth is about three times larger than that of our closest primate relatives, so a large cerebral cortex is often considered to be a distinguishing feature of humans. Curiously, brain volumes can be reduced to a third of normal in human patients with a neuro-developmental disorder known as autosomal recessive primary microcephaly [111]. Affected individuals are characterized by a smaller head with a sloping forehead (due to considerably reduced brain growth *in utero*) and typically display mild to moderate mental retardation [112]. Of the genes known to be mutated in primary microcephaly, both *microcephalin* and *ASPM* appear to have been subject to strong positive selection associated with increasing primate brain size [113–115], leading to suggestions that such adaptive changes contributed to human brain evolution. However, we now know that accelerated evolution of *ASPM* occurred in various other primate lineages, with positive selection reportedly associated with major changes in relative cerebral cortex size rather than whole-brain volumes [116]. Since the relative size of the frontal cortex was described as similar in humans and other great apes [117], further work may be required to clarify the roles of associated genes in human evolution. Caution is also needed in extrapolating such findings to defining features of humanity, both to avoid erroneous suggestions that patients with primary microcephaly are anything other than fully human and in light of a poor correlation between brain size and cognitive abilities in healthy human adults [118].

Meanwhile, language has been proposed to be a unique feature of human beings [119]. Assuming this distinction, the FOXP2 transcription factor became a focus of attention when it was found that a couple of amino acid substitutions in the human lineage distinguish this highly conserved protein from that of closely related species [120]. Since a familial point mutation in FOXP2 had been associated with severe speech and language difficulties [121], it was suggested that the two amino-acid replacements described as specific to humans might be involved in the evolution of language. However, subsequently available sequence data shows how one of

the residues previously considered as unique to humans is also conserved in dogs, cats and horseshoe bats, despite more rapid FOXP2 evolution in the latter [122]. Nevertheless, altered behavior and vocalization has been observed in mice following introduction of both amino acid changes associated with humans [123]. Furthermore, multiple genes that are known to be differentially expressed in human and chimpanzee brains are also differentially expressed following the addition of human versus chimp versions of FOXP2 to neuronal cells otherwise lacking the protein [124]. Therefore, at least one of the substitutions affecting FOXP2 in humans may have had profound effects on human evolution, although a precise role in the development of language remains obscure.

In any event, linguistic impairments had not prevented affected individuals from being recognized as human [121], and it may be questionable to what extent language in humans is a qualitative boundary separating us from other species. For instance, certain nonhuman species have been shown to be capable of mastering both semantic and syntactic information in artificially taught languages, including a bonobo [125] and bottlenose dolphins [126]. Contrary to earlier speculation that recursion might be a feature of language unique to humans [127], the ability to recognize complex recursive grammars in acoustic patterns has also been described in starlings [128]. In addition, our closer ape relatives can communicate context-dependent meaning through extensive use of brachiomanual gestures, which are thought to have served as a stepping-stone for the evolution of symbolic communication in language [129].

So what exactly makes humans unique? The answer is surely not any one factor in isolation but rather a suite of different characteristics. Despite the accumulating list of similarities between humans and other species, it is nonetheless clear that various cognitive differences remain [130], albeit more quantitative than qualitative in nature. If the degree to which we differ from other animals is therefore not limited to singular characteristics of isolated individuals, it could encompass everything that our species as a relational community has been endowed with to manipulate nature and exert authority over the natural world [35]. This appears to include not just large and intelligent brains coupled with manual dexterity but also the ability to cooperate in a society (facilitated by language), among other things. Although we may only have the earliest glimpse of a genetic basis for the relevant multiple traits and how they fit together, the remarkable ability of humans to manipulate nature certainly remains hard to dispute. Indeed, without this very human ability, it is doubtful that our discussions about the creation of interspecies chimeras would necessarily arise to begin with! However, if many of the characteristics associated with our species are already shared to some degree with other animals, can humans be defined in a way that distinguishes us from all human–nonhuman chimeras?

15.5 Interspecies Entities and Human Identity

Although the power exhibited by humans over the natural world clearly sets our species apart, this is not obviously reducible to any one physical characteristic or gene and can only be understood in a broader relational context. Consequently, if

one naïvely depends on isolated criteria (such as brain size or linguistic competence) as defining features of humanity, then this may necessitate concomitant treatment of some divergent species as if they are human, whilst also excluding individual members of our own species from equal treatment as if they are somehow “subhuman” (or even nonhuman). Such a discriminatory approach may have disturbing ethical and legal ramifications, notwithstanding the moral significance of cognitive capacity and sentience in any species [131]. These difficulties may echo attempts at determining moral status based on various conceptions of personhood, which have been complicated by multiple definitions [132–136] and notable disagreement regarding their merits [137, 138]. Alternatively, one might contend that the percentage of human DNA or of coding sequences in a cell’s nucleus is necessary and sufficient for determining species identity. However, this could then imply that various nonhuman species with shared sequences are effectively human, whilst human blood is considered to be primarily nonhuman (because of the absence of any nucleus in mature red blood cells), which is clearly nonsensical from a biological perspective! If human identity depends merely on properties shared to some degree with other species, then determining whether or not some interspecies organisms should effectively be treated as members of the human species may be even more challenging.

Given the possible complexity, some have suggested that the concept of “species boundaries” makes little sense anyway, if “species” are just snapshots of a dynamic process [139]. Nevertheless, some notion of species identity is required in order to maintain that species are thus in flux, even where boundaries established over the eons are blurred by shared evolutionary origins. For example, a concept of species boundaries is still maintained in the case of ring species [140–143], which comprise local populations able to interbreed with neighbors in a connected series that gradually encircles a barrier, such that the terminal forms coexist but have become reproductively isolated from each other. Moreover, we implicitly acknowledge that species represent genuine biological distinctions by recognizing that the extinction of species is a genuine loss [144]. So rather than rejecting species boundaries as meaningless, the problem therefore appears to be a question of how species in general, and the human species in particular, should be defined.

Attempts at identifying humans in terms of species membership may also be complicated by multiple species concepts [145–147], requiring that the definition employed should incorporate criteria appropriate to the relevant context [148]. In the case of extant sexually reproducing organisms (which includes humans), the most widely accepted definition of “species” appears to be Ernst Mayr’s biological species concept [149], according to which “species are groups of interbreeding natural populations that are reproductively isolated from other such groups” [150]. Whereas a comprehensive view of our species in terms of a relational community [35] implies far more than merely sexual reproduction, the criterion of reproductive isolation may appear at first glance to be at least partly consistent with this. Consequently, it would seem that most human–nonhuman entities would not be members of the human species if such creatures were otherwise fertile but unable to reproduce successfully with humans on reaching sexual maturity and thereby produce fertile offspring.

The presumed lack of viability of some interspecies entities (such as human–cow cybrids) would therefore seem to rule out their classification as human based on Mayr’s definition, though concerns regarding bestiality [151] would also preclude determination of species membership based on sexual reproduction. However, the aforementioned biological species concept is really a special case of the evolutionary species concept [152], which defines a species more broadly as a single lineage of organisms (comprising populations of ancestors and descendants) that maintains its identity from other such lineages and has its own evolutionary tendencies and historical fate. Using this broader definition may rule out the classification of some chimeras as human because of their overall lack of common ancestry, especially if only a relatively small fraction of cells is entirely human. But how many cells must be of human origin, and in which bodily organs, in order for a creature to be considered as essentially human?

These issues seem to be reflected in comments by a former UK Minister for Health [153], who stated: *“In the case of an embryo in which the brain might be predominantly animal, it is worth reminding ourselves what we mean by ‘predominant’. We refer not only to the percentage of the DNA but also to its location and functionality. If that entity had a human brain, that could clearly have a predominant function so, by definition, it would be at the human end of the spectrum...”*

Whilst such comments strive to capture what some might consider to be significant characteristics of humanity, they also point towards a potential difficulty in confidently predicting when exactly a “predominant functionality” would necessarily arise. Despite recommendations that human–nonhuman chimeric embryos should be destroyed prior to formation of the nervous system [154], it remains unclear what scientific benefit there would be in creating chimeras in which observations of the fate of transplanted human cells in a developing organism were extremely limited. For instance, in a paper describing early development of implanted human–mouse chimeric embryos [75], the authors highlighted the need to examine later developmental time points and indicated that live chimeric animal models would be more valuable as a research tool.

Rather than advocating either absolute prohibition or acceptance of all conceivable human–nonhuman mixtures, each kind of proposed experiment may need to be evaluated separately in a manner that seeks to uphold both the highest standards of animal welfare and truly beneficial scientific advances [35]. Where uncertainty surrounds the potential outcomes of experiments involving human–nonhuman chimeras, work may either be preceded by analogous experiments involving transplantation of stem cells from other species [154], or preliminary experiments that carry less risk of significantly altering higher brain functions may be prioritized [78].

The prospect of creating various chimeric animals with the potential for what may be viewed as hitherto uniquely human faculties raises profound questions concerning rights and responsibilities, yet many traits of humans are already shared to some extent with other species. As it is hard to identify isolated qualitative features unique to all humans at an individual level, multiple



Fig. 15.2 “Affe mit Schädel” (Ape with Skull, c. 1893) by German sculptor Hugo Rheinhold; bronze cast on display at the Ashworth Laboratories, University of Edinburgh. This intriguing statuette, depicting a pensive female chimpanzee atop a pile of books, has been open to diverse interpretations. One of the books is simply titled “DARWIN” (possibly referring to “The Descent of Man”), while the open book at her feet displays the Latin text “ERITIS SICUT DEUS” (“...and you shall be as God...”) translated from Genesis 3:5, although curiously omitting the ensuing words “...scientes bonum et malum” (“...knowing good and evil”) due to an apparent tear in the page.

characteristics may need to be considered together to appreciate the quantitative differences that cumulatively distinguish our species. Whilst some conceptions of human uniqueness may seem to be challenged by various interspecies mixtures [2], it is also possible that the underlying assumptions could already be questioned on the basis of existing knowledge about the abilities of other species and their relationship to humans (Fig. 15.2). Instead, the real ethical issues seem to arise not so much in the mere creation of an organism as in its expected treatment, though these issues may converge if one intentionally creates an organism with both the potential for significant cognitive status and severely compromised welfare.

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Chapter 16

Chimeras and Hybrids – How to Approach Multifaceted Research?

Gisela Badura-Lotter and Marcus Düwell

Abstract Chimeras and Hybrids – How to Approach a Multifaceted Research?

Since a couple of years now, the creation of chimeras and hybrids has left the realm of myth and legend and entered into the reality of scientific research. The more it advances towards the creation of human-animal mixtures, the more the need for ethical and legal regulation is claimed. In our article, we will clarify different biological definitions concerning mosaics, chimeras and hybrids and contrast them with a new concept for an ethical evaluation of these highly diverse research fields. The proposed concept is illustrated by an example and some fundamental ethical reflections should briefly map the actual debate as well as the needs for further ethical reflection.

Keywords Bioethics • Chimeras • Hybrids • Research ethics • Species integrity

16.1 Introduction

It's already been a few years since the creation of chimeras and hybrids has left the realm of myth and legend and entered into the reality of scientific research. The more it advances into the creation of human-animal chimeras, the more there is a need for ethical and legal regulation. In its sixth framework program, the European Commission (EC) asked for an evaluation of the diverse and broad research field of chimera and hybrid research; this chapter has been generated within the framework of an EC-project and is dedicated to answering some of the questions surrounding chimera and hybrid research.¹ We will first try to clarify inconsistently used

¹CHIMBRIDS, see <http://www.chimbrids.org>. J. Taupitz/M. Weschka (eds.), CHIMBRIDS – Chimeras and Hybrids in Comparative European and International Research: Scientific, Ethical, Philosophical and Legal Aspects, Berlin/Heidelberg: Springer 2009.

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biological terms (16.2) and will propose some distinctions in order to facilitate a structured, interdisciplinary analysis of the field (16.3). The proposed concept will then be illustrated by an example (16.4), and some fundamental ethical reflections, aiming at highlighting needs for further ethical debate, will conclude the chapter (16.5).

16.2 Definitions

The term “chimera” is not unambiguously defined. It is part of a field of definitions concerning different types of chimeras and chimerism, whether on the levels of eukaryotic organisms, viruses or even proteins. Natural blood chimerism occurs in man and other mammalian species (first described by Owen [1]). Chimeras and hybrids can be created technically on the DNA, cellular, tissue, organ or embryonic level. In this article, we will focus on human–animal chimeras and hybrids.

Mosaic. A mosaic is “an organism that consists of cells of more than one genotype. The strict definition requires that genotypically different cells are all derived from a single zygote” [2]. Quite often, the term “mosaic” is applied to any organism with genotypically different cells (see below). In biological research, mosaics are of considerable importance in the study of gene function. By manipulating not all, but merely a subset of cells within an animal, the function of a target gene and its role in development can be assessed. In mammals (mainly mice), the creation of mosaics became an important tool for studying gene function once techniques such as homologous recombination and site-specific recombination were established and could be combined with stem cell research [3, 4].

Chimera. A chimera is usually defined as an individual that contains (genetically distinct) cell populations derived from *different* zygotes [2]. That means that any (successful) introduction of genetically different cells or organs into an existing organism or aggregation of genetically different embryonic cells leads to the formation of a chimera.

Hybrid. Generally speaking, a hybrid is something of mixed origin or composition. In biology, a hybrid is usually defined as an offspring of parents from different species or subspecies produced by gamete fusion. Two classical examples are the mule and the hinny. Entities created by nuclear transfer (NT) techniques are generally referred to as “nucleo-cytoplasmatic hybrids” but are sometimes also classified as chimeras (e.g., [5]). Since derived cell-types are mainly uniform within the body (i.e., cell-specific differences are within the “normal” differences every mammal exhibits), we suggest using the term hybrid for organisms derived by nuclear transfer techniques, although it is argued that they do not represent “real” hybrids because nuclear material stems from one donor only (see, e.g., [6]). The term nucleo-cytoplasmatic hybrid is related to a broader definition of cytoplasmic hybrids, also termed *cybrids*, meaning the viable cell resulting from the fusion of a cytoplasm (enucleated cell) with a whole cell – a technique currently also used in reprogramming experiments (see, e.g., [7], and the example in Sect. 16.4).

16.3 Structuring the Ethical Debate

Chimeras, mosaics, and hybrids can be obtained by many different procedures and on very different levels. Depending on the specific techniques, placing the resulting entity into one of the above-mentioned categories is sometimes far from being self-evident, especially when new options arise. In order to structure the ethical debate about different possibilities to create entities that in some way are crossing the “borderline between humans and animals,” the biological classification is, however, not very helpful. This is because the ethical questions one may distinguish in this field of discussion do not coincide with the different biological categories; by contrast, the same questions apply for different forms of chimera research.

In the following, we will, therefore, use the looser definition of mosaics given by Rossant and Spence as “any organism of mixed genotype, whatever the initial cause” [2]. In doing so, chimeras and many other genetically altered animals become a subclass of mosaics. We will propose distinctions concerning different levels of the ethical debate about mosaic and hybrid research. They are mainly of heuristic relevance for the distinction of different levels of ethical debate, but will also help to understand the kind of questions the ethical debate will have to deal with:

1. Intra- versus interspecific hybrids and mosaics
2. Sources, donors, and introduced material
3. Recipient species and (experimental) setting
4. Developmental stage of the recipient/transplantation side
5. Research aims, possible uses, side effects

In our overview, we will try to identify the most relevant questions within the debate of human–animal chimeras, especially those for which we have reason to assume that they are specific for this topic. How the different questions will be answered, and how much weight will be given to them, depends, of course, mainly on the assumptions that are made on the level of ethical theory.

16.3.1 *Intra- Versus Interspecific Hybrids and Mosaics*

At first glance, one can discriminate between *inter-* and *intraspecific hybrids and mosaics*. Intraspecific (or intraspecies) means that body parts of another individual of the same species are introduced into an organism. Thousands of intraspecific hybrid and mosaic animals are produced each year in biological and medical research. In particular, experiments within the context of gene function in developmental biology and the creation of animal models for human diseases in biomedical research are of essential basic importance. Of course, research interests differ widely in these fields, and range from seeking basic insights into developmental biology to the development of new therapeutics for specific human diseases. In humans, intraspecific mosaics are created in transplantation medicine and the creation of hybrids and mosaics is discussed in the context of cloning and gene therapy. All of these activities raise a set

of ethical questions. We will, however, only refer to interspecific hybrids and mosaics in order to address specific questions more precisely.

Interspecific hybrids and mosaics are created on different levels, e.g., between viruses (reviewed in [8]), and mainly in research on xenotransplantation and developmental biology. Creating interspecific hybrids and mosaics in bio-medical research is in general less common, and in scientific [5, 9] as well as public² debates, more challenged than creating intraspecific hybrids and mosaics. There are strong intuitions that morally relevant differences between the creation of inter- and intraspecific mosaics and hybrids exist, and within the ambit of the catchphrase “crossing species boundaries” one can already find a variety of recent publications related to the new technological developments (see [10] and comments within the same journal in [11]). However, a philosophically coherent and strong justification of the possibility that something like a species boundary can be violated remains to be demonstrated.

16.3.2 Sources, Donors, and Introduced Material

The materials used to form mosaics and hybrids as well as their origins often justify a separate ethical evaluation. However, the ethical problems related to donors/sources and materials are, for the most part, not specific for mosaic and hybrid research, and related problems are only treated briefly.³

The material transferred might be significant for an ethical evaluation of certain approaches because: (1) The material can be more or less dangerous in a clinical application. In some cases we may not know how a specific material will react after the transfer; (2) it can be more or less (morally) problematic or expensive to obtain, to maintain, and to adopt it to the specific purpose for which it is needed; and (3) it can be a more or less powerful tool for chimera formation, which is the only point specific for the research at stake. Three major tools are currently at hand to transfer foreign biological material: viruses, somatic cells (nuclei, tissues, and organs), and embryonic as well as germline cells (nuclei, tissues). All of these face major medical risks and problems when they are envisaged for clinical use. Embryonic cells are considered quite powerful in their capacity to form chimeras, especially when placed in an embryonic environment. This capacity might be of moral significance, when the amount of “alteration” is considered as a valid quantitative criterion to judge specific research areas. With the use of embryos and embryonic cells, all moral questions concerning the status and use of human embryos arise.

²See, e.g., Amanda Onion: Mixing humans and animals for science, ABC news 7.02.2005 <http://abcnews.go.com/Technology/Health/story?id=465202>.

³Legal and moral conflicts arise in more and more cases alongside an increasing tendency to patent biological findings. They cover an amazingly wide field of cases – from singular and collective plaintiffs concerning improper informed consent and misuse of tissue for personal enrichment (see case discussed by Gitter [12]) to violations of native populations genetic autonomy (Tsosie [13]).

16.3.3 *Recipient Species*

For the ethical evaluation of hybrid and mosaic research, it can make an important difference whether the recipient of xenogenic material is a human being or an animal. If the recipient is a human being and the use of xenogenic material is part of a medical treatment or clinical experiment, special attention has to be given to the (long-term) well-being and interests of the patient, and eventually third persons. This includes medical, as well as psychological, social, and other aspects. The situation is different if a human embryo is used for research purposes only, i.e., if it will be destroyed during or after the experiments. In this case, other fundamental problems must be taken into account (see [Sect. 16.4](#) and 16.5).

With regard to *non-human recipients*, one should point out that experimental settings can involve considerable pain and killing of animals; for all ethical approaches considering suffering or killing of animals to be morally relevant, at least a careful assessment of the very aims and careful conduction of each experiment would be demanded. The questions that may be raised concerning the consequences for the animals involved are, however, not different from the questions that are generally discussed in the debates on animal experimentation. With regard to this, it is not very likely that research concerning chimeras and hybrids will confront us with very new questions.

A third category of ethical problems arises, however, concerning entities of which the biological categorization of the recipient cannot easily be determined, and the associated topics are actually in the center of public concern: What happens if the transfer of material between humans and animals alters the nature of the created individual in a way that the application of our traditional biological categories becomes doubtful? What will we call a biological entity if its intellectual or behavioral characteristics are significantly changed or if we do not know whether those changes have taken place? How can we know, for example, if a mouse with 90% human brain cells⁴ might be able to acquire human-like capacities when we have no real means to prove it, because no educational experience with a humanized mouse exists? We might assume a substantial change in disposition, but how should we ethically assess it? Doing such experiments by trial-and-error could be in itself morally questionable if the results revealed that the entity really exhibits human-like capacities. For the ethical debate, the first important question is whether and to what extent the ontological and moral status of the created being depends on the source used to create it or, on the recipient species (see [Sect. 16.5](#)).

16.3.4 *Developmental Stage of the Recipient and Transplantation Side*

In general, the amount of migration, propagation, differentiation, and overall participation in body formation of foreign cells or DNA introduced into an organism

⁴The debate arose following the publication of Muotri et al. [14].

depends on the age of the recipient, the transplantation site, and the cell-type/vector used to transfer the genetic material. One can say that the earlier in development the recipient, the larger (in general) the contribution of introduced cells/DNA to body formation. For example, human stem cells transplanted in the developing primate brain (e.g., in experiments by Ourednik et al. [15]) or human chromosomes in mouse ESC to generate trans-chromosomal mouse strains [16] might contribute to a greater extent to the formation of the organism than, for example, adult stem cells would do in the tail of an adult animal. The age of the recipient is especially important if human beings are concerned; an adult can, at least, be asked for his informed consent and can judge costs and benefits of such an intervention according to his own interests and values (risks for third persons are to be considered in a non-individual, legal framework). This is not the case for human embryos and fetuses.⁵ Apart from medical risks and the possible psychological and social burdens for the developing individual, the genetic information might be passed on to future generations. With these questions we are unavoidably entering the debate about embryonic engineering and “enhancement.”

Furthermore, the transplantation site could be of moral relevance because of the different symbolic values, i.e., the different importance for our human self-understanding that we ascribe to certain body parts. The alteration of the human brain or, still, the heart, for example, raises more intuitive concerns than the alteration of the kidney.

In summary, we consider the transplantation site to being ethically relevant in light of: (1) insecurities and risks concerning the medical consequences; (2) symbolic and ethical values; and (3) psychological, social, and other effects.

16.3.5 *Research Aims, Possible Uses, Side Effects*

For an ethical evaluation, it seems important to analyze explicit and non-explicit *research aims* scrupulously. Since the production of mosaics and hybrids almost always raises ethical questions, the evaluation of research aims (and possible non-intended outcomes and uses) is necessary to judge possible benefits, risks and threats that may be ethically relevant even when no profound moral barriers stand against a specific experimental setting.

16.4 Example

We will now show how to use our proposed criteria for a first assessment of hybrid and mosaic research on a case-based approach. The selected case is based on Chen et al. [19].

⁵For risks and hurdles of *in utero* transplantation trials see, e.g., Chen et al. [17]. For work on fetal chimeras, see, e.g., Almeida-Porada et al. [18].

Short description: Human somatic cells (from the male foreskin and female facial skin) were obtained from donors at ages of 5, 42, 52, and 60 years. The cell nuclei were transferred to enucleated rabbit oocytes by cell fusion to test rabbit oocyte cytoplasm as a means to reprogramming human somatic cell nuclei. The *interspecific hybrid embryos* developed to the blastocyst stage and the inner cell mass cells were removed and cultured on mouse embryonic feeder cells to form ESC. These possessed most of the properties and phenotypes of conventional human ES cells: they retained a normal karyotype, expressed typical marker genes, and were capable of multi-lineage cellular differentiation. The capacity for tumor formation has not been tested.

16.4.1 Intra- Versus Interspecific Hybrids and Mosaics, as well as Recipient Species and Experimental Setting

In the experiments of Chen et al. [19], human–rabbit interspecific hybrid embryos were created. The ontological and moral status of the hybrid embryo – created and destroyed for research purposes – is far from clear. Should we regard them as merely human embryos, since only about 1% of the cells' genome comes from the rabbit? In this case, they should be treated as “fully human” embryos, i.e., they fall under the same legal regulations and ethical considerations as the latter. If it is considered to be an entirely new life form, we have but few moral criteria at hand to judge research on it. It is often argued, however, that such interspecific hybrid embryos are most probably not viable in the sense that they could develop to term, because it is highly unlikely and has not been achieved so far. However, uncertainty remains just until the converse is proven. This argument is meant to rule out the so-called potentiality argument for a moral status of potential agents, and hence to promote the view that those embryonic entities cannot be regarded as having a moral status at all (or a very reduced one). It is, however, not clear that from the assumption of the non-viability of a later fetus or adult individual, there follows the right to create a (living!) embryo and to destroy it after use. In this regard, it is interesting to note that the authors talk of NT-units rather than hybrid pre-embryo or embryo. The effect of the scientific language is, in general, a desired exclusion of morally charged terms. A side effect is that confrontation with associated fundamental ethical problems is avoided, yet the illusion of a purely scientific, morally neutral action is evoked when it comes to the public perception of the research communicated – whether voluntarily or not.

Since the recipient species is a rabbit, this might strengthen the biological basis of the non-viability argument because of the comparatively distant biological kinship. If, by contrast, the oocyte stemmed from a great ape, the question of viability would be much more difficult to assess.

16.4.2 Developmental Stage of the Recipient, Transplantation Side

Looking at the developmental stage of the recipient (oocyte), new questions arise. What is the influence of the enucleated egg on the development of the hybrid

embryo? With only the mitochondrial DNA being of rabbit origin, can we consider this entity to be merely an interspecific mixture? What if mitochondria are replaced by human mitochondria? Some additional points will be discussed in [Sect. 16.5](#).

16.4.3 Sources, Donors, and Introduced Material

In this case, both “compound parts” involved (the oocyte and the human skin cells) do not seem to pose direct moral problems in themselves. Since the “donor” of the egg cells is an animal, mainly well-known questions from animal ethics debates arise. They concern the killing or suffering of the involved animals or the conditions of husbandry. Since these considerations are not specific to mosaic and hybrid research, we will not further outline possible argumentations. As for the human material, it is mentioned that discarded material from surgery was used, and that informed consent was given (although not specified for the special situation of the child). The procedure seems, at first sight, not controversial. However, everything depends on the exact nature of the information given to obtain consent, such as: are research aims revealed in detail? Who decided for the child, etc.?

16.4.4 Research Aims; Possible Uses; Chances, Risks and Side Effects; Alternatives

Three explicit research aims were stated in the paper of Chen and colleagues:

- a. To provide a proof of concept: the possibility of deriving NT-ESC from interspecific nuclear transfer hybrids using human somatic cell nuclei.
- b. To develop a system to solve the problem of immune incompatibility in (possible) future cell transplantation, avoiding the need of (medically more appropriate) human eggs.
- c. To develop a tool for the study of reprogramming and differentiation processes of somatic human cell nuclei.

Not mentioned, and even unintended uses, might be:

- d. *In vitro* experiments to study the influence of the animal oocyte (cytoplasm and mitochondrial DNA) on the development of the hybrid embryo even beyond the blastocyst stage.
- e. *In vivo* experiments to study developmental processes (transfer of hybrid embryos into the uterus of an animal).
- f. Advancing technical standards for reproductive cloning in animals and men.

If the setting of the experiments, as described above, does not impose special harm on involved animals and human beings, the evaluation of the experimental protocols depends to a large extent on the answer to the question of whether we owe the hybrid embryo moral respect or not (unless one is against any animal experimentation and

against any use of human cells for research purposes). Apart from this fundamental question, other possible ways of assessing the research approach can be used.

With regard to the research aims, one can identify some substantial conceptual and medical problems, some of which we will just briefly outline.

16.4.4.1 Transplantation Medicine

It is not clear whether ES-cells are at all suitable for transplantation medicine, since so far no long-term integration and physiological as well as therapeutic functioning of the inserted cells has been shown for any disease in humans. Furthermore, there are considerable risks associated with ES cells that will presumably remain even if the problem of immune rejection might be solved: the risk of tumor formation in the host and risks of culture contamination and DNA changes associated with culture conditions [20, 21].

As to the circumvention of immunological responses, some conceptual questions are still open, too, because the influence of the animal cytoplasm and mitochondrial DNA on cell (surface) characteristics is not yet clear and prevention of immune rejection has not yet been shown. So far, unknown problems may occur after transplantation of these artificial cells. It has been shown, for example, that SCNT in monkeys results in a high degree of genetic instability in the resulting cells [22]⁶; the case is not yet clear for human cells, but might affect their use in transplantation medicine.

Furthermore, it is a general problem in research on cell transplantation as well as gene therapy trials in animals that long-term follow-up studies are rare and insight into possible (side-) effects therefore have hardly been achieved so far. This is surely due to the persisting predominance of experiments still aiming at elucidating basic mechanisms of engraftment, integration and primary function of introduced cells and organs, indicating at the same time that most of the research in this field still faces initial hurdles and questions. Furthermore, the often-criticized but nevertheless growing pressure on scientists to publish as fast as possible, renders difficult and risky long-term experimental settings not very attractive. However, some long-term studies do exist and they sometimes reveal different outcomes than might have been predicted from short-term experimental successes or drawbacks (see, e.g., [24, 25]).

Finally, alternatives can be envisioned. Scientists from a large variety of research fields try to develop medical solutions for the diseases currently “on the list” for possible future ES-cell-based therapies (such as cardiac failure, diabetes, liver diseases and others), ranging from the use of somatic stem cells to genetic, drug-based or mechanical approaches. The recently derived induced pluripotent stem cells seem to be the most promising approach to individualized cell-based

⁶Although research in NT-ESC is progressing, and a greater comparability to “normal” ESC has been shown, e.g., in mice (see, e.g., Brambrink et al. [23]), many questions remain open.

transplants [26, 27]. However, it is not foreseeable which path will yield the best results. Moreover, some of the envisioned diseases are so-called “life style”-related, so that other than medical solutions could be envisaged.

With regard to the more basic research applications, it must be said that using interspecies systems to study “normal” biological processes *in vitro* as well as *in vivo* is most often inferior to intraspecific models. Only some very specific questions might be better addressed by interspecies models.

16.4.4.2 Tentative Appraisalment of the Case

The ethical judgement concerning the experimentations done by Chen et al. [19] depends, first of all, upon the answer to the question of whether the created hybrid embryo has a moral status that would be a reason to avoid destructive experimentation. If the answer is yes, then the creation and destruction of such an embryo is morally unacceptable. One can also argue that the creation of life that is a substantial mixture of animal and man is ethically wrong, independently of the specific status of created individuals. That would as well rule out all research creating hybrid embryos. Both arguments are briefly outlined in Sect. 16.5.

However, if one assumes that the created entity does not have a moral status that entails certain moral respect, the experimentation as such cannot be judged as being *prima facie* morally wrong – given that the rights of involved persons have been respected and that animal suffering is negligible. However, taking the conceptual weaknesses of underlying research aims as well as the manifold alternatives into account, one can question the scientific and medical value of this research branch as such (the proof of principle of the derivation of rabbit–human NT-ESC, which is even with regard to the state of the art in 2003 not to be regarded as a conceptual or even practical breakthrough). It might in this context also be of some importance, that the public concern about creating human–animal chimeras and hybrids as well as possible future developments rising from it, already leads to a certain damage of the image the related research has in society. And even if one doubts that there are reasons for the public to be alarmed, this should at least be a reason to establish a convincing regulation of the research on human–animal chimeras before such an experiment is allowed.

16.5 Fundamental Ethical Problems and Open Questions

16.5.1 Animals and Species

Research on chimeras may result in suffering and killing of the animals used, or lessening of their well being. It is clear that substantial suffering and killing of animals is involved in many areas of chimera research, e.g., in animal models of human diseases and especially in xenotransplantation trials involving genetically

altered pigs (held in pathogen-free husbandry conditions) and primates as recipient animals,⁷ making some research aims highly questionable. However, it is not very likely that the experiments will have significantly greater impact on the well being of animals than many other research methods. Therefore, new ethical arguments are not likely to apply, here. By contrast, new arguments referring to the integrity of the whole species may arise. This aspect can be regarded in different ways [29, 30].

1. The first and most common one is founded on the ontological assumption that “species” do exist and that the nature of these different species is not contingent upon but rather the product of either environmental (natural) selection or the will of God (or an intelligent designer) and should, therefore, not be altered in a way that obviously violates observed rules and principles. Both approaches hold that “nature” is an instance that justifies the moral regulation of human action in certain fields. These views have been challenged (and supported) by several arguments, and not only since the appearance of applied bioethics in the 1970s. An extended debate already exists in this field. One example of this opposition is those who maintain that the fact that humans exercise a willful, engineering interference on nature is regarded as one of its major species characteristics or human “universal” (anthropinum). The cultivation of nature is even regarded as being a divine assignment. In short, the opponents of a moral normativity of nature disclaim the fact that the “nature” of a particular species is an entity worthy of moral protection.

It is likely that this dispute might remain unsolvable in a strong argumentative sense. But of course, other normative relevant considerations might be taken into account to judge certain attempts to create hybrids and mosaics. In some cases, it might pose an ecological risk if mosaics/hybrids enter into the natural environment. Taking into account the complex ecological functions of different species, we cannot expect to master, or even overlook, all relevant implications of a substantial change imposed on the biological basis of a species. We have at least strong precautionary reasons to take the “integrity” of a species into account. A precautionary argumentation would, however, not refer to a specific ontological status of the species but on epistemic limitations of our knowledge of ecological circumstances. This specific argument would only be valid in case of experiments where the created entity would enter the natural environment.

2. A second approach to the species’ integrity aspect might ask for the impact that chimera and hybrid research might have on our social and cultural self-understanding and self-construction as human beings. Instead of referring to a specific ontological status of “species,” one can regard the concept of species as a human construction, invented merely to handle the complexity of the environment surrounding us – a tool for categorization that faces persistent conceptual problems and challenges reflected in the many definitions that are historically and currently used to define “species.” As a consequence, we can bestow to “species” no greater value

⁷See, for example, the report of the bioethics council of New Zealand (Toi te taiao [28]).

than men are willing and able to do. However, since the distinction of animal and men, of, for example, *Gorilla gorilla* and *Homo sapiens*, is a fundamental, if not constitutional element of our self-perception and recognition, we might not be as free in our choice as might be hoped or feared by this finding. Crossing species' boundaries and creating new interspecies individuals and maybe new species, challenges our own species integrity and hence our identity, and raises new fundamental questions as to our capacities to discriminate us as human beings. In the following, it forces us to reconsider our moral obligations against humans, animals, and human–animal combinations. In theory, this is an open process that might even lead to an expansion of the moral community, a greater tolerance and acceptance towards different life forms, etc. (taking an optimistic starting point). However, it is hard to assume that we will be able to cope with the cataclysm that such a process would involve. And as to the image of mankind evolving to even more virtuous beings, considering every life form as worthy of respect, we have to come back to the real mosaic and hybrid research, as a very gloomy starting point: scientists are creating interspecies-individuals (or someday new species) with the purpose of using them as resources for the interests of (some) “purely” human beings. Therefore, it is necessary that they are to be regarded as having a minor moral status. This might not encourage hopes that we are heading for men's greatest virtues. Looking at a concrete case, the example of xenotransplantation trials in animal models might be illustrative: placing a pig's heart into the abdomen of a (healthy) baboon and letting it die from (predictable) hyperacute reaction and multi-organ failure represents a brutal act – whether justifiable or not (see, for example [31]).

But even if one does not want to forecast a dystopian or utopian future, it is hard to deny the relevance of references to the human species for all kinds of moral convictions and legal regulations. Legal regulations sort out something concerning the “human being” (human rights are, for example, ascribed to “human beings”). We can and probably should deny that the species-membership can provide the grounds for ascribing rights to human beings, because it would be speciesistic [32], but even then we have to refer to an idea of what is human that integrates the role of the biological species in order to apply the human rights framework.

In this context, one should also think about the relevance of esthetics for our self-understanding as human beings. One might regard some of the existing and many of the imaginable experiments in mosaic and hybrid research as simply abhorrent. The relationship between esthetics and ethics is, of course, too complex to allow simple normative conclusions from such arguments [33]. The fact that people react with disgust is as such not a normative argument. But is it possible that these feelings are not just yucky feelings but articulate an important part of the esthetic experience of other human beings? In interpersonal relationships, it is important that human beings do not meet each other as pure intellectual entities but as human beings who perceive each other by their senses and who perceive each other always in esthetic categories as well. For this sensual and esthetic experience, it is – so far – important to experience each other with some kind of similarities. Through our experiences, we are able to deal with differences between human

beings of another gender, another skin color, or with beings who have some kind of abnormalities, such as having no arms, no legs or the like. But we are able to recognize human beings with the face of a human being, and so far that is valuable for us. If we would live in the world of Star Trek, things would be different and experiences may differ in the future as well. Until now, a theory of the relevance of the biological species for the application of moral or normative categorizations has been missing. The relevance of the species for our self-understanding as human beings, as beings that articulate respect to each other and that interpret themselves as biological beings, seems to be an important topic for ethical considerations. The creation of a human–animal chimera may affect this self-interpretation.

This shows, perhaps, the most challenging aspect of the topic for the ethics of the life sciences: the relationship between biological and normative concepts becomes more and more contested and vague. To talk about “the human” has always been a statement, where biological and evaluative aspects were clearly related. We can talk about “the human” as a biological species, like “the mallard.” But at the same time we talk about “acting like a human,” “humanitarian,” or we talk about “the formula of humanity.” In the last, we are using an evaluative or normative vocabulary. In the past, the relationship between the biological and the evaluative concept of the human was not perceived as being particularly problematic. When Kant developed his “formula of humanity” he did not have in mind that it could be particularly difficult to identify human beings in the biological world. But in the course of discoveries in the life sciences, the relationship between both areas becomes increasingly difficult and contested. A comprehensive theory of the moral relevance of the human species is necessary.

16.5.2 The Use of Human Embryos/Fetuses

The actually most struggling ethical concerns related to mosaic and hybrid research might be linked to the formation of embryonic or fetal hybrids and mosaics and the use of human embryos for this research in general. Since the developing embryo/early fetus lacks immune responses, the transplantation of “foreign” cells, mainly early embryonic cells, embryonic stem cells and somatic stem cells, is less complicated than in later developmental stages, especially after birth. Experiments with human–animal embryonic (and fetal) hybrids and mosaics face a specific dilemma between scientific interest and ethical problems concerning long-term studies, especially studies of post-natal development, could provide important scientific and clinical findings and are clearly required, but they would imply the creation of substantially chimeric and well-developed human–animals.

When addressing questions about the moral status of the early phases of human development, we face an advanced ethical debate. The ethical positions concerning the use of human embryos for research purposes are controversial [34] and there is no possibility of ignoring or compromising this disagreement. It is impossible to make any normative statement in the area of embryo research without presupposing

a substantial ethical position concerning the moral status of the embryo. One specific aspect might gain significant relevance especially for hybrid and mosaic research: since many research proposals aim at involving the use of embryos (or stem cells) at the developmental stage before implantation (zygote to blastocyst stage), it is especially important to determine whether or not the distinction between embryos and pre-embryos⁸ is morally significant. The term first appeared in the discussion on human artificial procreation and embryo experimentation in 1982 (see the Warnock report [35]) and his invention was motivated by the scientific need for clear legal regulations in these fields. The concept, still contested in philosophical debate, is based on specific criteria of individualization and bestows a lower moral status on early human embryos (if at all) (see [36], for example). In the case of chimera and hybrid research, however, this issue is somehow reversed. As explained above (1.3) the creation of human–animal chimeras and hybrids might – from a moral point of view – be even more problematic in (early) embryonic stages than in later ones. Such a view might be justified because hybridization in these early developmental stages (until the blastocyst stage) affects the entire organism and, therefore, might represent a more fundamental manipulation of an individual than later interventions. The crucial question is, at which point do quantitative changes turn into qualitative changes?

To complicate things further, we have to deal not only with the unsolved problem of the moral status of (purely) human embryos, but with the status of hybrid embryos. Some hold that interspecies embryonic hybrids (e.g., nucleo-cytoplasmatic hybrids derived from nuclear transfer experiments) are most probably not viable and the so-called “NT-units” can, therefore, not be regarded as embryos. Viability of interspecific NT-hybrids has only been achieved for closely related species (e.g., dog-wolf, or wild felids-domestic cats [37, 38]). It seems plausible that the degree of kinship between the donor and recipient species might be of crucial importance. However, as mentioned above, does the assumption of non-viability justify the creation and subsequent destruction of an – actually living – embryo that would, according to the argument, otherwise not be justified? If one day an NT-hybrid, e.g., between two different primate species, would be born, would that influence our moral perceptions of NT-hybrid embryos established from human somatic cell nuclei? Of course, any attempt to demonstrate the proof of principle by bringing human–animal hybrid embryos to term would mark the crossing of a substantial moral border and would face strong moral objections.

There are many other open questions surrounding topics such as the availability, manipulation, commercialization, and destruction of early human life. Some fear problematic changes of attitudes towards early stages of human life and the growing power to engineer mankind in a substantial physical and irreversible way – without

⁸The term pre-embryo applies to human embryos during the first 14 days of development. It is a purely philosophical and political definition. The term is, in this sense, rarely, if ever used in most biological textbooks and original research papers. The philosophical argument refers to the notion of an “undividable individual” as a morally relevant criterion. Biologically, the embryo is regarded as definitely individual after 14 days of development, when the primitive streak has formed.

having any authority to judge desirable and non-desirable characteristics. We might not be able to prevent some of the developments, but we should at least be prepared for the manifold possibilities they will provide.

16.6 Conclusion

The creation of human–animal chimeras raises a broad field of ethical questions. This chapter aimed at an inventory of important levels of discussions. Animal–human chimeras evoke questions such as those concerning the aims of concerned research strands, but it forces us, on a more fundamental level, to reconsider moral questions related to the use of human embryos, and to analyze the moral relevance of the “*species homo sapiens*” for our self-understanding as human beings. The way the term “human” is used as a biological as well as an evaluative term represents a fundamental challenge for ethical reflection. When biological research questions the biological borderline of the human species, it affects our whole thinking about the human being – that is, ourselves.

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Chapter 17

Chimeras + Hybrids = Chimbrids: Legal Aspects

Jochen Taupitz

Abstract The chapter tackles the legal issues concerning scientific research involving chimeras and hybrids. It discusses domestic and international law, the regulatory needs and challenges, as well as offering recommendations for further implementation. Especially the Council of Europe and the EU should consider appropriate methods of governance. A definite requirement concerning chimbrids related research lies in the prior review by a qualified independent body.

Keywords Human dignity • Balancing of interests • Legal regulation • European Union • Council of Europe

17.1 Introduction

Scientific research involving chimeras and hybrids raises not only scientific questions, but also questions about ethical, social and legal implications that challenge our understanding of what it is to be a member of the human species.

National, European and international concepts and strategies concerning the legal framework of this research are still largely missing, even though they are absolutely necessary in order to use the potential of chimera and hybrid research effectively and efficiently for the benefit of science and society. Accordingly, dynamic development of chimera and hybrid research creates insecurity on the part of decision makers and society at large. Public concerns, a general lack of knowledge of the scientific development in this area, and a lack of understanding of the

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potential consequences of the mixing of human and animal biological material, generate the need for both reflection and discussion in relation to the complex legal questions arising from the creation of human-animal mixtures.

One particular problem in this area is the need to clarify the terminology used. Whichever definitions are employed, the terms used will be value-laden, and this will have a deep impact on the legal aspects, since the terms that are used determine the applicability of regulatory frameworks. The traditional definition of a chimera is that of a biological unit containing cells of distinct entities. Apart from that, a hybrid is considered to be the result of interbreeding between two different species, i.e. an ovum from one species is fertilized by the sperm of another species. In contrast to a chimera, the cells of a hybrid all have the same genome. However, there are cases, such as somatic-cell nuclear transfer cloning, in which the applicability of these definitions is problematic.

A research group, coordinated by the Institute for German, European and International Medical Law, Public Health Law and Bioethics of the Universities of Heidelberg and Mannheim [1] tackled this issue and created the word “CHIMBRID” as a comprehensive term to cover chimeras, hybrids and similar genetic mixtures that are not directly covered by one of the aforementioned definitions¹.

The need to clarify the terminology used also includes common terms such as “human being” and “embryo,” our understanding of which is being challenged by new scientific developments. The term “human” is, on the one side, used as a *biological term*: so, when we speak of “human material” we mean biological material coming from a human being. However, if we speak of “human dignity” or “human rights,” we are using an *evaluative term* that grants a specific status to a being, with specific features that are typically seen as being possessed by members of the human species. In this respect, a human is someone to whom we owe respect. For chimbrids, there is the crucial problem with identifying a being as “human” or “animal” in a biological sense. We can either focus on the *biological sources* used for the creation, in which case we see that material from humans and animals are involved. Or if we start with the *features of the product*, the chimbrid entity must be evaluated in the light of an ethical theory.

First of all, it depends on whether our ascription refers to the *sources* that are used for the experiments or to the *product* as the result of the experiments. But even more important is the question concerning the kinds of criteria that we could use to determine the legal and moral status of this entity as “human” or “animal.” All regulatory frameworks are, however, essentially presupposing a dichotomy between “animal” and “human”; there are different legal regulations for humans and animals. This means, in general, that the *evaluative term* “human” (with all the moral and legal implications attached to it) is ascribed to entities that are identified by *biological* characteristics. Since the biological identification of these entities is, at least to some extent, difficult (if not impossible), the question is: “What does the evaluative term

¹Whereas chimbrids can be either interspecies or intraspecies mixtures, the project only dealt with human-animal chimbrids. Therefore, in this project, and in accordance with it, in the article at hand, the term “chimbrids” refers only to human-animal mixtures unless otherwise specified.

‘human,’ in terms of owing respect, refer to?” Chimbrids experiments therefore pose an obvious and fundamental problem in respect to the relationship between biological and evaluative notions of “human” and “animal.”

It is necessary to reflect on what the more general implications of all kinds of regulations are when considering the legal regulation and moral debate surrounding chimbrids. One can, to some extent, make smaller adaptations to the existing legal frameworks, but there is a danger of the coherency of the law being undermined if the biological and evaluative notions of “human” are treated as interchangeable. This problem goes far beyond the scope of the regulation of chimbrid experiments.

17.2 Legal Aspects

17.2.1 *The Regulatory Needs and Challenges*

17.2.1.1 Interests and Values Concerned

Chimbrids-related activities may take place in many different forms and for many different purposes. It is clear that they may in individual cases concern a wide spectrum of interests and values that are generally considered deserving of judicial protection. These include the freedom of research and the improvement of scientific knowledge, protection of public health and safety, protection of the person (autonomy, privacy/personal integrity, etc.), human dignity, and the genetic identity and heritage of future human generations. Other relevant interests to consider are related to animal welfare, species integrity, environmental sustainability and biodiversity. The appropriate balancing of such important and sometimes conflicting interests, in a manner that can satisfy basic prerequisites for the rule of law, would by necessity require the involvement of some kind of legal regulation. To the extent that pre-existing legislation does not satisfactorily cover the chimbrids area, new regulation is needed.

The issues that need to be addressed include both the permissibility of creating different types of chimbrids on different levels (cellular to living creature) and the appropriate standards for treatment and protection of the interspecies organisms and individuals that are actually created (i.e., when to use animal standards, human standards, or even a *sui generis* standard).

17.2.1.2 Some of the Challenges

The determination of appropriate criteria for the distinction between the legal concepts “animal” and “human” constitutes the principal challenge in the regulation of chimbrids. Such criteria could be based on the source of the biological materials used to create the chimbrid, the resulting biological or genetic proportions, as well as specific qualifying properties or characteristics that can be observed in the chimbrid

that was created. To what extent should different types of chimbrid creations be placed within the regulatory framework applicable to humans or that which is applicable to animals? Or should new legal standards be created for some chimbrid categories? Achieving a well-balanced regulation of this complex issue, which may also have implications for fundamental perceptions of our identity as human beings, must be recognized as a demanding task. It could be argued that we should not put ourselves in a position where uncertainty may arise as to whether the chimbrids that are produced should be defined as human beings or animals.

Another difficulty, although a more general one, arising in many regulatory projects dealing with rapidly developing areas, concerns the lack of knowledge regarding both the potential future value of, and the risks involved in, chimbrids' activities. The necessary balancing of interests will therefore (to some extent) amount to a balancing of two unknowns, making it virtually impossible to reach any well-founded conclusions on proportionality. Rapid development also calls for flexibility, which could mean that the legislation should be subject to regular or even continuous revision, based on step-by-step consensus on scientific knowledge and sufficiently informed public debate.

Since the field of biomedicine is highly internationalized, the consequences of scientific tourism (i.e. researchers avoiding restrictions by moving their activities to more liberal countries) also need to be observed. Even so, it would prove very difficult, if not impossible, to reach an agreement on a uniform chimbrids regulation acceptable to all countries concerned. In matters so influenced by the cultural and religious pluralism of values and norms, any more widespread consensus on all chimbrids-related issues would seem highly improbable within the foreseeable future, should it be considered desirable. The diversity of national legal approaches also points to, and is influenced by, the pluralism of legal traditions; while this issue is of less fundamental importance, it may still prove problematic in certain areas.

Terminological ambiguity has already rendered the task of determining the extent of both agreement and disagreement in this area problematic, since basic concepts such as "embryo," "pre-embryo," "chimera," and "genetically modified organism" are by no means interpreted or used in a uniform way. This problem, however, is one that can be addressed and handled. Even without any normative consensus between different jurisdictions, it should be possible to clarify the relevant definitions in order to avoid misunderstandings and facilitate the identification of relevant legal differences.

17.2.2 Regulatory Tools and Strategies Applied

When the need for new legal regulation is considered, there is always the question of legal competency and the appropriate level of the regulation that must be answered first. The level of the regulation of a certain issue decisively depends on the level of public interest in it. Therefore, an issue might be of such a global interest, that its protection might be warranted only on the level of the international public law. For other issues, a supra-national, national regional or even lower level of

protection might be sufficient and more appropriate. Accordingly, the level of the protection would depend on the chosen regulatory instruments that often have very differing legal effects.

17.2.2.1 Public International Law

At present, no binding documents in public international law explicitly regulate chimbrids. The areas of assisted human reproduction and scientific research involving humans, including embryo protection, are subject to some international regulation (primarily European/regional), as are the environmental aspects on genetically modified organisms and issues related to animal welfare. In principle, these regulatory documents focus either on humans or on animals and do not specifically address human-animal mixtures.

Generally there is no universal consensus on chimbrids-related research. The only practice that is being considered contrary to human dignity, and therefore reflected in a number of different international instruments [2-5], calling for the member states to prohibit it, is the reproductive cloning of human beings. Even though these regulations were passed having the human-human cloning constellations in mind, they may be applied to interspecies constellations as well.

There are also numerous non-binding international declarations, guidelines and other soft-law documents that deal with scientific research, genetics, human reproduction, etc., but for the most part, these do not address chimbrids issues either. Xenotransplantation is, however, an exception: several international and European policy documents have been produced on this area [5].

17.2.2.2 EU Regulation

Explicit chimbrids legislation is also lacking at EU level. This is not very surprising, however, since the regulatory competency of the EU is restricted in many ways and in principle does not cover areas such as national health care policies (including assisted reproduction) or ethical aspects of scientific research – for example, the use of human embryos for research purposes. Nevertheless, chimbrids-related activities may still be covered by more general EU legislation concerning, for example, clinical trials, medicinal products, public health, protection of the environment, animal welfare, etc.

17.2.2.3 Domestic Law

From the country reports and comparative reports produced within the aforementioned CHIMBRIDS project [1], it is clear that the developments and contents of national law in the area of chimbrids vary considerably.

Looking at the countries that do have chimbrids specific legislation, quite a few of them would seem to have applied a step-by-step or ad hoc approach, resulting in sometimes detailed, but not necessarily comprehensive regulation. In others, e.g.,

Japan, a more systematic approach can be seen. Lack of chimbrids-specific regulation, on the other hand, may in some cases also be attributed to deliberate strategies, based on a wait-and-see approach or no-need/non-exceptionalistic policies. Nevertheless, it is clear that to some extent, the omission of addressing chimbrids issues is also due to ignorance or lack of foresight on the part of policy-makers.

The existing chimbrids-specific domestic regulation can be divided into those addressing xenotransplantation and those related to other ways of creating chimbrids, e.g., by the involvement of gametes. The crucial definition of human vs. animal has not been legally regulated in any of the participating countries, nor the standards according to which a chimbrid creation should be treated.

Legislation on xenotransplantation seems to exist only in France [6] and Switzerland [7]. A number of countries have investigated the matter, and in several there are formal, non-binding guidelines, for example, in the UK, the USA² and Israel,³ whereas others have reached no conclusive decision (e.g., Sweden). Accordingly, in most countries xenotransplantation is only covered by general legislation concerning, for example, patient safety and public health, transplantation, scientific research involving humans, animal welfare, or genetically modified organisms. In general, the attitude towards xenotransplantation must be considered to be cautious rather than liberal, primarily due to unknown risks related to public health.

Several countries have legislation that to some extent explicitly addresses the creation of chimbrids by direct or indirect use of gametes – for example, Canada, Germany, Japan, Spain, Switzerland, and the UK. In China, governmental guidelines exist, whereas no explicit regulation at all has been found in Austria, the Czech Republic, France, Sweden, Israel, or the USA. In these countries, such activities may still be covered by general, more or less detailed, rules on research involving human gametes or embryos, as well as animal welfare legislation, etc.

Many countries thus have protective legislation concerning the use of human embryos, and there are also domestic laws prohibiting or restricting the creation of hereditary genetic modification in future human generations. The approach in these regulations is in some cases very restrictive (for example, in Germany) whereas in others more liberal (such as the UK and Sweden).

However, the definitions of fundamental concepts such as “human,” “fertilized ovum,” “embryo” or “functional embryo” are not always clear, and may still be decisive as to whether or not a certain procedure is lawful.

²In the USA, xenotransplantation is governed by the Food and Drug Administration (FDA) through case by case decisions. FDA-promulgated non-binding regulations “Source Animal, Product, Preclinical, and Clinical Issues Concerning the Use of Xenotransplantation Products in Humans.” Available from: <http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Xenotransplantation/ucm092707.pdf>^f

³In Israel, the Director-General of the Ministry of Health issued a circular on xenotransplantation in August 2000, followed by detailed guidelines promulgated in February 2002 and expressly designating xenotransplantation as a clinical experiment, which is subject to the regulatory regime established by the Public Health (Medical Experiments on Humans) Regulations (1980).

Although certain chimbrids-related activities may be explicitly prohibited in some countries, the most common regulatory approach would seem to involve framework legislation with complementing procedural rules, where different agencies are authorized to make decisions based on case-by-case assessment. The countries represented in the CHIMBRIDS project show a large diversity of such decision making bodies entrusted with – in some cases considerable – discretionary powers to decide on chimbrids-related matters. They thus range from the national to the ministerial and local level, and may be appointed to address very specific issues, such as the use of human embryos, or far more general areas of research or environmental issues. Although in most countries, chimbrids research is subject to prior review by several different agencies that protect interests related to humans, the environment, or animals respectively, the decision making bodies do not seem to be equipped to address and balance all the relevant interests.

17.2.3 Conclusions

Chimbrids activities constitute an area of biomedicine where fundamental interests are at stake and where our traditional perception of human identity is challenged. In such a field of research, it is of the utmost importance that any development take place in openness, in order to increase general knowledge and awareness of the potential benefits and risks involved, and to stimulate public debate. Appropriate public discussion and consultation is also a requirement laid down in Article 28 of the Council of Europe Convention on Human Rights and Biomedicine, with regard to fundamental questions raised by the development of biology and medicine.

Although there are obvious obstacles to any comprehensive international consensus in the field of chimbrids-related activities, the discussion of chimbrids issues should be brought to the international arena and the possibility of international action assessed. Since the international regulatory tools available range from binding international law to documents serving only as sources of inspiration, international organizations may also play an important role in education and public debate. The Council of Europe Recommendation Rec (2003)10 on xenotransplantation is an example of such a non-binding document that could serve as a model for regulation. Due to the partly voluntary character of public international law, the varying traditions of implementation in domestic law and the limited access to effective international sanctions, however, the responsibility to offer appropriate judicial protection in the area of chimbrids will obviously be found at the national level.

The European Union has limited competency and legitimacy to regulate in the chimbrids area, with the exception of issues related to, for example, the fundamental principle of free movement, consumer safety, and the protection of public health. For example, the upcoming Directive on advanced therapies deals with chimbrids' products for human application, and it is possible that cross-border public health issues could be raised by xenotransplantation. Nevertheless, the legal regulation of chimbrids research cannot primarily be seen as a task for the EU. However, this

does not exclude the possibility of the EU using other forms of governance to influence research development in this area, for example, by way of funding requirements.

At the national level, it may be appropriate to prohibit certain chimbrids-related activities, with or without the possibility of exemptions (by way of special authorization, in exceptional cases, for certain purposes, etc.). Detailed regulation may be needed with regard to issues involving particular risks (such as human reproduction, xenotransplantation and medicinal products). In other areas, it may be considered sufficient to make the activities subject to certain restrictions or conditions (notification, procedural assessment, approval or licensing, or substantive conditions such as a specified purpose). In order to provide sufficient flexibility, the regulation should not focus on certain techniques or methods, but primarily on results and risks to be achieved or avoided. It would seem to be an appropriate minimum requirement, however, that all chimbrids related research be subjected to some kind of prior review by an independent body qualified to address both general and chimbrids' specific considerations.

Regulation primarily focused on procedures rather than fixed material rules will clearly provide more flexibility. Although this may be considered an advantage, a very generous delegation of powers could endanger democratic values in a way that is particularly problematic in areas where important interests may be at risk. Too wide a margin of appreciation may result in poor predictability and thus conflict with the rule of law. It is also more difficult to achieve uniform application if case-by-case assessment takes place at the regional or even local level. This means that the discretionary powers left to lower-level decision making bodies should be very carefully considered and not only restricted by appropriate legislative frameworks, but also complemented by official guidelines, etc.

With flexible rules, the qualifications and legitimacy of the bodies making decisions in individual cases thus become increasingly important, and the need for public oversight and openness in the decision making procedure, as well as the possibility of appeal. Overlapping competency between different decision making bodies may result in rivalry or, quite the opposite, leave so-called orphan issues.

A regulatory system must also provide tools for monitoring and controlling the development in chimbrids research and applications. Such tools traditionally include, for example, the appointment of supervisory agencies, requirements for notification, follow-up, reporting of adverse events, etc., as well as appropriate legal sanctions to be applied in case of unlawful activities.

17.3 Relationship Between Ethics and Legal Regulation

It seems quite clear that not all kinds of moral considerations can be directly implanted into legal regulations since not everything that is morally problematic must directly be prohibited by law, and not everything that is legally permitted is morally unproblematic. Nevertheless, the legal order in modern democratic states

presupposes that there are fundamental starting points that are morally acceptable. For example, the moral codification of human dignity, individual rights and the intrinsic value of the animal all represent the legal order directly referring to fundamentally important moral principles or values. It is therefore a necessary part of the discourse concerning the legitimacy of the legal order to explain and justify their fundamental starting points. Therefore, while the ethical debate is of central importance to the legal regulation, a simple model of the law as a straightforward codification of morality should be avoided.

17.4 Recommendations of the CHIMBRIDS Project

The participants of the aforementioned CHIMBRIDS Project formulated the following practical recommendations that might be help for decision makers [1]:

- Chimbrids research should only be conducted following careful consideration of its scientific merit, human research ethics, animal ethics, legal aspects and societal and environmental implications.
- States should initiate public discussion and conduct public consultations regarding the complex ethical and societal issues raised by chimbrids research and application. States should also examine their existing regulations to evaluate the adequacy of current laws or guidelines. States should then assess whether there is a need for further legal regulation.
- Because of the international dimension of chimbrids research and application in biomedicine and biotechnology, there should be an assessment of the need and possibilities for action at the international level, including regulation. In particular, the Council of Europe and the European Union should consider appropriate methods of governance within their respective competencies.
- Members of the scientific community should actively engage in a public discourse concerning their work. They should also organize a discussion amongst themselves, on an international level, with regard to chimbrids research concerning the aims, motivations and implications of their work, including ethical and societal ramifications.
- Research projects that aim to create chimbrids should be subject to an independent examination by an interdisciplinary body. Careful attention ought to be given to the composition of these review bodies to ensure that they are competent to assess the project based on consideration of its scientific merit, human research ethics, animal ethics, legal aspects and societal and environmental implications. States should determine to what extent this review should be legally required or binding, and whether exemptions might be justified for specific subcategories of chimbrids research on the grounds that they present no significant issues in terms of the considerations mentioned above.
- When considering chimbrids research, there must be a systematic examination of the way in which the terms “animal” and “human” are used in regulatory frameworks.

There is an ambiguity in these terms. For example, on the one hand, “being human” is used to describe morally relevant characteristics or other evaluative aspects (a normative term), while on the other, this term is used to describe the biological origin of specific material (a biological term). The distinction between these uses must be transparent and unequivocal.

- Assessment of chimbrids experiments should take into account the origin of the biological material and the procedure as well as the attributes of the resulting entity. The characteristic ethical issues raised by chimbrids research concerns the nature of the entity resulting from the experiment.
- The ethical issues surrounding the incorporation of animal biological material into an existing human organism depend on the degree to which alteration might have effects on features of the existing or future person concerned, insofar as they are typically considered to be human (appearance, behavior, cognition, intellect, emotion, senses, abilities, etc.). Likewise, the ethical issues surrounding the incorporation of human biological material into an existing animal organism depends on the degree of possible “humanization” of the existing or future animal; the greater the probability of “humanization” of animals and “animalization” of humans, the stronger the need for restrictions. If the relevant knowledge is not available, this would be a reason for exercising precaution. As the humanization of animals or the animalization of humans is problematic, the creation of entities that will express such effects must also be governed by these principles. Although there are certain cases in which a prohibition is required, circumstances can be imagined where such a prohibition has to be reconsidered and regulatory frameworks have to provide mechanisms for reconsideration and/or exceptions.
- With regard to animal-into-human-xenotransplantation, the Council of Europe Recommendations on xenotransplantation should be followed.
- Whenever chimbrids create risks similar to those involved in xenotransplantation, equivalent safeguards should be applied.
- Chimbrids created for and/or used in a reproductive context raise additional issues compared to other uses; this should be given appropriate consideration.
- Because of the gravity of the ethical and legal issues involved in chimbrids research when embryonic stages of humans are involved, such projects, whenever permitted, should be subjected to legally required supervision.
- Projects which include the incorporation of animal material into human embryos, fetuses or post-natal beings that are likely to affect the genome of descendants should be prohibited. If scientific evidence becomes available that demonstrates that the risks are predictable and if the risks are ethically justifiable, the prohibition should be reconsidered.
- Careful monitoring is required for projects in which the incorporation of human material into animal embryos, fetuses or post-natal beings is likely to affect the animal’s germline because of the potential risks to, for example, human health and the environment, and the specific risk of a possible development of human gametes in an animal.
- Accordingly, given the principles laid down in the previous recommendations, the following cases need special consideration:

- Incorporation of human pluripotent cells into an animal blastocyst or into its preliminary embryonic stages;
 - Incorporation of animal pluripotent cells into a human blastocyst or into its preliminary embryonic stages;
 - Mixing human and animal gametes;
 - Mixing of animal and human totipotent cells/embryos.
- The application of the principles laid down in recommendation 8 suggests that the subsequent transfer to a foster mother (human or animal) or equivalent means of gestation be prohibited.
 - The insertion of a human cell nucleus into an enucleated animal egg, followed by the transfer to a foster mother (human or animal) or equivalent means of gestation is a type of reproductive cloning and therefore should be prohibited.
 - The insertion of an animal cell nucleus into an enucleated human egg should be prohibited if followed by the transfer to a foster mother.
 - The transfer of a human embryo into an animal should be prohibited.
 - The transfer of an animal embryo into a woman should be prohibited.

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Part IV
Stem Cell Banking for Translational Stem
Cell Research or Stem Cell-Based
Therapies

Chapter 18

Stem Cell Banks: Reality, Roles and Challenges

Glyn Stacey

Abstract Human embryonic stem cell lines have the capability to develop into cells representative of the three germ layers of the human body, which has raised the possibility of exciting new opportunities for *in vitro* research and the development of therapies for the future. However, it is vital that in these pursuits researchers are aware of certain critical issues in sourcing cell lines to ensure that their work provides data of a high standard and does not lead to confusion and that any future use of the cells – such as clinical application – is not compromised by the conditions under which the cells are developed. Stem cell banks have been much discussed in the literature and would appear to have a significant role to play in this important area of endeavour. This chapter places the current development of stem cell banking against the history of bioresource centres and culture collections. It also identifies the different types and roles that stem cell banks may have and as a case study describes the particular remit of the UK Stem Cell Bank and how this influences its policies and procedures. The author goes on to explain the importance of careful development of cell line procurement procedures, the need for standardised banking protocols and the challenges for appropriate supply of pluripotent stem cell lines. In conclusion, the author outlines the importance of international collaboration between the developing network of stem cell banks giving examples of standards (e.g. www.stemcellfoun.org/) that may develop from such activity.

Keywords Cell banking • Quality assurance • Governance • Stem cell lines • Quality control

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18.1 Introduction and Historical Perspectives on Collections of Biological Cultures

Human embryonic stem cell lines have the capability to develop into cells representative of the three germ layers of the human body, which has raised the possibility of exciting new opportunities for *in vitro* research and the development of therapies for the future. However, it is vital that in these pursuits researchers are aware of certain critical issues in sourcing cell lines to ensure that their work provides data of a high standard and does not lead to confusion and that any future use of the cells – such as clinical application – is not compromised by the conditions under which the cells are developed. Stem cell banks have been much discussed in the literature and would appear to have a significant role to play in this important area of endeavor.

The work of a European project, hESCreg, has shown that internationally there is now thought to be a resource of hES cell lines in excess of 600 (www.hescreg.eu) [1], which should service the needs for most areas of research using these cells. However, as identified in the hESCreg work, not all of these lines are published, the methods of derivation vary, and the degree and nature of characterization varies significantly between cell lines. Also, the culture of animal and human cells *in vitro* is prone to the adverse effects of microbiological contamination, cross-contamination, or culture switching and instability [2, 3]. In the selection of human stem cell lines there is a range of criteria that should be addressed to ensure that cells used in research are of an appropriate quality or, in other words, are fit for purpose. The justification for these criteria and recommended approaches to deal with them have been dealt with elsewhere [4], and this includes the recommendation for researchers to obtain their cell lines from qualified sources such as bioresource centers or “stem cell banks.” In this chapter the author will review some of the key challenges for stem cell banks and what role they have to play in support of the regenerative medicine community and other researchers.

18.2 What Is a Stem Cell bank?

The principal of obtaining qualified biological cultures from central qualified sources has been an industry standard for the reliable and efficient delivery of biotechnology products for many years, and establishment of the first “bank” or collection of microbial cultures is widely accredited to the Czech scientist Kral in the late 1890s. Over this extensive history of development, certain principles and best practices have been developed for the establishment and operation of “cell banks,” and today there are international networks of culture collections and bioresource centers that serve various aspects of research and development, such as the World Federation of Culture Collections (<http://wdcm.nig.ac.jp/wfcc>),

ECCO (www.eccosite.org), the International Society for Biological and Environmental Repositories (ISBER, www.isber.org), and the Federation of International Mouse Resources (www.emmanet.org). Physically, a cell bank can be described as a stock of cells frozen in a viable state, which can provide reproducible stocks of cells for future use. This has been developed in industry as the master-working bank principle (see Fig. 18.1), whereby an early passage master bank is established, which is extensively characterized and safety tested, and then subordinate stocks called working cell banks are established from the master cell bank to provide a reliable stock of starting cultures for manufacturing processes [5]. The types and level of work involved in establishing and maintaining a cell bank of mammalian cell lines will vary significantly depending on the remit of the host laboratory and the intended use for the cells, although there are fundamental elements of best practice that apply in most cases [6, 7]. Banks of cells intended for clinical applications or commercial manufacturing purposes will have very different requirements and resource issues compared to those for general research purposes as illustrated. Even banks of cells provided for research will vary, depending on the number of labs to be supplied and whether the bank is intended to be a local, national or international resource. The effect of these differing roles means that while there are often what appear to be common

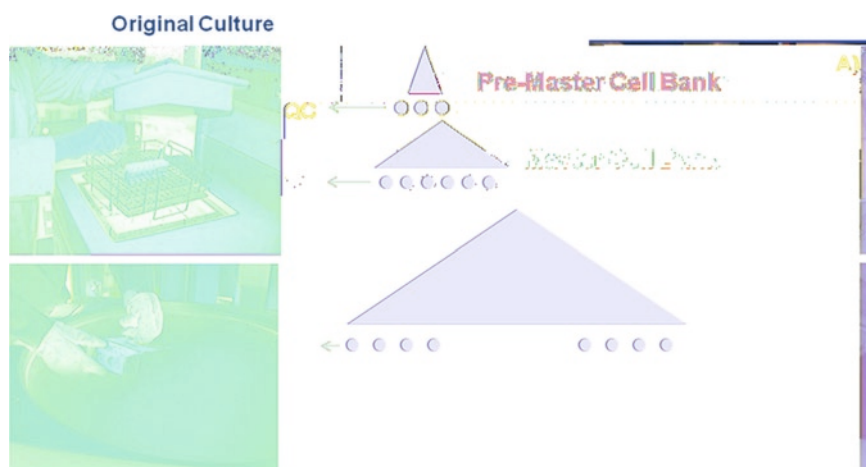


Fig. 18.1 The tiered master- working-cell bank system. The size of each cell bank (i.e. number of containers of cells) are often different ranging from 5 to 10 for a pre-master stock to 100s for a master or working cell bank. The level of quality control and characterization will also vary between the different banking levels; typically with minimum testing on the pre-master (e.g., viability test, contamination checks), extensive testing on the master cell bank and an intermediate level of testing on the working cell Bank. During the preservation process batches of consistently prepared aliquots of cells must be cryopreserved under the same conditions and transferred to a stable ultralow temperature environment (vapor phase of liquid nitrogen). In (a) a bank of cells are about to be cryopreserved in an automated controlled rate freezing device, and (b) shows transfer of the cryopreserved cells into a liquid nitrogen refrigerator for long term storage (Pictures and diagram courtesy of G Stacey, NIBSC-HPA)

elements to the activities of different banks, the level and nature of these activities vary significantly, depending on the range and requirements of customers as illustrated in Fig. 18.2. Accordingly, the aims of each “bank” need to be absolutely clear at the outset, otherwise “mission drift” could lead to the bank’s output being incompatible in terms of quality and/or capacity, with the intended use of cultures supplied, and ultimately to failure.

In the case of the UK Stem Cell Bank (UKSCB), its ethos was initially established through a series of fundamental operating principles:

- Directing transparent operation (through its code of practice and review by its sponsors and oversight body – see below)
- Being responsive to requests for information from the public and media
- Sustaining close working relationships with leading scientific groups and regulators, and
- Avoiding conflicts of interest that could arise from engaging in fundamental stem cell research and near market product development.

The Bank has also considered it vital to establish high-level strategic goals based on the value of the bank to its stakeholders, which state that the UKSCB will be:

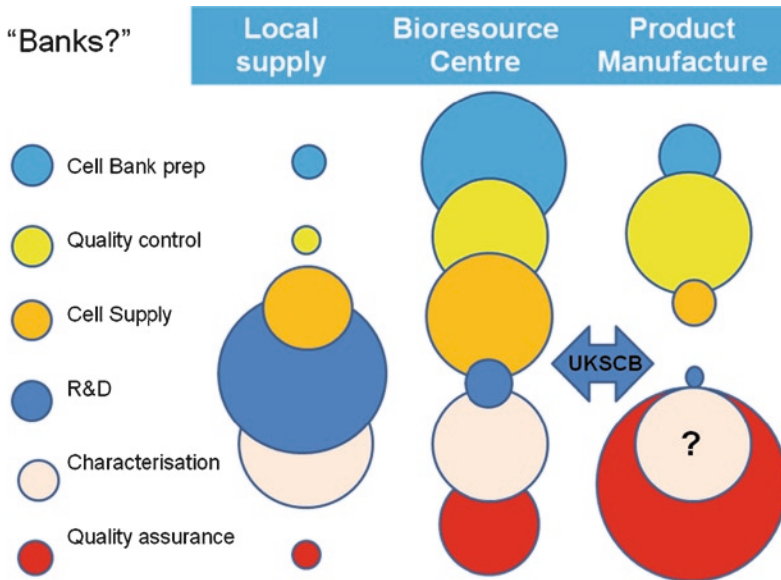


Fig. 18.2 Common functions of stem cell banks. Some of the key core activities within banking centers are shown with a generic estimation of the level of staff activity and resources applied for each in Banks with different remits. The resource available for R&D becomes reduced as the group increases its focus on distribution which requires increasing quality control and overall quality assurance. For banking aimed directly at products there is a similar trend but here it is driven by the need to provide cells of a certain specification into a repeated manufacturing process rather than providing consistent quality cells to a wide range of different users. In both cases it is important to address the need for appropriate quality standards to assure the reliability of output

- An efficient, customer-focused reference laboratory providing the most useful resource in the world for quality-assured stem cell lines, materials for their growth and characterization, and related information
- The primary international source of expert advice and the development of best practice in cell banking processes
- An independent, ethical organization that has demonstrably facilitated the clinical development of stem cell therapy, and
- Acknowledged by all stakeholders, including the public, as having achieved these aims.

These goals have been translated into a 10 year strategy (see Appendix) that forms the over-arching structure under which the Bank formulates its work programs and initiatives. These specific modes of operation, goals and strategy for delivery have been key to giving the Bank staff, collaborators and others a clear direction on how the Bank can operate and where it is going, and to promote its development as a focused and successful neutral partner in a highly dynamic environment of stem cell research and regenerative medicine.

The governance applied within the UK Stem Cell Bank has been driven by the conditions of its inception, which required a system that would address specific public concerns relating to the use of human embryos for research and the need to facilitate public confidence in the ethical robustness of stem cell research. Other centers distributing stem cell lines operate different principles of governance, although it is possible to identify core consistencies within the different models used (see International Coordination below). It is helpful for customers to make informed choices if each operates under its own published standards of operation or a Code of Practice (e.g., [8]).

18.3 “Quality?”

Where a bank is supplying many different kinds of customers for different purposes, the perceptions of quality are inevitably diverse and for many researchers will focus on speed of delivery and ease of growth of the cells. It is important that each bank establish its own specification for the quality of the cells it provides and makes these clear to customers. There is sometimes a misunderstanding that “quality” and “quality control” are much the same thing. Quality control refers to a series of checks and tests performed at different points in any process to ensure that an acceptable product arises from the process. The specific measures of these features are captured in a range of quality control, characterization and safety tests applied to samples from the cell banks, and these have been reviewed elsewhere [9, 10].

However, this is only one component of the overall quality assurance for stem cell banks, which includes:

- Evaluation and quality control measures for cells and critical reagents coming into the laboratory, which includes traceability for appropriate informed consent for the donor of the original tissue,

- Control of the laboratory environment, equipment and procedures,
- Control of data arising from cell culture, and
- The delivery of research materials, including cells, to other laboratories.

Where a stem cell bank intends to supply for use in humans, it will be expected to ensure that appropriate validation has been performed for all facilities, processes, cleaning and analytical methods. This will require careful specifications for the expected output from each of the elements to be validated.

For human stem cell lines, there are certain core technical quality criteria to address for general biomedical research purposes and these revolve around the fundamental characteristics of purity (absence of contamination with microbial organisms or other cells), and identity (the genetic identity of the cell line is consistent with its purported cells of origin).

It is clear that “quality” is not a clear and universal set of characteristics of stem cell lines since it depends on the perspective from which it is viewed and the intentions of the individual bank in relation to the type and level of quality assurance to be provided.

18.4 Models for Provision of Stem Cell Lines

Naturally, the early provision of hES cells for research has been led by the originators of the lines. NIH funding promoted the early exchanges of the cell lines from different suppliers of cells posted on the NIH registry. The UK Stem Cell Bank was established in 2003 at the National Institute for Biological Standards and Control by the Medical Research Council and the Biotechnology and Biological Sciences Research Council to provide a public service collection for sourcing human stem cell lines (www.ukstem-cellbank.org.uk). WiCell, which held the grant for a national repository until 2009, is a long-standing supplier and continues as a private bank to distribute hESC and induced pluripotent stem cell (iPSC) lines (www.wicell.org). Other organizations with substantial numbers of cell lines, such as Cellartis (www.cellartis.com), provide cells on a commercial collaborative basis.

It is likely that these models will continue to operate in parallel, but it will probably be difficult for any organization to sustain a business model primarily based on supply of cells; each stem cell bank will need to provide other added value activities that enhance the value of the cells themselves for specific purposes or deliver services and products that can find a market in the research, health service or commercial communities.

18.5 Benefits of Stem Cell Banks

It is only natural that the scientists deriving cell lines will want to facilitate exchanges of cells in their early collaborations. However, in the longer term, as a broader spectrum and larger user group develops, significant benefits can be had by both originators

and collaborators through provision of cell lines from resource centers specifically established for the purpose of enabling access to quality controlled stem cell lines. These benefits include:

- Safe storage of cells for depositors in case of local storage failures,
- Depositors released from the burden of culture expansion, preservation and distribution and technical backup,
- Standardized transfer agreements that can save time for depositors and recipients, avoiding separate negotiations for each request for cells,
- Consistent banking and quality control measures to enhance reproducibility and standardization between research groups,
- Management of the cell banking process is not at risk of compromise due to competing pressures to sustain a research output,
- A growing body of knowledge on the performance and features of a range of stem cell lines to be provided as advice to users of the bank, and
- Patent repository facilities (such as an international depositary authority registered with the World Intellectual Property Organization Budapest Treaty (1977) (<http://www.wipo.int/treaties/en>))

These are in addition to the potentially significant role that stem cell banks can play in acting as custodians of the ethical status of the cells they curate as already discussed.

18.6 Assuring Appropriate Procurement of Cell Lines

Established stem cell banks will also have an obligation to manage any issues that arise from the nature of agreements under which they have obtained the cells, any constraints on the use of the cells including those imposed by the donor of the original tissue, and for international transfers to ensure that cells are supplied within the receiving laboratory's local legal and regulatory requirements. A commitment to ethical review of donor consent and for some banks, such as UKSCB, to review recipients' use of the cell lines is also an important factor in enabling stem cell banks to give public confidence in research within this field. This has been and will continue to be an important factor in sustaining public, and therefore political, support for stem cell research. In the USA, the NIH has recently established new criteria and a review process for registering hESC; lines as acceptable for use in federally funded research (<http://stemcells.nih.gov/research/registry>). The criteria are broadly consistent with generic standards for consent established in the UK for hESCs [11].

In the case of the UK Stem Cell Bank, both processes of depositing and obtaining embryonic stem cell lines from the Bank are reviewed from an ethical and technical perspective by an overseers' body, the "Steering Committee for the UK Stem Cell Bank and for the Use of Stem Cell Lines" (the process is summarized in Fig. 18.3). This process, which applies to all hESC work in the UK (not just the UK Bank), is intended to assure the public that any stem cell lines derived from human embryos are both derived from sources with appropriate informed consent and that they are only used in projects that meet UK standards of ethical review. Having established

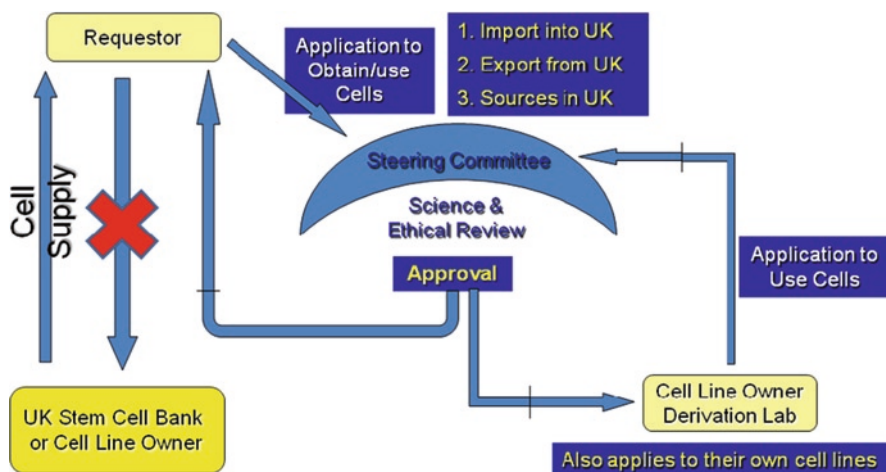


Fig. 18.3 An example of bank oversight on cell line deposit and release. Role of UK Stem Cell Bank oversight body in evaluation of hESC cell lines deposited in the UKSCB and any cell lines moved between Institutes or projects. The “Steering Committee” reviews all applications to deposit cell lines and determines if the consent procedure is consistent with current UK standards. It also reviews applications to obtain cell lines from the Bank to ensure that the intended use is focused on serious human health issues and has been subject to appropriate ethical review. The Steering Committee also approves any transfers of the cell lines including import and export and reviews all new project work including that proposed by the originators of the cells (Courtesy of C Hunt, UKSCB, NIBSC-HPA)

Steering Committee approval for applications to deposit or access cell lines, the Bank is then charged with ensuring traceability relating to informed consent for each lines and also to obtain feedback on use of the cells, which is a requirement of the access agreement signed by all recipients (Fig. 18.4) (www.ukstemcellbank.org.uk).

18.7 Standardization of the Banking Process and Good Cell Culture Practice

A primary technical aim of any stem cell bank should be to minimize the likelihood that the banks own culture and preservation processes do not cause any change in the cell line. It is therefore to characterize the cell lines to assess any change in the cell cultures, genetically (e.g., chromosomal stability) or phenotypically (e.g., consistent expression of stem cell-associated markers), but also to attempt to minimize variation within the cell culture environment by standardizing the performance of equipment and culture processes. In particular, variations in the gaseous environment and temperature or in the way cells are manipulated can significantly affect their growth and characteristics.

The documentation of procedures under standard operating procedures (SOPs) are an important element in reducing this variation, as is the review of procedures

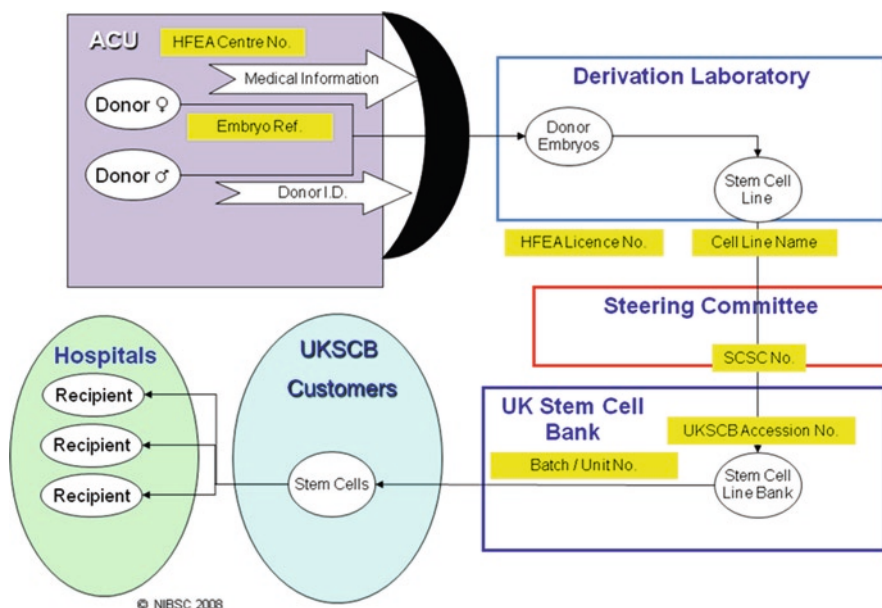


Fig. 18.4 Two-way traceability for hESC lines. The UK Stem Cell Bank Assures traceability of all cell lines to fully informed consent through a series of reference numbers shown (shaded) in the diagram. This system operates an anonymized link at the stage when the embryo passes out of the assisted conception unit (ACU) which prevents donor identity and certain detailed medical information from being made available to others (black crescent). This system also permits recognized incidences of post donation disease (such as TSE) to be tracked to prevent a cell line derived from their donated cells from being released to future clinical trials. In a similar way serious adverse events involving cell lines used in clinical trials could be tracked back through the banking procedures and theoretically feedback to the donors via the anonymized link (Figure Courtesy of C Hunt, UKSCB, NIBSC-HPA)

through the auditing of records and quality control data. As already indicated above, validation of methods, equipment and processes will also be required under regulatory requirements for clinical applications.

18.8 Challenges for Appropriate Supply of Stem Cell Lines

Some banks may wish to implement classification of users for charging policies or allocating different types of transfer agreement. However, such classifications can be difficult to determine in some cases, as academic use may be closely associated with spin-out companies operating in the same laboratory environment. Some uses, which do not appear at first evaluation to be “commercial,” may be involved in a commercial process such as product testing. This is an active area of research using

pluripotent stem cell lines, and while early method development falls under the umbrella of general research, the use of cell lines in commercial testing procedures for testing commercial products or in their development may be considered to be “commercial” applications.

Further complications arise where a cell line supplied under an existing agreement with a depositor changes ownership or where new regulation or law comes into place that might potentially invalidate the transfer agreement for its intended purpose. It is therefore important that the stem cell bank should address the need to review its agreements with depositors and its transfer agreement for recipients to evaluate any external changes that may impact on them.

Another generic issue for the supply of stem cell banks is the potential for adverse patents on the cells, or a process that a recipient of the line wishes to use the cells for. It is not possible for a small organization to be able to resource robust patent searches on a routine basis and it is beyond the scope and resource of many organizations, and is certainly true for an individual stem cell bank, to assure that there are no patents that could affect the users of the cells at the time of supply or in the near future. It is wise for the stem cell bank to address this issue in their agreements with the recipients of cell lines as they should be aware that this could potentially impact on their work. However, at least in some countries, there are specific arrangements to avoid this impacting on basic research with no commercial intent, as established in the USA.

18.9 International Coordination and the Future

Resource centers disseminating cells to other groups have a responsibility not to distribute cells that are unfit for research purposes (e.g., cross-contaminated, infected with microorganisms, absence of appropriate donor consent) and should meet the basic requirements of current best practice. A collaboration between more than 100 scientists and stem cell banks (the International Stem Cell Banking Initiative, www.stemcellforum.org) has produced an international consensus on best practice for banking hESC lines, and its fundamental criteria are generally applicable to any other type of pluripotent stem cell line [12] for public biological resource centers. This focused on the procedures required for procurement, banking, testing and distribution, but also emphasized the need for banks to promote access to stem cell lines and dissemination of current best practice. A further important theme in this guidance was that it is especially important for stem cell banks to sustain their operations in the light of current best scientific practice as exemplified by outputs from international collaborations such as the International Stem Cell Initiative [13, 14]. Guidance at the patient therapy level is beyond the scope of this chapter but is developed at the national level and is also contributed to by international organizations such as scientific and professional societies’ (e.g., AATB, EBMT, ISCT, ISSCR) criteria.

18.10 Appendix 1. Strategic Aims of the UK Stem Cell Bank

The mission of the UK Stem Cell bank is to assure the quality of stem cells for the scientific and clinical community and the strategy to achieve this will be to:

- “Focus on banking and supplying tissue specific and pluripotent human stem cell lines that are required, or are likely to be required, for stem cell research and development;
- Provide associated reference reagents and materials to assist with their growth and characterization;
- Store all embryonic stem cell lines submitted to the Bank as required by UK legislation;
- Develop and make readily available a repository of useful technical information to assist the stem cell R&D community;
- Implement and maintain quality assurance systems that are fit for...
 - Cell lines with significant potential for clinical development, to ensure as far as possible that they are developed in a way that will not compromise their future use for sustained human therapy,
 - Other cell lines, to ensure that they are consistent, safe to use in research, and sufficiently well characterized to be of use to recipients.
- Maximize efficiency and minimize bureaucracy related to deposit and release of stem cells while ensuring compliance with steering group/other guidelines and legislative requirements;
- Provide expert training in technical matters relating to the growth and characterization of stem cells, including GMP cell banking;
- Carry out high quality scientific research on methodologies for...
 - Improving consistency and propagation of stem cells,
 - Developing and improving diagnostic tools for stem cells,
 - Improving in vitro safety tests for adventitious agents and pathogens,
 - Preservation, storage and distribution of stem cells.
- Promote regulatory mechanisms that facilitate safe and effective development of stem cell therapies;
- Charge for materials and services fairly and appropriately to help defray costs.”

UK Stem Cell Bank Management Committee, May 2008

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Chapter 19

Broad Consent

Linus Broström and Mats Johansson

Abstract The requirement of informed consent is central to research ethics. Translational stem cell research is one of those areas, however, where the choice to donate biological material is not likely to satisfy the criteria for a truly informed consent, due to the uncertainties about possible future research applications. The question arises whether so called broad consent, where the individual authorizes research usages that are specified only in rather broad terms, may morally legitimize the relevant research. This chapter argues that in order to settle this question, one first needs to address certain other questions, in particular what the moral reasons are for requiring informed consent in the first place.

Keywords Autonomy • Biobanks • Broad consent • Informed consent • Research ethics

19.1 Introduction

As traditionally understood, the requirement of informed consent is that individuals be enrolled in research only if they have voluntarily consented to participate, after first having been informed about the purpose of the relevant research, what their participation involves, what the risks and benefits are, and more. Sometimes, however, as when people consider donating biological material for possible future

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translational stem cell research, the available information is very limited. In such cases, the potential donors are asked not to consent to participation in a particular study, but to a variety of more vaguely described possible future research usages. The question is whether such so-called *broad* (or general) consent can play a role in legitimizing these usages? And if it can, will it be able to legitimize research in the same way, and to the same extent, as traditional informed consent? In this chapter we will explore some issues that need to be settled in order to be able to answer these moral questions. As will become apparent, these issues involve theoretical complexity as well as empirical challenges. Our aim is to shed some light on the problems rather than to solve them.

19.2 Broad Consent in Perspective

In order to understand the ethical challenge of making sense of broad consent, it may be instructive to begin by recapitulating parts of the history of informed consent. Since World War II, considerable measures have been taken to protect the rights and safety of research participants. The background is all too familiar, and is best exemplified by the horrific studies that were carried out in the concentration camps during the Nazi regime. Since the Nuremberg trials, several attempts have been made to ethically regulate scientific research. This has resulted in international declarations as well as national legislation. Central to these rules and recommendations is the doctrine of informed consent – a doctrine according to which research on human beings should only be allowed if they consent to take part in the research, after first having been provided with relevant information. Just what information should be considered relevant may vary with context. However, a common interpretation is that the information should include the aims of the research, the methods to be used, sources of funding, the anticipated benefits and potential risks, and more [1]. So conceived, the doctrine has come to be perceived as a cornerstone in medical research involving human beings. It is not, of course, the only thing of moral importance. Central to research ethics is also the requirement to minimize (the risk of) harm to research participants, and that various conditions regarding the researcher's competence, regarding documentation and monitoring, etc., are met. The informed consent requirement thus operates in the context of additional moral requirements.

Although immensely influential, the doctrine of informed consent is occasionally sidestepped, and this with the blessing of ethicists. This is the case, for example, in research on humans who lack the capacity to understand the relevant information or are incapable of making a decision. Such research is normally believed to be justified only if it has significant value, there is no better alternative way to achieve the desired goal, and the participants are not exposed to any significant risks. Although these conditions are necessary, they are not sufficient. This means that other conditions may need to (and arguably should) be met in order to ensure the

safety of the participants. However, none of the suggested conditions center on participants' informed consent.¹

There are other examples, too, where the traditional requirement of informed consent is circumvented. What is striking in these cases is that the participants are capable of informed decision making. In certain psychological experiments, for example, informing the participants of the true purpose of the study will ruin it. In Stanley Milgram's [2] famous work on the human tendency to uncritically obey authorities, the participants were, among other things, fooled into thinking that the study they were participating in had a purpose different from the stated one. In this case the study design itself was incompatible with the doctrine of informed consent.

This chapter will focus on yet another field of research where the doctrine of informed consent is at risk of being sidestepped. This research is conducted with the help of biobanks, i.e., collections of biological material (such as blood or tissue) and associated information. Such biobanks can contain vast amounts of scientifically useful information. The problem is that at the time when the material is collected, *no-one* knows how one might want to use it in the future, and this undermines the possibility of obtaining informed consent from the donors. Later, of course, researchers are able to provide detailed information about the studies subsequently designed, at least in theory. The practical problems with doing so, however, should not be underestimated. When it comes to research involving a large group of people (perhaps thousands of individuals) it can be practically impossible, or at least exceedingly costly, to ask the donors to consent to each and every study. And even in small groups it might be very difficult to meet the requirements of informed consent, as traditionally formulated. Just tracking down and coming in contact with a single donor may be highly time-consuming.

Translational stem cell research is characterized precisely by the features that make much biobank-based research problematic in relation to the requirements of informed consent. The field is likely to undergo changes. Some of these may be dramatic and involve scientific breakthroughs. But even in the absence of radical scientific progress, there will be enough uncertainties about future research to make it impossible to provide donors with detailed information about how the sample will (may) be used in the future. Also, and of great importance, the research is typically believed to result in highly valuable information – information that may lead to cures for serious illnesses.

Given this tension between the requirement of informed consent and the realities of translational stem cell research, one could, of course, bite the bullet and not allow the relevant research. Needless to say, this is an unsatisfactory solution, as there may well be a significant price attached to not allowing such research. Hampering scientific progress can lead to a relative loss of valuable knowledge, and this can literally lead to death and suffering of patients who might otherwise be helped. Therefore, we have strong reasons to at least look for other kinds of solutions.

¹The closest one gets to this is the negative requirement that the person does not object to participating.

What, then, are the alternatives? One option would be to allow this research when its utility is expected to be high, and participants are at no significant risk of being harmed. The assumption, however, that informed consent could simply be replaced by some other protective measures, with no significant loss, has no immediate plausibility. Why make the moral legitimacy of research conditional upon informed consent, one might wonder, if researchers could just go on with their research when they believe that the scientific gain is sufficiently high and the risk of harm to participants is sufficiently low? In any case, this is not an avenue that will be investigated in the following.

Another option, and the one central to this chapter, meets the challenge by introducing the notion of *broad* consent. As indicated, the idea is that potential donors could be asked not to consent to participating in a specific research project, but to participating in unspecified possible future usages of the donated material. Information about these future projects could range from the fairly specific to the completely general (“blanket” consent). Clearly, broad consent introduces flexibility into the procedure, and is by some regarded as an ethically acceptable solution to the problem at hand [3–6]. Others believe this relaxation of the information requirement to be problematic [7, 8]. After all, should not the information be specific? Does it otherwise make sense to speak of donors as being informed? Is broad consent anything other than a cynical solution to a practical problem? To address these and related questions, a systematic approach is required.²

19.3 Morally Justifying Informed Consent Procedures: Three Steps

If there is a take-home message in this chapter it is this: In order to assess whether the relaxed information requirement of broad consent will do, one first needs to address the issue of *why* there ought to be an informed consent requirement in the first place. Only then will it be possible to determine how the informed consent procedure needs to be designed. And it is only after having settled that issue that it will be possible to determine whether the relevant field of research allows for these procedures. In other words, there are three different steps that need to be taken (in the right order) if we are to be in a position to determine whether a particular consent procedure will contribute to morally justifying certain kinds of research:

²Certainly there are more complex options, too. For example, the idea of broad consent could be combined with the idea of conditional consent, where the potential research subject is offered the opportunity to prohibit certain uses of donated material. Discussing this particular option is beyond the scope of this chapter, but the general point that we make in the following is equally applicable to this more complex approach.

1. Identify the moral foundation for allowing medical research only if research subjects have consented to participate in that research.³
2. Specify the conditions that the consent procedure has to meet, given this moral foundation.
3. Determine whether these conditions can be met in specific research projects (or in certain types of research).

Consider the first step. What moral foundation could we possibly be looking for? Some commentators seem to believe that there is an easy answer to this question, one that doesn't really need to be argued for. This is noteworthy because at the same time there is little agreement on how to understand these moral underpinnings. Some believe that it ultimately has to do with respecting the *autonomy* of research participants [9]. Others believe that it serves to protect individuals against *harm* [10]. Also, there are those who appear to believe that virtually every interest worth protecting is protected by informed consent [4] (and, of course, those who do not even ask the question about what ends informed consent is ultimately supposed to achieve). As we will see later on, this is only the beginning of the complexity of making sense of the moral foundation.

As for the second step, for any given moral foundation that has been identified, it can be asked what informed consent must be like in order to protect the relevant rights, interests, or values. For instance, if informed consent is important because it enables choices that reflect individuals' considered values and beliefs (a form of respect for personal autonomy), this would seem to demand fairly rich and individually adjusted information. If, on the other hand, the informed consent procedure is seen as a minimal safeguard against outright coercion, the information requirements could perhaps be less strict. Further illustrations of this point will be offered in what follows.

The third step concerns whether the required procedure is possible in the actual relevant field of research. Is there, in this case, anything about translational stem cell research that would make it difficult to design consent procedures that satisfy the relevant conditions? Only thorough reflection on the moral foundations of informed consent, on what information and consent involve, on the characteristics of the relevant field of research, and on the interrelations between these issues, could give us the answer to the question of whether broad consent is an acceptable way of dealing with the ethical challenges of biobank research. We will now turn to some of these questions, in order to get a glimpse of the difficulties embedded in morally justifying a particular consent procedure.

³It seems natural to first look into the moral foundation of more traditional forms of informed consent. After all, broad consent is typically believed to replace, or at least partly do the job of, these more traditional requirements. Having said this, it is important to emphasize that none of the claims or arguments in this chapter depends on this comparison. It may be the case that broad consent is morally distinct from what we here describe as traditional informed consent, in the sense that it could be justified in an entirely different way. Even so, the three steps still need to be taken, in the right order.

19.4 The Moral Foundation(s) of Informed Consent

Today there is wide consensus that informed consent is morally required when conducting medical research on humans. It remains to be settled, however, *why* it ought to be required. As has already been mentioned, we will not try to answer this question or even offer a complete list of possible answers. Rather, we will show how controversies about broad consent can only be settled if one first addresses this key issue. And to show this, we need at least to sort out the most plausible options.

19.4.1 *Protection Against Harm*

As mentioned, one basic idea is that the moral purpose of informed consent is to protect individuals against *harm*. But what counts as harm, and how does it relate to informed consent? With the Nuremberg trials in mind, the kind of harm that first comes to mind is perhaps *physical* harm, i.e., harm that involves death, pain, and bodily damage. Although we no doubt need protective mechanisms against such harm, and despite the fact that the informed consent procedure certainly may be conceived as *one* such mechanism, it does not seem very plausible that this is the reason for requiring consent (broad or specific) to the kind of participation that is here of interest. It is simply very difficult to see how the donors are at risk of being physically harmed (unless, of course, they happen to be involved in the research in other ways, too). The potential donors of biological material in, for instance, translational stem cell research, are more likely to need protection against other kinds of harm, such as violations of privacy or personal integrity. Also, there are various kinds of psychological harm that could be associated with virtually any kind of research, such as the worry that information will be accessed by the wrong people.

Is there any reason to think that informed consent procedures will offer some protection against harm? One reason for thinking that they will is that, as a general rule, no one seems better equipped to protect a person's interests than that very person. We may not only be in the best position to say what could harm us, but we may also be the ones most motivated to protect ourselves – or so the argument would go, somewhat resembling John Stuart Mill's case for liberty [11]. For example, informing potential research subjects about potential risks associated with giving others access to personal data, and letting potential donors make a decision on this basis, allows them to decide if participating conforms to their own feelings about what would pose a threat to their privacy, if it conforms to their own assessment of the likelihood of such harm, and their own stance towards taking personal risks for the common good. Basically, this justification of informed consent constitutes an instrumental justification of respect for personal autonomy.

19.4.2 Promoting Autonomy

In addition to this supposed instrumental value of respecting autonomy, autonomy can also be conceived as something intrinsically good. An autonomous life – one in which a person shapes her future in accordance with her own values and beliefs – could, for instance, be seen as better than a non-autonomous life, without one life being, say, happier than the other, or less likely to harm the individual. It is therefore important, or so the argument could go, that people be allowed to make autonomous decisions. This requires more than freedom to act [8, 12]; it requires that people be given a reasonable chance to make decisions that qualify as truly autonomous. There is, of course, much to say about this idea of the value of autonomy in bioethics [13–15], but some commentators do assume the intrinsic value of autonomous decision making to be a basis for allowing people to make up their own minds about participating in medical research.

19.4.3 Promoting Trust in Science

Another suggested possible benefit of informed consent procedures is that they promote trust in science. The idea is that such procedures make the participants feel more involved in the research. As a result, it becomes less likely that people consider science as something that they have no control over. Informed consent could invite a sense of shared responsibility for the research that actually takes place. If these things facilitate research, and (good) research is valuable, the promotion of trust could thus be important. One could of course question whether informed consent really promotes trust in this way, but even on the assumption that it does, one need not maintain that promoting trust in science is the main ethical purpose of informed consent procedures. In most instances, it could be argued, the primary justification for informed consent is to be found elsewhere. Still, promoting trust in science could be conceived as an important *additional* value. And if it is, it could also be that this value is what counts in research where there is no significant risk that the participants are harmed. So, even if trust in science won't help us to fully make sense of the doctrine of informed consent in medical research, it could be what explains why broad consent ought to be required when collecting material for biobanks.

19.4.4 Beyond Utility: Deontological Constraints and More

That informed consent may serve to protect potential research subjects against harm, to promote the (intrinsic) value of personal autonomy, or to promote trust in science, are distinct possibilities. What these approaches to justifying informed consent have in common is that they locate the value of informed consent procedures

in the presumed *consequences* of adopting them. One does not have to appeal to consequences, however, to morally make sense of informed consent procedures. For one thing, these procedures could also be analyzed from a so-called *deontological* viewpoint, where certain actions are morally prohibited, required, or permitted, regardless of consequences. It could be argued that there are absolute moral *constraints* on what we are allowed to do to other human beings, including constraints on how to conduct research.⁴ For example, it is typically believed that I have not just a legal right, but also a moral right to limit others' access to the property that I own, and that others have a duty to respect that right, even if it should turn out that all parties involved actually would be better off if my property were simply confiscated and used for purposes determined by others. Likewise, it could be argued that individuals have a right not to have parts of their own bodies (e.g., stem cells), or personal information (e.g., about health status) become the object of research, and that researchers, correspondingly, have an obligation not to seize and make use of such material – regardless of whether the relevant research would benefit or harm the individuals enrolled.

Deontological constraints against acting in a certain way are by definition such that they will not be overridden by there being sufficiently beneficial consequences of acting that way. This, however, does not mean that such constraints cannot be “lifted” at all. For example, if there is a deontological constraint against killing another human being, no appeal to the benefits of nonetheless doing so would lift that constraint, but acting in self-defense may well do so. And when it comes to constraints concerning what we are allowed to do to a person in research, it is usually believed, we need that person's *permission* in order to be able to conduct the research without violating the constraint. It is here that informed consent procedures come into the picture. Such procedures may ensure that deontological constraints against using humans merely as a means for research are not violated.

Another non-instrumental approach to informed consent would take as its starting point not so much rights-based prohibitions against certain actions, but rather on the characteristics of a *moral person*. Asking a potential research participant for consent before including that person in a study may simply turn out to be inevitable for anyone who has a healthy view of other human beings and on her own standing in relation to others. The consequences of successfully implementing informed consent procedures may or may not have desirable outcomes, but it could be that for anyone who acknowledges the research subject as a *person*, for example, there is no other option than to relate to her in certain specific ways, which involve making research conditional upon consent [17].

⁴ To give a radical example taken from philosophy: it does not seem as if we are morally allowed to kill someone in order to take that person's organs, even if we thereby will save the lives of several other persons in need of transplants [16].

19.5 Implications

Each moral foundation, once it has been articulated in detail, will yield a list of features that informed consent would have to possess in order for it to serve its moral purpose. The extent to which these lists will coincide or overlap is an open question, one that can be settled only after careful examination of all the competing foundations. Whether consent could be *broad* and still legitimize research is one of the things one could expect to be elucidated by this kind of examination. Our aim is not to examine every conceivable line of justification, but only to illustrate how different approaches may require different features of the informed consent procedures. Let us begin by looking at what happens if we choose to focus on the *consequences* of consent procedures.

19.5.1 *Broad Consent as Protection Against Harm*

Assume that informed consent procedures are justified because they protect participants against harm, and that the mechanism by which this protection is achieved is the individual's allegedly superior ability to judge what is in her own interest. Broad consent will then do the job if this ability can be exercised on the basis of less specific information about the possible research usages. Is it likely, then, that the potential research subject is able to protect her own interests if the information about risks, benefits, etc., is scarce? Obviously this depends on many things, such as just how scarce the information is, what her interests are, whether she is able to imagine possible but unspecified consequences, or whether she is risk-prone or risk-averse. However, it seems that broad consent will be less likely than fully informed consent to protect participants against harm, given that the protection is believed to be achieved by the participants' expertise.

Prudence requires that participants be able to imagine the possible personal consequences of their participation, to assess the likelihood of these consequences, and to judge whether probable outcomes will either further or frustrate their own goals. In order to enable such reflection, however, potential research subjects need *information* about the relevant research, as otherwise their preferences or values could not relate to anything. Broad consent – depending, of course, on just how broad it is – may primarily protect those conservative and cautious individuals who on the basis of uncertainty about future research choose not to participate. Hence, in such procedures, potential participants are not consulted to the same extent as the experts they are here presumed to be.

19.5.2 *Broad Consent and Autonomy*

As was previously indicated, if one believes in the intrinsic value of autonomy, then one has a reason to facilitate autonomous decision making. Let us say that a person

for religious reasons does not want to take part in some kinds of research, such as research that “violates the creation of God.” Whether or not this preference is based on controversial (or even absurd) beliefs, it may play an important part in that person’s life.⁵ Thus, a consent procedure must provide that person with an opportunity to make an informed decision that relates to *her own* values and beliefs. Arguably, this would need to include some information that would reveal whether future research will, according to the person’s subjective view, violate the creation of God. Just what information would be required would not be easy to determine, but it is far from clear that broad consent in general could be trusted to work for this purpose.⁶

19.5.3 *Broad Consent and Trust in Science*

What if the moral reason for requiring informed consent is to promote trust in science – will broad consent suffice for this purpose? The short answer is that it is difficult to tell, especially in the long run. It is obviously an empirical question just how this aspect of the consent procedure affects trust in science. Perhaps trust in science will not decrease at all by the use of broad consent, as long as other important measures are visibly taken. Perhaps broad consent will affect trust in science negatively because it will be perceived as scientists’ narrow-minded and cynical attempt to pursue their pet projects merely by acting as if they are taking moral responsibility. Perhaps broad consent procedures will affect trust in yet some other way. Certainly, too, it will affect people’s trust differently, depending who they are and in what particular contexts consent is sought.

19.5.4 *Can Broad Consent Lift Deontological Constraints?*

For the sake of argument, assume that there is a moral constraint on conducting medical research involving human beings. Assume also that this constraint can only be lifted if the research subjects consent to participate. The crucial question is then if *broad* consent will serve this purpose, or if only more informed consent will do.

⁵Obviously one needs not refer to religious beliefs to make the point. It might be the case, for example, that a person does not, on ethical or political grounds, want to participate in research funded by certain companies or other interests.

⁶It could be argued that this need not be a serious problem. After all, a person who is uncertain about whether participating will be in conflict with her considered values and beliefs, is free not to participate. That is, her autonomy is not violated by anyone. Still, this approach will not *help* her make an autonomous decision. And it is enabling autonomy that we are interested in here.

The answer will depend on the details of the deontological approach adopted, how exactly the relevant constraint is formulated, and why exactly consent will lift it. On the face of it, however, it appears as if broad consent may well suffice. Seemingly, the most important constraint is the one against using the relevant biological material without the individual's *permission*. Such permission (we have assumed) is critical, but it could still be up to the individual to decide if she wants to know what specific use will be made of the material. In fact, one could even think of it as the individual's *right* to judge how much information she needs in order to make up her mind. Just as there appears to be a deontological constraint against our taking your money without your permission (i.e., without your giving it to us), you are free to give your money away with either no preconditions ("Buy whatever you want!") or by letting us have it only if we will use it for certain specified uses. Likewise, broad consent could morally justify storing and then using blood or tissue for research purposes, even if there would otherwise be an absolute prohibition against doing so.

From a theoretical viewpoint, there is thus no apparent reason why even blanket consent could not serve to justify medical research. In real-world settings, however, things might be different. It could, for instance, be argued that consenting will not do the trick if the person consenting cannot, or cannot be expected to, imagine the possible outcomes. Consider again the money analogy. If we say to you that you could have our money, to use it "for whatever you need it," that indeed gives you the freedom to buy a very broad range of things, to make various investments, to give it away for charity, and more. But does it give you the freedom to, say, destroy the money in front of our eyes just for the fun of it? Comparing biobank-based research to such scenarios may seem silly. The analogy does illustrate, however, how we make assumptions about what applications those who ask for consent could possibly have in mind and, in this context, how our decision whether or not to participate depends on what research applications we can and cannot conceive of. In other words, what is morally authorized by broad consent may to some extent depend on the scope of one's imagination. The uncertainty surrounding the future possibilities of stem cell research may call precisely for this kind of caution when interpreting a seemingly broad consent.

What is also important to remember is that from a deontological perspective there may be other relevant constraints in addition to the one against enrolling individuals in research without their permission. Assume, for example, that there is a fundamental constraint against treating people merely as "means," a constraint underlying people's right not to be involved in research unless they consent to this. From this very basic prohibition, other constraints can be derived, too, among them constraints against threatening, lying, manipulating, or merely taking advantage of being more knowledgeable. Applied to research settings, the possibility of there being such further constraints means that particular (implementations of) broad consent procedures, depending on how they are designed, may still fail to respect the rights of participants, even if they avoid violating one central constraint by permitting research.

19.6 Further Implications

There are more general lessons from turning one's attention to ethical foundations, in addition to the specific implications regarding broad consent. As the previous section illustrated, the plausibility of any instrumental justification of informed consent requirements depends on the details – details that more often than not are highly speculative. On the face of it, referring to good consequences may seem as an appealing and transparent way of justifying informed consent procedures. However, we might agree that a certain procedure *would* be justified *if* it leads to something good, but at the same time reject the claim that the procedure has those consequences. And typically it remains to be shown that a certain procedure has this or that effect. Furthermore, even if we are able to provide empirical support for the claim that a procedure has some favorable consequences, it does not follow that we should rely on that procedure. Other procedures, or modified versions of the same procedure, might be available that may have even greater benefits. In other words, anyone who wants to justify informed consent procedures by reference to what can be achieved by these procedures needs to pay close attention to the empirical issue of whether, or under what conditions, they really do have the desired effects.

Understanding informed consent as something that lifts deontological constraints does not raise these kinds of empirical issues, but it certainly gives rise to some difficult questions. First, of course, is: Is there really such a deontological moral constraint on, for example, using donated stem cells in future research projects, without first obtaining the informed consent of the donor? If not, why is that? Again, notice that the question needs to be dealt with within a deontological framework. That is, simply referring to the insignificant risks of being a donor of tissue or blood for research will not do.

If indeed there is a deontological constraint against enrolling research participants, this has implications not only for broad consent, but also for other suggested safeguards. For example, the protection that could be offered by ethical review boards could do nothing to relax the relevant constraint. Likewise, informing research participants afterwards, as in the “debriefing” that is common in psychological research, would not be an option if one accepts the kind of deontological framework discussed in this chapter. The reason is that the constraint must be lifted *before* the research takes place. More pertinent, perhaps, neither is it likely that one could rely on “opt-out” solutions, such as informing people that a study is going on, and in this way providing them with an opportunity to choose not to participate.

A deontological approach could possibly have more radical implications, too, for what research should and shouldn't be allowed, than what is typically imagined. For example, what would this kind of approach have to say about participants who are incapable of decision-making? Biological material for research is often taken from infants, for instance, who lack the capacity to understand what is happening. Regardless of what the law might allow, is it reasonable to assume that their parents have a right to lift a moral constraint that normally would have to be removed by

the person most immediately concerned? In a more liberal vein, why wouldn't it follow from the assumption that broad consent is enough to lift a deontological constraint against enrolling individuals in translational stem cell research that we could relax the information requirement for all kinds of research? Are we comfortable with this implication? In this and similar ways, the chosen moral foundation for our position on broad consent to translational stem cell research commits us to considerably more than what is obvious at first glance.

Finally, an approach to research ethics that focused on the characteristics of the *virtuous* scientist, rather than rules that need to be satisfied in order for research to be morally (just) acceptable, would bring with it new and more demanding considerations for how to treat people when involving them in research. From this perspective, where the issue of how one could cultivate the appropriate attitudes towards research subjects takes center stage, even the question of whether broad consent is sufficient to legitimize research may seem to be the wrong question to ask.

19.7 Concluding Remarks

Everyone knows that one cannot evaluate a means without doing so in relation to some end. Just as trivially, it is impossible to evaluate a procedure without referring to its purpose. Nonetheless, bioethics is full of examples where procedures are assessed without a clear and explicit idea about what they are supposed to accomplish. The discussion concerning broad consent is but one example.⁷ This is the reason why in this chapter we have emphasized the fairly obvious, that broad consent only can be evaluated given its moral purpose. It is less obvious, of course, what this purpose is. We have seen how different approaches can lead to radically different answers to the question of whether broad consent is able to replace the traditional requirement of informed consent in research involving biobanks. Not only does it matter whether we understand this requirement as harmonizing with a basic right, or whether we understand it instrumentally as protection from harm. It also matters how we describe this supposed right, or the mechanism that supposedly gives rise to sufficient protection. The details are not purely of academic interest, but actually fundamental when it comes to judging whether research can be justified based on broad consent.

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⁷We have elsewhere [18] argued that this is the case in the discussion surrounding surrogate decision making, and the substituted judgment standard.

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Chapter 20

Banks, Repositories and Registries of Stem Cell Lines: The Challenges to Legal Regulation

Mette Hartlev

Abstract Stem cell biobanks can be understood as repositories of stem cells and stem cell lines including the data associated with the biological material. Such biobanks have widely differing extents, characters and purposes. Some are very large and serve broad-spectrum purposes. Others are smaller and have more distinct aims. Some are public, others are commercial. Altogether the area of stem cell banks is highly complex, which implies a regulatory challenge for legislators. This paper aims at clarifying how stem cell banks are regulated in international law with a special focus on the balancing of interests of different stakeholders. Tissue donors may have various interests in regards to stored stem cell samples, especially an interest in self-determination and privacy protection. These interests must be balanced against the interests of other actors, not least those patients who can benefit from the development of new treatment but also the interest of society at large in development of new knowledge. New issue arises in situations where stem cell research is moved from the laboratory to the clinic. The change of purposes associated with the translation of research into clinical practise gives rise to special regulatory concerns and challenges, especially in regards to tissue donor's right to self-determination. The paper concludes that the international regulation only to a limited extent refer explicitly to how the transition from laboratory research to stem cell treatment should be dealt with, and that there is a need for clarification in international law of how to tackle this challenge.

Keywords Stem cell banks • Legal regulation • Patients rights • Privacy • Right to self-determination

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20.1 Introduction

For several decades medical practitioners and researchers have been storing human tissue in collections which, over time, have developed into large systematic collections, often referred to as “biobanks.” The purpose of storing human tissue samples was originally primarily to use it for research and teaching. However, biobanks have also developed to have other functions, where the tissue is kept with a view to providing subsequent treatment, either for the donor of the tissue or others.

Biobanks have widely differing extents, characters and purposes. Some are very large and relate to population groups. Thus, many countries retain blood samples of all newborn children, both to test for phenylketonuria (PKU) and to form the basis of subsequent research. However, most biobanks are less extensive and are based at hospitals and research institutions. Biobanks may serve either broad-spectrum or more specific purposes. The pathological collections of hospitals often serve more general purposes. They contain tissue collected in connection with surgical operations and postmortems, kept with a view to carrying out quality control and later diagnosis and treatment of the patient, as well as with a view to research. Donor biobanks, such as blood banks, sperm banks and bone marrow banks, are established specifically with a view to treatment, and thus have more clearly defined purposes. The same applies to biobanks set up specifically with a view to research; this can either be in connection with a specific research project, or for research more generally in a given area. Even though a biobank may be established for a specific purpose, it may later be found that it can be used for other purposes. For example, research biobanks may be found to be useful for treatment, and donor biobanks may be found useful for research.

Stem cell banks also have different characters. Some are public; others are based in institutions or are commercial. Stem cell banks include both the biological material (the stem cell or stem cell line) and the data associated with it. The term “stem cell bank” could also refer to registries containing information about stem cell lines, including scientific data and information about the origin of the cells [1]. Stem cell banks may hold stem cells, or stem cell lines that are donated directly for stem cell research, but they may also store stem cell lines based on tissue from other biobanks that has not been donated directly for stem cell research.

Altogether, the area of biobanks – including stem cell banks – is highly complex, and this complexity is in itself a regulatory challenge. There are different considerations and interests involved, depending on whether the question concerns the use of biobanks for research, treatment of the donor, treatment of others, or some quite different purpose. Since biobanks can have several different aims, and since these aims can change – for example, from basic research to translational research – this prompts special regulatory considerations.

In this chapter, I will give an overview of the regulatory challenges associated with translational stem cell research in connection with existing stem cell biobanks. In this context, a stem cell biobank is understood to be a biobank that stores human tissue or registries that hold data on stem cell lines specifically with a view to stem cell

research. This can be embryonic stem cell research, or research into adult stem cells. The stored material can both be stem cells (e.g., blood, bone marrow, fetal tissue, umbilical cord cells, and fertilized eggs), or it can be in the form of stem cell lines. I begin by discussing whether there is a need for legal regulation of stem cell banks, and what interests and concerns should be protected by any such regulation. Afterwards, I look at the international regulations that are relevant to stem cell banks and stem cell research. Finally, I discuss what effect a change of purpose for the use of stem cell lines can have from a regulatory perspective.

20.2 Why Regulate Collections of Stem Cells?

Let us consider, for a moment, another field of research – biological research. In connection with research, biologists may collect biological material from nature, and some of this material is derived from or consists of living creatures. These creatures can be insects, fish, single-celled organisms, etc. When researchers collect and keep such material in special collections, there are either none, or very few formal legal rules. Hence, it is normally possible to build up a collection without having to think about permits from a special authority or registration, and it is possible to carry out research without undue concern about the creatures which the materials come from.

Does it make any difference if researchers instead collect material from humans, whether living or dead? Obviously, one cannot capture humans in the same way one can capture butterflies, displaying them in glass-front cabinets. But this is not the situation in connection with stem cell research. This only concerns material that the living person can, or dead person could, do without and that – consequently – is not of vital importance for the donor. So the question is whether there are any particular concerns associated with the use of tissue samples taken from the body of a living or dead person.

Some will argue that biological material that is removed in a surgical procedure, or that is taken in connection with a postmortem, can be considered either as the property of the hospital or as refuse that can freely be taken possession of and used, unless it is infectious, etc. This has previously been the view that has characterized attitudes toward biobanks. Hospitals, doctors and researchers have taken possession of materials that are of interest to them, and stored them in collections without the permission of the person from whom the material was taken, or from special authorities. This has even been the case in regards to aborted fetuses and fetal material [2]. According to this position, human tissue has no special legal status compared to other kinds of biological material.

However, more recently, much more attention has been paid both to biobanks and to the right of individuals to determine what happens to their own bodies and body parts. This is primarily due to two particular developments. First, within the last 20 years, there has arisen a general recognition of patients' rights in relation to

health services, and respect of patients' right to self-determination have become a key concept, which has also affected the view of how human tissue should be dealt with. This development has been in line with the greater awareness and recognition of fundamental human rights. Second, developments in biotechnology have intensified the general awareness of ethical issues, and this has led to concerns about both the risks associated with and the ethical acceptability of such technologies. Thus, developments in gene technology have led to increased awareness of the need to protect genetic privacy, and the potential applications of the new technology have prompted people to reflect upon personal, as well as societal, ethical limits. In line with this development, there have been both national and international initiatives for legal regulations to ensure respect for human rights, such as the right to privacy, self-determination, and integrity of individuals [3]. Apart from dealing with questions of risk assessment and ethical acceptability, this regulation has also served the purpose of achieving greater acceptance of the use of these new technologies.

20.3 What Interests Should Regulation Protect?

In the preceding section I have briefly referred to some of the considerations that are relevant to the formulation of regulations in this area. When one contemplates social regulation, it is both necessary to consider the private interests and self-determination of individual citizens, and to take account of the interests of other citizens and society at large in promoting new knowledge or developments. It is thus necessary to distinguish between consideration for the donor of the stem cells, and consideration for others – for example, persons suffering from serious diseases who may benefit from stem cell research and treatment, as well as the overall interests of society.

The *donor of the stem cells* can have various interests in what happens to their stem cells. First and foremost, it is important to be aware of the interest in *self-determination*. Some people are indifferent about what their tissues are used for, while others want the opportunity to decide this if, due to ethical, religious, cultural or other reasons, they do not want their tissues to be used for just anything. This interest is generally recognized, and among other things it is reflected in the requirements for “informed consent,” which is a basic principle in connection with patient treatment and human research, and for which there is both national and international regulation [4–7]. However, as I will discuss in later sections, it is possible to ensure consideration for self-determination in many different ways. Consideration for the *privacy* of the donor is also an interest worthy of protection that is generally recognized in both national and international regulations [4–8]. Human tissue samples contain information about the donor, and this information is often of a sensitive nature. It is therefore important to ensure adequate protection of the privacy of donors.

However, in relation to research and development in the area of health and the treatment of illnesses, it is also necessary to be aware of *other factors*. This refers

not least to those patients who can benefit from the development of new treatment methods. It is generally recognized that society has a responsibility to ensure that individuals have access to health services and treatment of the highest quality. In its constitution, the World Health Organization clearly states that “The enjoyment of the highest attainable standard of health is one of the fundamental rights of every human being without distinction of race, religion, political belief, economic or social condition,” and “health” is defined as “a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity” [9]. The right to health is also recognized in a number of international conventions, including the UN’s International Covenant on Economic, Social and Cultural Rights [10].

Finally, society at large has interests in research and development in this area. Both consideration for economic welfare and public health can be arguments for promoting research, and databases and biobanks may be very valuable in this regard. Hence, for many years it has been recognized that epidemiological research benefits greatly from having well-established collections of tissues and data.

As can be seen, there are a number of different considerations and interests that are relevant, and these interests do not always coincide. The donor’s right to self-determination may, for example, clash with society’s interest in promoting research. If regulations are to be adopted, it will usually be necessary to prioritize and weigh these different interests. Basically, it is necessary to weigh the interests of tissue donors on the one hand against the interests of other individuals and society at large on the other. In Sect. 20.5, I will look in more detail at how this has been dealt with in international regulations and at the challenges that arise as a result of developments in this area. Before I go into this discussion, it is useful to look briefly at the relation between research and treatment.

20.4 Research or Treatment – Does It Make a Difference?

Translational research crosses the line between basic research and treatment. Hence it is necessary to consider how this affects the legal interests involved. In national and international regulation, it is generally recognize/d that the use of human tissue for research involves slightly different considerations than the use of human tissue for treatment.

If stem cell material is taken from a person with a view to *treatment* of that person (autologous treatment), there are not many particular legal concerns. The patient must give informed consent to the treatment, but apart from this there are no special legal requirements. It is different where a person donates stem cell material for the *treatment of other persons* (allogenic treatment). Since donors do not themselves obtain any direct benefit from the donation, there are normally a number of stricter requirements laid down in the legal regulation [4, 11]. Among other things, a living donor has to consider the potential risks associated with the donation, and the legal regulation is sensitive toward any undue influence on

donors' consent – for example, in situations where organs are donated to family members.

If one makes a donation for the treatment of other persons, this benefits only the recipient. For some donors, it will be important that they be able to help a person who has an actual need for treatment. Thus, in connection with the donation of human tissue for treatment of another person, a special legal relationship is established between the two, where it is necessary to consider the mutual rights, such as the anonymity of the donor and the recipient. In comparison, the situation is different when one donates stem cell material for research. Here, the donation is not made with a view to provide direct benefit to another person, but with a view to the development of knowledge that can be useful to many people. This establishes a different legal relationship that in cases of donation for treatment, and the legal regulations in the research situation are concerned with ensuring that the interests of donors/research subjects are not ignored in the context of the general interest of research in finding new knowledge. This is clearly expressed in Article 2 of the Council of Europe's Convention on Human Rights and Biomedicine, which states that "The interests and welfare of the human being shall prevail over the sole interest of society or science" [4].

Consequently, the interests and concerns involved in relation to the donation of human tissue for treatment and donation for research is not identical and it is necessary to be aware of this when research crosses the line from being at the level of basic research to the clinical stage.

20.5 International Regulation of Stem Cell Banks

20.5.1 Introduction

Normally, tissues in stem cell banks will be donated directly for the purpose of stem cell research by the person from whom the tissue comes. For example, couples who are receiving IVF treatment can be asked to donate one or more fertilized eggs for stem cell research, or mothers can be asked to donate umbilical cord cells to research. However, it is also possible to use tissues that are kept in other biobanks for stem cell research, and depending on the legislation in the country in question, researchers will be able to have access to stem cells from existing biobanks without having to have direct contact with the person or persons who donated the stem cells.

The rules on donation and access to stem cell material for research differ from country to country, and they can also depend on the kind of stem cell material concerned. Normally, the rules will be more restrictive in the case of fertilized eggs, and possibly also with tissue from aborted fetuses, than in the case of blood cells or bone marrow cells [12].

There are several international sets of rules that can give an idea of the basic legal principles that are important in connection with human biological material

that is donated directly with a view to, or is in fact used in, research. UNESCO and the Council of Europe have been particularly active in this area. The EU has also formulated rules that are relevant to this type of research. In this section, I will examine the most important of the basic principles that can be derived from these sets of rules in relation to research on biological material, including stem cells and stem cell lines. I begin with the interests of donors, not because they are necessarily more important than those of the other actors, but because it is a useful starting point for structuring the discussion. The question of the commercialization of stem cells is not discussed here, as this is dealt with elsewhere in this book.

20.5.2 Balancing Consideration for the Individual and Consideration for Research and the Interests of Society

In general, all the international sets of rules are concerned with the balance between consideration for the rights of research subjects on the one hand, and consideration for the interests of research and society at large on the other. Article 2(d) of UNESCO's Universal Declaration on Bioethics and Human Rights emphasizes the importance of recognizing "freedom of scientific research and the benefits derived from scientific and technological developments, while stressing the need for such research and developments to occur within the framework of ethical principles set out in this declaration and to respect human dignity, human rights and fundamental freedoms" [5]. As referred to above, the Council of Europe's Convention on Human Rights and Biomedicine also states that the interests and welfare of the human being should prevail over the sole interest of society or science [4]. This is also repeated in the preamble to the Council of Europe Recommendation Rec(2006)4 on research on biological materials of human origin [13]. As can be seen, the UNESCO Declaration puts regard for freedom of scientific research first, while the Council of Europe's Convention emphasis is on the primacy of the human being. However, both legal instruments stress that it is necessary to strike a balance and to be aware both of regard for research and the fundamental rights of individuals.

One can discuss whether it makes a difference that stem cells and other biological material is separated from its human originator, and that it is not the donor herself who is subject to research. In other words, should stem cells be covered by the same protection as an individual who is a subject of biomedical research, or can one accept a lower level of protection, such as that often recognized in connection with research into personal data? It is not possible to give an unambiguous answer to this. Some forms of biological material can have a special value, and this can trigger special research restrictions. For example, there have been significant controversies and concerns about research using human embryos, and this is an area where there are significant regulatory differences internationally. Some countries prohibit research on human embryos, while others have no rules or restrictions [12]. This disagreement is clearly seen in connection with the United Nation's attempt to adopt a resolution against human cloning. A number of

countries either voted against the proposal or abstained, some because they feared that the resolution could be interpreted as a prohibition of research into embryonic stem cells, and others because they did not think the resolution went far enough in its restrictions by not including a prohibition on research into embryonic stem cells [14]. The Council of Europe's Convention on Human Rights and Biomedicine deliberately refrains from deciding on this question, and instead it merely states what restrictions should apply to human embryo research if it is allowed in the country in question (Article 18) [4]. This also means that a number of the Council of Europe's legal instruments exclude human embryos, and sometimes fetal tissues, from their scope. This is the case with Council of Europe Rec(2006)4 on research on biological materials of human origin [13]. There are also other examples where human biological material is given a special status. In Article 4 of UNESCO's International Declaration on Human Genetic Data, human genetic data have a special status compared to other personal data because they can be used to predict the genetic predispositions of individuals, and they can also be significant for family members and for the particular group to which the donor belongs [15].

In conclusion, one can say that dependent on the contexts, some stem cells will be ascribed a special value and will thereby be covered by research restrictions that do not apply to other kinds of human research. Other kinds of stem cells will be subject to fewer restrictions than when doing research on humans. However, as I will return to later, there will normally be more restrictions in regards to research on human tissue than when doing research into "ordinary" data.

20.5.3 Respect for the Self-determination of the Individual

Respect for individual freedom and self-determination is fundamental, both in the area of human rights law and health law. This is clearly reflected in the international sets of rules, in which self-determination and informed consent are basic principles. There must generally be informed consent for any form of medical intervention, and there are special rules for the protection of persons who are not in a position to give informed consent (for example, children and persons who are not able to make autonomous decisions). Informed consent is also a fundamental principle in biomedical research, which was clearly stated in the Nuremberg Code in connection with the post-World War II criminal tribunals [16] and later confirmed in the World Medical Association's Declaration of Helsinki [7].

The rules on informed consent are primarily formulated with a view to persons who are subject to direct intervention. For example, it is normally not permitted to make surgical intervention or take a tissue sample from a person without his prior informed consent. Research involving biological material differs slightly from the usual medical research situation, as it is not the person him- or herself, but rather part of his body that is the subject of research. Once the biological material has been removed from the person's body, that person may not necessarily take further

part in the research. The question is whether or not this influences the need for informed consent with regard to stem cell research.

If the biological material is taken explicitly with a view to research, the informed consent must also cover the use of the tissue taken for the purpose of research. This is emphasized in Council of Europe's Convention on Human Rights and Biomedicine which stipulates in Article 22 that part of a human body may "... be stored and used for a purpose other than that for which it was removed, only if this is done in conformity with appropriate information and consent procedures." Article 8 of UNESCO's International Declaration on Human Genetic Data (2005), is in the same line, requiring that "prior, free and express consent" must always be obtained in connection with the collection of biological material and its subsequent processing, use and storage [15]. However, it is accepted that the consent can be more or less precise. Thus, it is possible that a person may give very broad consent for their biological material to be used in any form of research in the future, though normally tissue will be taken in connection with a specific research project and the informed consent will state some parameters for what the tissue can be used. Article 10 of the Council of Europe's Recommendation Rec(2006)4 on research on biological materials of human origin emphasizes that both the information and the agreement or permission to collect the biological material must be as precise as possible in relation to the foreseeable research use of the material [13]. There is a corresponding requirement for precision in the European Group on Ethics Opinion No. 11 on the Ethical Aspects of Human Tissue Banking [17]. This can be a challenge in connection with research in areas where it is difficult to predict the results and where it may be necessary to initiate follow-up projects. The question then arises as to whether it is necessary to obtain renewed consent for further research. This is discussed below.

In some situations it will not be necessary to obtain biological material from a research subject, as it may be possible to use material that has been collected previously and stored in a biobank. This can also be the case with stem cell research, where there may be a need to have access to a biobank with, for example, fetal tissue, umbilical cord material or human embryos. In this situation, the same question occurs – as to whether this requires new informed consent from the donor of the material. It is not possible to answer this with certainty, as this is regulated in different ways in different countries. If one looks at the international rules, such further or secondary use depends on, among other things, the scope of the consent that was given when the material was originally taken. For example, if the biological material is part of the pathological collection of a hospital, and there has been no specific consent for research, one cannot say that consent has been given for the later use of the material for research. However, the Council of Europe Convention on Human Rights and Biomedicine accepts that it may be sufficient that the donor has not expressed explicit opposition, provided that the donor has been duly informed in advance about the possible use of the tissue [4, 18]. Article 22 of the Council of Europe's Recommendation Rec(2006)4 on research on biological materials of human origin stipulates that an attempt should normally be made to obtain informed consent for further or secondary use from the donor of the biological material. If, with reasonable efforts, this is not possible, then the biological material

can be used, as long as the new project fulfills a number of criteria, including the requirement for the research to be of significant scientific interest, that it would not be possible to obtain results without the use of the material, and that there is no evidence that the person concerned has expressed opposition to the use of the biological material for research purposes. It is also required that the research project be subject to impartial scientific assessment by a competent body. However, as stated above, this Recommendation does cover research on human embryos or fetal tissue, so international regulation does not give an answer to secondary use of such biological material.

All in all, it seems that the international regulation provides some guiding principles, but only few clear rules in regard to the use of stem cells for research purposes. The informed consent of the donor is a general principle that transcends the legal landscape in regard to medical interventions and research participation. However, there are less clear indications when it comes to research on and further use of tissue that has been separated from the donor's body [18].

20.5.4 Protection of Privacy

Another fundamental principle concerns the protection of the privacy of individuals. This is emphasized in a number of the international patients' rights rules referred to above and is also subject to separate regulation [8]. Human tissue samples contain information about the donor that prompts the question of privacy protection in connection with stem cell research. Some of the international legal instruments explicitly cover human tissue samples whereas it is more uncertain whether other instruments cover the tissue sample or only the data that may be extracted from the tissue [19].

The requirement for the protection of privacy should be seen in conjunction with the right to self-determination, and it raises some of the same questions. In themselves, the limits on the use of biological material that have been formulated in connection with the donation of material for research give some protection of privacy, as they set limits to the group of persons and institutions that can have access to the material. Furthermore, the requirement for privacy protection also means that the donor of biological material may not be identified in connection with the publication of research results unless there is explicit consent to do so.

It is not always necessary to do research using material that is identified with the donor. Some rules state that if it is possible to carry out research using anonymous material, the material should as much as possible be made anonymous in order to protect the donor's privacy [13]. If the biological material has been made anonymous, the normal requirement in regards to consent, etc., will normally not apply. According to Article 23 of the Council of Europe's Recommendation Rec(2006)4 on research on biological materials of human origin, such material can usually be used in research projects, provided it does not breach any restrictions that the donor stipulated in connection with collection of the material [13].

However, it could be discussed whether it is in fact possible to anonymize biological material, since its DNA will be able to help identify the donor [19].

20.6 Translational Research

The purpose of stem cell research is to develop knowledge that can be beneficial in the treatment of patients. Thus it is hoped that at some point the research results can be used in the treatment of specific patients. Before this point is reached, it will normally be necessary to conduct clinical trials, where the results of laboratory tests are tried on patients. In connection with this, it can be necessary to use stem cells or stem cell lines that have originally been collected and developed in connection with research projects. This prompts the question of what concerns this translation gives rise to from a regulatory perspective.

First, it must be said that it is in the nature of research that the knowledge acquired is put into practice to the benefit of society. UNESCO's Universal Declaration on Bioethics and Human Rights explicitly refers to the sharing of benefits resulting from scientific research in article 15 [5]. In many situations there will not be any special ethical concerns about using knowledge obtained from research projects in connection with patient treatment. However, with stem cell research, it may not only be the knowledge that is used, but also the biological material. This may cause problems, as it cannot be assumed that a person who has consented to the use of his biological material for the purpose of basic research will also accept the use of the same material in direct patient treatment. In this context, that person changes from being a research subject to being a tissue donor. As an example, a couple who has donated an embryo for stem cell research may not be willing to accept that the embryo is used directly for patient treatment.

On the basis of the rules on self-determination, referred to in [Sect. 20.5.3](#), what is decisive is whether the consent of a person who has acted as a research subject also covers consent to their stem cells or stem cell lines that have been developed being used for other research, including clinical research. If this is the case, it will normally be in line with international law to use the stem cells in clinical research. Also, in the end, the material is used for the same purpose – gaining new knowledge of stem cells and their potential use in patient treatment.

On the other hand, when the scientific knowledge reaches the stage where stem cell treatment is implemented in daily clinical practice and stem cells are used for patient treatment outside a research situation, there will be slightly different concerns. As referred to in [Sect. 20.4](#), for some people it can make a difference whether their biological material is used for research, which can benefit society as a whole, or whether it is used for the treatment of a specific recipient. The UNESCO International Declaration on Human Genetic Data refers to this problem in Article 16, which deals with “change of purpose.” According to this, biological material

that is collected for a specific purpose – for example, stem cell research – may not be used for some other purpose “that is incompatible with the original consent,” unless voluntary informed consent has been given to this secondary use, or unless it may be used in accordance with the law and is for an important public interest reason [15]. This provision reflects a principle that is also expressed in Article 6.1(b) of 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. According to Article 6.1(b), personal data may not subsequently be treated in a way that is incompatible with the purpose for which it was collected. Furthermore, Article 10 of the Directive provides for member states to derogate from the normal provision in connection with the use of personal data for research purposes. However, according to Article 13.2 of the Directive, if member states have derogated from the general provisions, the data can only be used for the purposes of research and there must also be guarantees that the data are not used for making decisions about individual persons. Thus, in regards to the use of data for research purposes, the Directive restricts secondary use to other kinds of research unless special legal requirements are fulfilled. The Directive does not explicitly deal with biological material, but this approach is also applicable to research using biological material. Some member states have decided to apply the rules of the Directive to biobanks, while other member states have chosen not to include biobanks within the scope of their personal data law, and may have adopted special legislation for biobanks [19]. The UNESCO Declaration and the EU Directive reflect concerns about the validity of informed consent in connection with change of purpose from one setting to another, and these concerns could serve as guiding principles in regards to translational stem cell research.

Altogether, it can be said that only to a limited extent do the international regulations refer explicitly to how the transition from laboratory research into stem cells to clinical research and then to stem cell treatment should be dealt with. Since stem cells donated for research can also be shown to have a clinical application, there is a need for clarification of how to tackle the transition from a research situation to a donation situation. In this connection, due attention should be paid to donors’ rights of self-determination.

20.7 Conclusion

Just as with other biobank research, stem cell research is an interesting area in which the research subject stands somewhere between “person” and “data.” The rules that regulate the use of people as research subjects or as donors of tissue for the treatment of others differ on some points from the rules that deal with research that is based purely on personal data, or the application of this research in practice. Thus, from a regulatory perspective, stem cells are a hybrid of person and data, and translational research could also be characterized as a hybrid between basic research and clinical treatment.

Making regulations for hybrids is usually a challenge [20]. However, it is necessary to meet this challenge. The uncertainty that characterizes this area is a problem because there is a risk that stem cells will be used in a way that gives rise to ethical discussions and controversy, and this may compromise the legitimacy of stem cell research. There is thus a need to establish greater regulatory clarity in this area. This requires one to navigate between the regulations governing the body and the regulations governing data. In some cases, this can involve the same considerations and interests that arise in connection with research using humans. In other cases, stem cell research can more resemble research using ordinary data.

An important principle in this context is the recognition of the self-determination of individuals. The more it is possible to give an advance account of the possibilities of using stem cells, the more it will be possible to ensure that persons who donate stem cells for research accept the secondary use of the donated cells. Moreover, if one combines this with the right for individuals to revoke their consent, if circumstances arise that that person cannot accept, one will to a large extent have created an ethically legitimate set of rules. One should consider whether the anonymization of stem cells or stem cell lines takes sufficient account of the interests of research subjects. I do not believe it does. It is true that measures have been taken to guard against exposing the privacy of such persons, but this is not necessarily the most important interest. For some, the possibility of deciding for themselves the use to which their cells are subsequently put is a matter of great importance, and this interest can only be taken account of by creating rules that recognize the right to self-determination.

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Part V
Translational Stem Cell Research and
Commercial Funding

Chapter 21

Proprietary Interests and Collaboration in Stem Cell Science: Avoiding Anticommons, Countering Canalization*

Matthew Herder

Abstract In this chapter I explore how proprietary interests and commercialization norms can impede collaboration in stem cell science. I begin by outlining three layers of property in stem cell science—stem cell data, stem cell materials, and stem cell patenting—and explain how they are intertwined in practice. I then present two stem cell research initiatives, the Cancer Stem Cell Consortium (CSCC) and Stem Cells for Safer Medicines (SC4SM). Using two conceptual frames, the “tragedy of the anticommons” and “patent canalization,” I analyze the extent to which the CSCC and SC4SM appear to address proprietary or commercialization-related impediments to collaboration. Whereas the anticommons frame, and empirical methodologies it has spawned to date, tends to capture costs imposed upon the scientific fields as a whole, patent canalization focuses on the individual scientist, hypothesizing that patenting and other commercialization behaviours may (re) constitute the scientific self. The chapter concludes by highlighting three intellectual property-related best practices intended to facilitate collaboration in stem cell science.

Keywords Stem cells • Patents • Commercialization • Anticommons • Data sharing

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21.1 Introduction

All collaboration is complicated. The decision to do so is informed by two principal considerations: how easy it is to collaborate, and how attractive it is to do so. The trouble is that those considerations are, in turn, contingent upon a number of other things that would-be collaborators may have little control over, imperfect knowledge about, or subject to the influence of. Take any field of scientific inquiry today: researchers must abide by ethical requirements, participate in the commercialization of their work, and compete to prove their expertise. Each is relevant to any question of scientific collaboration.

Recent forecasts for collaboration in stem cell science – the field that I will explore here – have leaned toward the negative. Some assert that inconsistent regulatory frameworks from one jurisdiction to the next make cross-border collaboration difficult [1]. According to David Winickoff, Krishanu Saha and Gregory Graff, the problem may be even more intractable. In their view, collaboration suffers because actors have failed to tackle, in an integrated fashion, the full range of issues – from the ethical, to the technical and proprietary – raised by stem cell science. As a result, Winickoff, Saha, and Graff conclude that this “exploding field...is characterized by a lack of any deeply collaborative architecture” [2, p. 57].

I agree with Winickoff, Saha and Graff’s contention that an integrated approach is needed. However, I want to investigate further into the proprietary dimension of stem cell science. In an age where commercializing publicly funded research is considered a legitimate goal, many have begun to ask whether that process, and the proprietary posturing it tends to command, is undercutting collaboration – that is, collaboration that would occur despite the added constraints of complying with ethical standards (which are well justified) and norms of scientific competition. Much of the focus at this point is upon figuring out if commercializing research actually poses a problem, and if so, exactly what the problem is. Is patenting bad in and of itself? Or is it more a matter of how patent rights are exercised or licensed? Are difficulties in obtaining biological materials a bigger concern? Maybe various challenges associated with experimental data – from storing it, to sharing and verifying it – that is neither published nor patented deserves more attention? The problem is tough to define. Yet architects of large-scale research initiatives are making decisions about these issues in real time while others still are beginning to ask whether those in a position to effect change are doing enough. Meanwhile, the science evolves.

Something, in my view, may have been missed in the debate over patenting in biomedical research – a novel type of cost that I term “patent canalization,” which speaks directly to patterns of scientific collaboration. To explain how this potential cost of commercialization differs from the “anticommons,” the dominant conceptual frame at the moment, I will analyze two nascent research initiatives – “Stem Cells for Safer Medicines” based in the United Kingdom, and the cross-border Canada–California “Cancer Stem Cell Consortium” – which escaped Winickoff, Saha and Graff’s survey. Before examining those two initiatives in detail, it is first necessary to provide some additional background about how different proprietary elements of stem cell science are intertwined.

21.2 Property Layers

Property rights, whether attaching to intellectual or more tangible objects, have been generating a growing amount of controversy in stem cell science. The bulk of the criticism relates to the sharing of stem cell materials, particularly, human embryonic stem cell (hESC) lines, and the entity, the Wisconsin Alumni Research Foundation (WARF), accused of inadequately doing so. WARF's (in)actions have attracted the most attention because it holds the patent rights over the process of the deriving of hESCs from human embryos,¹ a technique pioneered by University of Wisconsin scientists James Thomson during the 1990s [3]. That many in the research community have condemned the way WARF has handled the distribution of hESC lines dovetails with recent surveys where researchers in the life sciences generally have expressed greater frustration with material transfer agreements (MTAs) than patents per se [4–6]. However, it is important to understand that the two proprietary means of maintaining control and extracting rents from users of hESCs – patent rights and the MTAs that frequently accompany the exchange of biological materials, including hESCs – are mutually reinforcing [7, 8]. Patent rights give WARF the power to police those who would otherwise try to create hESCs on their own. MTAs preserve WARF's interest in any future technologies developed using the hESCs that WARF provides to others.

WARF's strategy is not exceptional. Coupling patent rights and MTAs is a common strategy among universities today [5]. Given the potential of hESCs to remedy human disease, however, the normal course is arguably not good enough. Learning from these experiences with hESC patents and materials exchange is in any event critical, especially as promising new directions of inquiry within stem cell science emerge, such as “induced pluripotent stem (iPS) cells” – a field that WARF also appears poised to control.² Equally integral to progress in stem cell science is access to stem cell “data.” Data is essentially information that is not necessarily eligible for patent (or copyright) protection,³ but that is critical to scientists' ability

¹ To my knowledge, these patent rights have thus far been granted in the USA only.

² In November 2007, two groups, one led by James Thomson at the University of Wisconsin and the other led by Shinya Yamanaka of Kyoto University in Japan, reported experiments in which they were able to genetically reprogram human adult cells into hESC-like, pluripotent stem cells (that is, these so-called “iPS cells” were similar to hESCs in morphology, proliferation, cell surface markers, pluripotent cell-specific gene expression, and telomerase activity) [9, 10]. While there are differences between the techniques and results of the Thomson and Yamanaka groups, the patent application filed by WARF on behalf of Thomson entitled “Somatic Cell Reprogramming” (US Patent Application No. 12/053,440 [with priority date 23 March 2007]) may encompass Yamanaka's work and thus assume priority. Yamanaka has already secured a Japanese patent on iPS cells and designated the USA, among other countries, for the purpose of his application under the Patent Cooperation Treaty (PCT/JP2006/324881). However, Yamanaka apparently failed to include an English translation with his application, allowing Thomson's application to assume priority [11–13].

³ In Europe, data or information can be protected as part of a database. This type of protection does not exist elsewhere, however.

to replicate the findings of others and work with well-characterized stem cell lines. The remainder of this background section thus explains these three overlapping elements – stem cell data, materials, and patents – in greater, but far from comprehensive, detail.

21.2.1 *Stem Cell Data*

Knowing the precise characteristics of a given stem cell population is fundamental to a researcher's ability to understand, utilize, and draw conclusions from any experiments involving those stem cells [14]. The researcher must not only have information about stem cell genomes, gene, and protein expression, but also information about the culture history of those cells, including “the particular growth factors that have been added to the media, the substrate of the cell culture,” whether any “implantable materials” or “genetic engineering vectors” were used, and the “duration of such events” [2, pp. 69–70].

Several groups and institutional actors have thus called for or sought to facilitate enhanced stem cell data sharing. For example, an international and interdisciplinary team known as the “Hinxton Group” issued a consensus statement in 2006 in which it encouraged researchers to “make cell lines and data...publicly available” [15]. The International Society for Stem Cell Research (ISSCR) has sought to advance the same goal through its journal *Cell Stem Cell* by making data (and materials) sharing a condition of publication [16]. Whereas the established registries or stem cell banks in the USA and the UK have failed to compile more than a list of the lines they hold, two more recent projects – the “International Stem Cell Forum” and the “European hESC Registry” – appear to be taking data collection for the purpose of stem cell characterization much more seriously [2, pp. 86–7].

Yet, to date, this invaluable data is seldom published or packaged together with samples of stem cells sent out by the various banks and registries that exist. The reasons are complex: there is a long history of poor data sharing in scientific circles for purely competitive reasons [17]. The data issue also intersects with disincentives associated with sharing stem cell materials and obstacles engendered by certain patent licensing practices (discussed below). In short, though, those in a position to leverage greater data sharing have neglected to do so. A lot of journals have not been proactive. The policy espoused by *Cell Stem Cell* appears to be the exception, not the rule, and a hard rule to enforce at that [2, pp. 61–2]. Moreover, research funding bodies which, apart from legislatures, have the greatest power to effect change, have essentially avoided the issue. Most notably, perhaps, the California Institute for Regenerative Medicine (“CIRM”), a body created by popular vote in 2004 with \$10 billion at its disposal, has so far failed to translate its broad endorsement of data sharing into concrete expectations that bind the recipients of CIRM grants [18].

21.2.2 *Stem Cell Materials*

That various stem cell registries and banks exist should, in principle, facilitate materials exchange relative to a world where researchers and institutions are left to negotiate the terms and conditions of material transfer on a case-by-case basis. However, the creation of an electronic registry or, more ideally, an actual bank of stem cell lines, will not by itself preclude materials-sharing problems from arising. It is simply a first step toward mitigating them and is likely to be ineffective without simultaneously tackling the intellectual property dimension of those materials. For example, WARF's participation made the creation of the US National Institutes of Health (NIH) "Human Embryonic Stem Cell Registry" possible, in principle, enhancing access to stem cell lines derived in accordance with the (now repealed) criteria established by former US President Bush [19, 20]. Even so, well after the creation of the registry, WARF was seen by many as imposing undue constraints upon the distribution of hESC lines by virtue of its patent rights and the licensing strategies it chose to pursue [2, 7].

In a positive move, WARF relaxed its stated policy positions in early 2007. Specifically, WARF (1) removed the stipulation embedded in the MTAs accompanying hESC samples sent to academic institutions that private sector sponsors of such research be required to negotiate a separate commercial license with WARF; (2) allowed inter-laboratory transfer of non-WARF hESC lines without WARF's permission; and (3) retracted its assertion that it was entitled to some portion of CIRM grants [21]. Moreover, the recent removal of former President Bush's restrictions around funding hESC research – restrictions that effectively strengthened WARF's control over the field by precluding the creation of new hESC lines with federal funds – may diversify the available sources of hESC materials.

However, several materials-related stumbling blocks remain. First, WARF's change in position "does nothing to change the fact that any entity seeking to commercialize hESC technology [in the USA] will have to negotiate a commercialized license from WARF" [2, p. 73]. To date, WARF has only entered into a handful of commercial licenses, and the broad set of exclusive rights that it has already granted to Geron Corporation, which funded Thomson's pioneering hESC work, may hamper any future negotiations [7]. Second, it is unclear what WARF has taken from its experience with hESC lines. Assuming it becomes the dominant patent-holder in respect to iPS cells and methods of manufacturing the same, there is a risk that WARF will seek to maintain a similar level of control, not only in the USA but also abroad, because iPS cells would not seem to trigger the same bars to patentability in Europe as hESCs [22]. Third, new avenues of stem cell research, most notably for my purposes in this chapter, research into "cancer stem cells" [23], require large-scale repositories of other kinds of biological materials (e.g., biopsied tumor samples). Unlike hESCs, those biological materials may not be in short supply. But optimizing them for research use nevertheless requires specialized facilities, highly qualified personnel, and complex information management systems, thus carrying a host of other costs.

21.2.3 *Stem Cell Patents*

As foreshadowed above, WARF's patent rights and the manner in which it has purported to exercise them is the dominant theme in the discussion around patenting stem cell technologies. That WARF might soon lay claim to foundational methods of making iPS cells as well as the cells themselves is likely a growing concern. However, as Karl Bergman and Gregory Graff have shown in depth, the global stem cell patent landscape is in fact radically more complex. Although the number of applications has declined in all three primary filing sites since 2001–2002, patenting of stem cell lines, stem cell preparations and growth factors remains “intense,” with ownership “fragmented across multiple organizations” [24, pp. 421–422].

Several questions follow from this. The first set of questions – the focus of most of the literature to date – concerns whether and to what extent this proliferation of patent rights is slowing progress in stem cell science. Winickoff, Saha and Graff, for instance, warn that “the complex set of technologies... necessary to control the early stages of differentiation... will not have many alternatives,” thus generating opportunities for patent owners to hold up and/or add a toll to the research and development process [2, p. 75]. In essence, the concern is that the transaction costs associated with getting access to the necessary stem cell data, materials, and patented technology will, sooner or later, trump scientific progress. This is the stuff of an “anticommons tragedy” – too much property begets underuse of a resource [25].

A second set of as-yet unrecognized questions asks not whether an overabundance of (ambiguous) property rights might impede the collaboration that we would like to see, but rather whether the fact of having a proprietary interest might lead those doing the science to collaborate less in the first place. If that proves to be true, the consequences for stem cell science may not simply be a loss of efficiency, but diminished quality. I term this second type of cost potentially associated with pursuing commercialization – perhaps only too soon or too much, not by definition – “patent canalyzation.” The next section of the chapter explains the concept in greater depth, contrasting it with the anticommons metaphor. As I will explain further below, it is critical to note that the evidentiary basis for patent canalyzation, like the anticommons, is minimal at present. Nevertheless, managing both risks makes sense.

21.3 Crowding Out Collaboration? The Tragedy of the Anticommons Versus Patent Canalyzation

Concerns about patenting in the upstream research space have been voiced for some time. Skepticism climaxed around one captivating hypothesis, “the tragedy of the anticommons” [25]. In essence, an anticommons can emerge when property rights

are many and messy. Michael Heller coined the concept after observing merchant kiosks thrive outside scores of empty stores on the streets of post-socialist Russia [26]. That the stores had been newly carved up by a variety of property rights was to blame. With the help of Rebecca Eisenberg, Heller cautioned that the same tragedy might befall biomedical research. The authors warned that the abundance of fragmented, overlapping, and ambiguous patent rights in the upstream research space “may lead paradoxically to fewer useful products for improving human health” [25, p. 701].

Evidence of such a tragedy has, however, been less than forthcoming. Using opinion surveys, John Walsh and colleagues have shown that academic researchers working in the fields of genomics and proteomics very rarely consider patents to be an impediment to choosing or pursuing a particular research project. Conversely, MTAs represent a bigger source of concern [4, 5]. In a study of stem cell scientists specifically using similar methodology, Timothy Caulfield et al. found essentially the same thing: minimal evidence of researchers’ experiencing patent-related problems [6].

What explains these findings, given the abundance of patent rights in both fields? The answer is surprisingly straightforward: in contrast to MTAs, which researchers tend to be highly aware of (because the costs of making materials in-house are often considered too high), researchers tend to have no immediate awareness of patent rights [5]. The situation may change in the future if either universities’ fear of liability or patent-holders’ willingness to test it increases [27]. For the moment, however, academic scientists largely ignore the possibility that they are potentially engaging in patent infringement. Thus, the absence of an anticommons.

The only evidence we have of an anticommons effect is indirect. Using a novel type of citation analysis, Fiona Murray and colleagues have shown that overall knowledge flows (measured by citations to a published article) decrease after a patent pertaining to the knowledge embodied in that same article is issued [28, 29]. The most recent and broadest study in this series, authored by Kenneth Huang and Fiona Murray and encompassing 1,279 human gene “patent-paper pairs,” showed that citations to the paper in each pair decreased by 5% post-patent grant – an effect that was exacerbated by an increase in patent thicket density, patent strength, and whether or not the genetic sequence in question was known to relate to some form of human cancer [29]. Even assuming these figures are something to worry about it, they remain puzzling. If most researchers are not cognizant of patent rights, why is a decrease in citations to published knowledge observed [30]? The findings of Murray and colleagues therefore merit cautious interpretation [27].

This is where patent canalization theory can step in and help fill the analytical void. The term is inspired by the work of Conrad Hal Waddington. In the late 1930s and early 1940s, Waddington began using the term “canalization” in connection with his notion of an “epigenetic landscape” – a metaphor that Waddington invoked to illustrate the deficiencies of biological discourse at the time. The terms “genotype” and “phenotype” only captured “differences between whole organisms... [and were in Waddington’s opinion] not adequate or appropriate for the consideration of differences within a single organism” [31, p. 156].

Following a series of experiments with amphibian embryos, Waddington proposed the concept of canalization to capture his inference that genetically mediated pathways dictate cell fate. Absent some sort of external “perturbation,” such fate could not be altered. It was entrenched or, as Waddington put it, *canalized*.

That basic scientific idea still holds some explanatory power,⁴ and I have chosen to steal from Waddington’s powerful notion of canalization in order to frame a novel cost potentially associated with patenting early stage research. As with canalization in the biological sense, the idea with patent canalization is that a researcher becomes increasingly locked into a particular line of scientific inquiry over time. The researcher can diverge from this path but it becomes progressively harder or, again, using Waddington’s language, requires an increasingly “significant perturbation,” to do so until the project’s fate is determined. This could apply regardless of whether a researcher condones commercialization of her work. Acceptance of a government grant carries an obligation to see the research project through. My theory, however, is that the process of commercialization (from disclosure of the invention to filing a provisional patent application, executing one or more licensing agreements, prosecuting the patent until it is issued, and attempting to generate new sources of revenue), whether realized in whole or in part, will exacerbate the level of canalization that we would otherwise see – assuming the researcher has some real-time awareness of the commercialization process. Researchers may be generally ignorant of patents held by others but they are presumably more familiar with commercialization activity tied to patents of their own.

This notion of patent canalization obviously borrows from another longstanding concept: path dependence. With the exception of Paul David’s work regarding how one sub-optimal technology (the “QWERTY” letter arrangement along the keyboard’s top row) became the industry standard [33], I am unaware of any scholarly work that invokes the concept of path dependence specifically to help explain observed quality tradeoffs in research or technology development. I therefore prefer to use this new term of patent canalization to underscore its focus upon the quality costs potentially associated with patenting, and to contrast those costs with the sort of transaction costs that anticommons analyses typically draw attention to.

Under anticommons analysis the impact, if any, of patenting upon other users of the knowledge that has been appropriated is the principal focus. In contrast, patent canalization is trained on the individual scientist(s) credited with the invention and tries to discern the impact of participating in commercialization pre- and post-patent grant. Does, for instance, the commercialization process lead researchers to become increasingly insular (observable, for instance, by citing

⁴For example, Shinya Yamanaka’s elegant experiment demonstrating how to induce adult stem cells into a pluripotent state through the manipulation of the cells’ transcription factors quickly calls to mind Waddington’s epigenetic landscape and notion of canalization – yet Yamanaka’s work was published in 2006. Yamanaka has himself subsequently adapted Waddington’s epigenetic landscape in order to explicate different models of iPS cell generation [32].

others less, or citing themselves or members of their own close-knit group more) in the course of their research? If so, what tradeoffs does that carry? In the context of molecular biology, for example, does it account for why so many of the studies done to date have been “underpowered,” that is, involve few patient samples and extend only short periods of time [34]? We know that current levels of experimental replication are exceedingly low insofar as “biomarker”-disease associations are concerned [35], and that many biomarker-disease associations have subsequently been shown to be spurious [34]. Does participation in commercialization make scientists less likely to experimentally validate or refute the work of their peers? If so, patent canalization may explain why most known biomarkers of human disease, genetic or otherwise, appear to be of questionable clinical validity [36].

Each of these tradeoffs is theoretical at present. The concept of patent canalization is only introduced here, not proven. And, directly contrary to what the preceding paragraph suggests, at least one group of scholars has suggested the exact opposite – that improving the clinical validity of biomarkers requires a further, although refined, embrace of patent rights [36]. My current research is aimed at empirically testing whether patent canalization, in fact, occurs. However, a few disparate pieces of evidence already exist that suggest that patent canalization may be real.

First, Jerry and Marie Thursby have found that researchers’ publications drop in years when an invention disclosure is made [37]. Second, Toby Stuart and Waverly Ding have shown that coauthor networks typically contract after an academic scientist transitions to a more openly entrepreneurial environment, either by founding a start-up company or becoming a member of a firm’s board of directors [38]. Third, Carlos Cosell and Ajay Agarwal have shown that the overall breadth of knowledge flows associated with university patents has diminished by more than 50% since the early 1980s [39]. Fourth, and finally, Tania Bubela and colleagues have demonstrated that although the stem cell scientists surveyed by Caulfield and colleagues may not report any problems relating to patents, there was a direct correlation between the number of patents a researcher held and how collaborative she was. Specifically, more patents equaled fewer co-authoring relationships [40].

Thus, as I have argued elsewhere, just because researchers are ignoring patents held by others, thereby avoiding the various transaction costs that paying attention to those patents would entail in the immediate to short term, does not mean that such a state of being is cost free (quite apart from the costs associated with patent prosecution, licensing, and any ensuing litigation) [41]. On the contrary, this more insular state of being *may* carry other costs – patent canalization costs – that could ultimately undermine the quality of one’s own scientific research and the quality of the field as a whole.

The remainder of the chapter examines how well two large-scale stem cell research initiatives – the Cancer Stem Cell Consortium (“CSCC”) and Stem Cells for Safer Medicines (“SC4SM”) – guard against both anticommons and patent canalization concerns.

21.4 Two Models of Stem Cell Commercialization

The CSCC and SC4SM initiatives differ in several respects. The origins of the former are bottom-up in that the CSCC was conceived by members of Canada and California's stem cell research communities together with leaders in their respective technology transfer and business communities [42]. Conversely, the SC4SM was born from the top down, growing out of a series of recommendations contained in the "Pattison Report" sponsored by the U.K. government [43]. And, whereas both initiatives are expected to foster commercialization, their philosophies differ sharply owing to the use they have chosen to put stem cells toward. The SC4SM's approach to commercialization is indirect: the initiative is designed to provide a "pre-competitive" space in which stem cells are used as predictive toxicology tools, streamlining the regulatory process for biopharmaceutical firms. In contrast, the CSCC is expected to directly yield a variety of commercial outcomes, including "build[ing] an exciting wave of new biotechnology companies based on CSCC discoveries" from the study of cancer stem cells [44]. Next, the specifics of these two different approaches are spelled out separately and then examined simultaneously under the lens of the anticommons and patent canalization.

21.4.1 *Cancer Stem Cell Consortium*

The CSCC was originally conceived as a cross-border research partnership between California and Canada with equal funding from both governments [45], but later shifted to a Canadian-based, -staffed, and -funded initiative [42]. A strong link with California-based researchers and institutions was, however, established in June 2008, when the CSCC concluded a three-year agreement with CIRM, the body charged with funding stem cell research in California, to formally explore opportunities for collaboration [46]. The first such opportunity was announced in February 2009, with the release of CIRM's request for applications for "Disease Team Research Awards" [46].

Before assessing the CSCC's collaborative potential and how the various strings attached to CIRM funding risk complicating the same, it is important to have a basic understanding of how the scientific focus of the CSCC differs from many other stem cell research initiatives. First, whereas hESCs and iPS cells are derived from embryonic and adult tissue sources, so-called "cancer stem cells" are isolated from patient tumor samples. Second, rather than trying to develop stem cells directly into therapies for a variety of degenerative diseases, the CSCC aims to increase our knowledge of the role played by such cells in the mechanism of various forms of cancer, in turn enabling scientists to identify diagnostic, prognostic, and predictive biomarkers to power more effective, "personalized" therapies of the future [44].

By virtue of its focus on cancer stem cells as opposed to the development of stem cell-based therapies, the projects the CSCC seeks to facilitate may avoid many of the "thoroughfares" that Bergman and Graff suggest could be complicated by

patent hold-up and anticommons issues [24]. Cancer stem cell-related research may, however, have patent thickets of its own to worry about, given the spike in applications of late [45]. The more immediate efficiency concern, discussed below, has to do with how different sources of funding promise to complicate general decision making related to intellectual property. In particular, ownership over any patent rights resulting from CSCC-funded research will be left in the hands of researchers' parent institutions [45]. This sets the CSCC apart from the second model of commercialization under scrutiny here, the SC4SM.

21.4.2 *Stem Cells for Safer Medicines*

The SC4SM, a public-private partnership founded with funding from five different governmental agencies and three multinational biopharmaceutical companies [47, 48], was established in late 2007 with the following objective: to enable the creation of a bank of stem cells, open protocols and standardized systems in stem cell technology that will enable consistent differentiation of stem cells into stable homogenous populations of particular cell types, with physiologically relevant phenotypes suitable for toxicology testing in high throughput platforms [49].

Like the CSCC, then, the SC4SM has no intention of developing stem cell-based therapeutics. Rather, the SC4SM initiative provides an interim, pre-competitive strategy, using stem cells as predictive toxicology tools in an effort to streamline the process of biopharmaceutical development. The SC4SM intends, in other words, to use stem cells to identify what we can call "toxicity biomarkers."

To accomplish this objective, the initiative creates something akin to a club or "protective commons." Entities participating in the initiative are entitled to utilize the intellectual property contributed by other participants as well as any new intellectual property generated as research projects unfold. Entities not participating in the SC4SM may get access to those resources, but they are not entitled to them *per se*.

To make this work, the SC4SM sets up two categories of intellectual property: "Background IPR" and "Foreground IPR" [50]. While ownership of Background IPR remains with each member of SC4SM, they are obligated to "grant to the [SC4SM] a royalty-free, non-exclusive, perpetual, worldwide and sub-licensable license of its Background IPR solely for the purpose and to the extent necessary for each Project to be undertaken and completed." The SC4SM is, in turn, responsible for sub-licensing such Background IPR to other "participants" in a research project to ensure its successful completion. If any new intellectual property results from the research project, that is, Foreground IPR, then the SC4SM will assume ownership of the same but must grant "a non-exclusive, perpetual, royalty-free, worldwide license" to use such Foreground IPR to each participant in that particular project as well as current members in the SC4SM more generally. Third parties that are external to the initiative may apply for and obtain a non-exclusive license to use such Foreground IPR, but subject to the SC4SM's discretion. In all three instances, Foreground IPR may only be utilized for "research purposes."

This framework has the potential to generate any number of inefficiencies. As explained next, however, the SC4SM appears to better manage those inefficiencies relative to those likely to be encountered by the CSCC.

21.4.3 Managing (Potential) Transaction Costs

A number of definition-related efficiency questions follow from the SC4SM's stated intellectual property policy. According to recent Court decisions, the distinction between "research use" and "commercial use" is increasingly difficult to draw. What exactly, then, does the term "research purposes" as used by the SC4SM encompass? Does it create ambiguity and thus set up future disputes? Moreover, is the distinction between Background IPR and Foreground IPR actually practicable? Or does it, too, suffer from ambiguity in the sense that some Foreground IPR will likely be unusable without access to related Background IPR as in the case of patented improvements?

A closer reading of the SC4SM's intellectual property policy reveals that these issues are, in fact, carefully addressed. While the definitions that delineate the boundary between research use and commercial use are somewhat circular, the distinction drawn is straightforward enough when read in light of the SC4SM's overall objective of fostering more efficient biopharmaceutical development by using stem cells as predictive toxicology tools. The default rule is that anyone holding a license to Foreground IPR cannot commercialize (i.e., sell, develop, dispose of, or authorize another party to do the same) stem cell technologies as predictive toxicology tools, but they can make full use of those technologies in their individual efforts to commercialize new diagnostics, drugs, and biologics. If they wish to undertake "direct exploitation" of Foreground IPR, which presumably includes commercializing some stem cell technology as a predictive toxicology tool, they must apply for a license from the SC4SM to do so. However, in order to ensure that any efficiency gains can be shared with other drug developers in the future, if granted, such a license must be non-exclusive. Similarly, in any situation where a party needs access to Background IPR in conjunction with Foreground IPR – whether in the course of an ongoing research project, to practice the Foreground IPR for research purposes, or, for the purpose of directly exploiting the same – the terms of SC4SM's intellectual property policy provide that such a license will be granted [50].

In this way, the SC4SM would seem to act as a clearinghouse of sorts, at least mitigating transaction costs that research institutions and companies would otherwise incur if access to such intellectual property had to be negotiated on a case-by-case basis. Whether the SC4SM is functioning as intended is not yet known. But an anticommons-type situation appears unlikely to arise, assuming the letter and spirit of the SC4SM's intellectual property policies are followed.

The SC4SM's uniform approach to intellectual property would seem to represent an advantage compared to the CSCC – at least for the time being. To reiterate, the CSCC does not intend to claim ownership over any resulting intellectual property. However, it does intend to play a supportive role as researchers and their parent

institutions begin to commercialize CSCC-funded research outcomes, specifically, by appointing a number of “Commercialization Officers” with “[cancer stem cell]-specific scientific knowledge and relationships with biopharmaceutical companies and investors in the [cancer stem cell] space” to “act as expert advisors and will be dedicated to working with [CSCC] funded researchers and institutions on a project (Research Team) specific basis” [44, p. 15]. Approximately 10% of the CSCC budget will be devoted to supporting commercialization in this, and presumably other, ways. This is significant. Many of the challenges endured by Canadian research institutions related to commercialization are due to a lack of resources, both financial and human. Nevertheless, the fact that several asymmetries in the laws and policies applicable to Canadian and California researchers and institutions – asymmetries that will be relevant in the context of any collaborative research project funded jointly by the CSCC and CIRM under the newly announced Disease Team Research Award program – remains a major potential problem.

To begin with, there are salient differences between the two jurisdictions surrounding “joint inventorship.” The request for applications recognizes this as an issue but fails to state what “specific arrangements as to Joint Intellectual Property” are to be made [46], leaving the issue open for negotiation (and thus potential delay and cost) until a research team comprised of Canadian and Californian scientists is awarded funding, and a situation involving joint inventorship actually arises.

Secondly, CIRM’s own intellectual property policies contain a number of provisions that are relatively foreign to typical technology transfer practice (in the USA as well as in Canada). In particular, for-profit applicants for funding are required to provide “plans” to ensure that Californians have affordable access to resulting stem cell technologies [51]. Moreover, all funding recipients must pay back to the State of California a predefined share of net revenues (from licensing and/or product sales) once certain thresholds are surpassed [51, 52]. Rather than clarifying whether and to what extent these and other requirements in force in California apply to Canadian researchers and institutions, CSCC authorities appear to have grafted loosely worded parallel obligations onto their own conditions of funding.

The request for applications, for example, states that the CSCC plans to “reserve March-in rights to ensure that [intellectual property] generated during the course of the project using CSCC funding can be fully exploited for the national benefit,” and require that award recipients “provide free access to the Canadian research community to all Publication Related Biomedical Materials generated during the course of the project” [46, p. 29]. While acceptable in principle, the present wording of these stipulations fails to capture several of the nuances built into CIRM’s intellectual property regulations around when March-in rights can be invoked, what precisely must be made available to the research community, and when. They are also potentially out of step with policies already applicable to Canadian research institutions in receipt of other funding from other bodies.

True, technology transfer officials are not unaccustomed to multi-institutional, if not also multi-jurisdictional, research initiatives. But that does not mean that research under the umbrella of the CSCC would not benefit from less complex intellectual property architecture. Indeed, efforts are already under way to develop an

“intellectual property framework,” serving as a tool to enable researchers and institutions to identify and work through various intellectual property issues depending on what sources of funding (and corresponding rules and expectations) are involved.⁵ If finalized and followed by recipients of funding from the CSCC, this framework may substantially negate the foregoing inefficiencies. If not finalized (or subsequently followed), the vaguely worded requirements applicable in Canada coupled with the more detailed requirements that govern in California may invite significant delays as technology transfer officials on both sides of the border wade through, interpret, and monitor compliance with the various policies that apply.

21.4.4 Minimizing (Potential) Costs to Research Quality

On the other hand, other features of the CSCC’s stated approach to commercialization may engender higher quality of research than the SC4SM, at least insofar as patent canalization theory holds. Both the CSCC and SC4SM are interested in biomarkers – diagnostic, prognostic, and predictive biomarkers in the context of the former and toxicity biomarkers in the latter. Also noted above, however, is the fact that the clinical validity of most biomarkers identified to date is suspect. There appears to have been a near-systematic failure to ensure that biomarker-disease associations are statistically robust and map onto meaningful clinical outcomes over time [36, 53]. The reasons for this are complex, spanning from gaps and ambiguities in regulatory frameworks, funding deficiencies, to the absence of the necessary large-scale bio-repositories and attendant information management systems [54] – the last of which the CSCC aims to address. But there is arguably also an intellectual property aspect to this quality problem, which I frame as patent canalization.

This concern may prove illusory. However, the important point for the time being is that the CSCC appears to be taking a proactive stance. Its architects have clearly recognized the impoverished state of the biomarkers field and, wittingly or not, have taken steps to negate the possibility of patent canalization, first by linking its genomics research program to “large-scale cancer resequencing programs such as the NIH-funded project called The Cancer Genome Atlas... project and the International Cancer Genome Consortium” – both of which aim to ensure “rapid and complete” release of data for use by all members of the global research community [55, 56]; and second, by stressing the importance of linking any identified cancer stem cell biomarkers with “clinical parameters such as patient prognosis and treatment outcome to firmly establish the clinical relevance of [cancer stem cell]s” [44, p. 9]. The CSCC, in other words, allows for experimental replication by independent research teams while at the same time directing the teams that it funds to strive for clinical validation. The CSCC thus

⁵ Personal communication with Angus Livingstone, Director, University-Industry Liaison Office, University of British Columbia.

promises to enable higher quality research than what would seem to be the *status quo* for biomarkers.

In contrast, the SC4SM, by seeking to claim Foreground IPR, is following the *status quo*. Again, this is a speculative concern. Because such newly generated intellectual property will effectively be treated like a club good, perhaps SC4SM partners will help each other with their homework, that is, validate the markers of biopharmaceutical toxicity that they each identify through stem cell modeling. The lack of language to that effect in the SC4SM's mission and policies is, however, somewhat disconcerting.

Given that the initiative is explicitly intended to be precompetitive, the more fundamental question is whether the pursuit of Foreground IPR is at all necessary to achieve its objective of streamlining the process drug discovery. Why not instead make the data available to all members, participants, and third parties via a "click-wrap" license or simply releasing the data into the public domain [57, 58]? Either mechanism would presumably be more cost-efficient than seeking Foreground IPR because it would shed the costs of patent prosecution and any resulting litigation as well as negate the need to review license applications for use of Foreground IPR. Instead, undertaking those (needless) responsibilities takes away from measures similar to those advocated by the CSCC to ensure biomarker quality.

21.5 Conclusion

To abstract a set of best practices around intellectual property and collaboration from the two models discussed here potentially obscures the importance of the broader cultural, political, and economic contexts in which any large-scale scientific research initiative is embedded. Three broad points nevertheless follow from the preceding analysis. The first two map primarily onto anticommons concerns whereas the third relates more to this new idea of patent canalization. All three should facilitate collaboration in stem cell science.

The first point is that inefficient distribution of all biological materials, not just stem cell lines, has long been recognized as a problem. Encouraging research institutions to adopt a model material transfer agreement has proven insufficient. Architects of research initiatives must therefore seek to make efficient materials exchange a stronger norm within the community of researchers and organizations that choose to participate in the venture. Instead of leaving MTAs to be crafted and negotiated by individual institutions as the need arises, standardized terms and conditions should be set by the initiative at the outset. Distribution of materials according to the same should, in turn, be made a condition of participation in the initiative with failure to do so triggering pre-defined consequences.

Secondly, all scientific research stands to benefit from greater levels of data-sharing. The more challenging decision for architects of a large-scale research initiative likely concerns how broadly data will be shared. Due to the perceived risk of "parasitic patenting" [57], several initiatives have opted to make data available

under click-wrap licenses rather than simply releasing the data into the public domain. Others contend that the risk of parasitic patenting has been exaggerated, and that broader data dissemination is a more immediate concern [58]. Research initiatives should specify a clear policy with respect to data-sharing while continuously re-assessing and balancing the risks of parasitic patenting versus incomplete, inefficient data sharing as research projects under their auspices unfold.

Finally, large-scale initiatives should promote a practice of not patenting “fundamental discoveries.” As several commentators have pointed out, and WARF’s rigid control of its hESC patented cell lines powerfully illustrates, patenting inventions that are considered foundational to a field of inquiry can hinder research progress. Patenting to ensure that an invention is commercialized, moreover, makes little sense when significant research and development is *not* required to “bring the invention to practical and commercial application” [59]. Such is the case where fundamental discoveries, especially research tools like cell lines, are concerned. Similarly, architects of large-scale initiatives should consider promoting a practice of not patenting biomarkers of unproven clinical validity, especially in the absence of counteracting measures and resources that promote replication and validation of scientific findings. If not, the vision of patent canalyzation that I articulate here may unfortunately materialize.

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Part VI
Patenting of Human Stem Cell-based
Inventions: Scientific, Ethical and
Regulatory Issues

Chapter 22

Legal Problems Raised by Patents on Human Stem Cell-Based Inventions

Paul L.C. Torremans

Abstract A non-expert audience may be surprised by the mere idea that inventions based on human stem cells can be patented. But the starting point is clearly that living material is patentable. Both US law, especially since the Supreme Court's decision in *Diamond v. Chakrabarty*, 447 U.S. 303 (1980), and EU law, where article 5(2) of Directive 98/44 on the legal protection of biotechnological inventions, [1998] OJ L213/13, reads 'An element isolated from the human body or otherwise produced by means of a technical process, including the sequence or partial sequence of a gene, may constitute a patentable invention, even if the structure of that element is identical to that of a natural element', have adopted this starting point quite a while ago. The fact that there is no exclusion in principle for living material does however not mean that any living material can be patentable in any circumstances.

This contribution looks therefore at how the conditions for patentability apply to human stem cell based inventions and in a second stage it considers the application of the morality clause to them.

In order for an invention based on human stem cells to be patentable the inventions needs to satisfy the requirements of novelty, inventive step and capability of industrial application. Novelty is always an issue when the invention is based on existing (human stem) cells, but the issue can be overcome if certain conditions are met. Inventive step similarly raises issues, but issues that can be overcome. Real issues arise in relation to the requirement of capability of industrial application. This requirement is traditionally somewhat undervalued, but potentially it can be crucial in an area such as patents for human stem cell-related inventions. In the United Kingdom, this potential was recently demonstrated in *Eli Lilly and Company v. Human Genome Sciences, Inc.*, (2008) R.P.C. 29.

And then finally attention turns to the thorny issue of the application of the morality clause in this area. The current approach is seen as unsatisfactory and an alternative approach is put forward.

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Keywords Stem cell • Inventions • Legal • Morality exception • Patentability

22.1 Introduction

A non-expert audience may be surprised by the mere idea that inventions based on human stem cells can be patented. But the starting point is clearly that living material is patentable. Article 27 (1) TRIPS¹ leaves no doubt about it and individual national patent law regimes cannot avoid this conclusion. In Europe Article 5 of the Biotech Directive² [1] confirms this by stating that human material in isolated form is patentable.³

But one should not derive from this straightforward starting point that patents on human stem cell-based inventions are completely non-problematic and straightforward. Even those inventions that come from an area of technology that is in principle patentable must meet the criteria for patentability – and here a rigorous application of these criteria is needed! Sufficiency in the disclosure of the invention may also be an issue.

These rules apply in all jurisdictions, even if local practices may differ somewhat. But especially in Europe one also has to consider excluded subject matter and issues of morality⁴ [2]. Under TRIPS, these areas of excluded subject matter are allowed on grounds of public policy and morality and both categories are hence tightly linked.

But before we examine each of these points in some detail, it is worth pointing out that one can, and arguably should, split the category of human stem cell-based inventions into a couple of subcategories. At the bottom end, one can distinguish between patents for the stem cells themselves and patents for research tools in the human stem cell area.⁵ At the top end, one finds treatments that involve human stem cells and products that derive indirectly from the use of human stem cell in their development. One could think of patents on induced pluripotent stem cells that include the products of cellular reprogramming such as the Yamanaka patent of the Japanese patent office. The way in which each of these requirements and points apply to each of these four subcategories may turn out to be rather different.

¹Agreement on Trade Related Aspects of Intellectual Property, administered by the World Trade Organisation

²European Parliament and Council Directive 98/44/EC on the legal protection of biotechnological inventions (1998) OJ L 213/13

³Non-naturally occurring living substances are patentable; this line is also clear in the US. See *Diamond v. Chakrabarty*, 447 US 303 (1980). In Canada see *Monsanto Canada Inc. v. Schmeiser* (2004) 1 S.C.R. 902, 2004 SCC34

⁴A. Plomer, P. Torremans, B. Knoppers, C. Denning, J. Sinden, and M. Levin (2006) *Stem Cell Patents: European Patent Law and Ethics Reports*, Report for the European Commission, available online at [www. Nottingham.ac.uk/law/StemCellProject/project.report.pdf](http://www.Nottingham.ac.uk/law/StemCellProject/project.report.pdf)

⁵In terms of USPTO practice one could think of the three early WARF patents and the Edinburgh patent

22.2 Novelty

Any invention that is to be patented should be new, i.e., it should not form part of the state of the art. In practice, few problems arise in relation to human stem cell-related patents on this point. Knowing that something, i.e., the stem cell, exists in nature is not enough to anticipate the invention. If human beings cannot access the existing material, it is not available to the public and in any case anticipation requires that what is applied for corresponds rather exactly to the potentially anticipating source. It is in this respect illustrative to recall the facts of the House of Lords' decision in *Asahi*⁶ [3]. The fact that the protein was known could not anticipate the patent for the genetically engineered version of the protein, i.e., a patent involving how to make the product. The legislature has also expressed a clear desire to patent first and further medical uses of a known substance or composition. What is envisaged emerges clearly from section 4A of the UK Patents Act 1977:

4A Methods of treatment or diagnosis

1. A patent shall not be granted for the invention of
 - (a) A method of treatment of the human or animal body by surgery or therapy, or
 - (b) A method of diagnosis practiced on the human or animal body.
2. Subsection (1) above does not apply to an invention consisting of a substance or composition for use in any such method.
3. In the case of an invention consisting of a substance or composition for use in any such method, the fact that the substance or composition forms part of the state of the art shall not prevent the invention from being taken to be new if the use of the substance or composition in any such method does not form part of the state of the art.
4. In the case of an invention consisting of a substance or composition for a specific use in any such method, the fact that the substance or composition forms part of the state of the art shall not prevent the invention from being taken to be new if that specific use does not form part of the state of the art.

Certain stem cell related inventions will also benefit from this kind of provision.

22.3 Inventive Step

Apart from being novel, the invention should also involve an inventive step, i.e., it should not be obvious to a person skilled in the art. Many earlier human stem cell-related patents have experienced problems in this respect. The use of known research techniques and tools and the fact that the claims remain relatively close to

⁶ *Asahi Kasei Kogyo KK's Application* (1991) RPC 485 (House of Lords, UK); see also *Kirin-Amgen Inc. v. Transkaryotic Therapies Inc.* (2003) RPC 31 (Court of Appeal, UK), (2005) 1 All ER 667 (House of Lords, UK)

the naturally occurring stem cells, makes it at least arguable that no inventive step is involved. This is a problem that occurs regularly in relation to early biotechnology patents and that also dominated the re-examination by the USPTO⁷ of the WARF⁸ patents that eventually lead to the patents being confirmed in slightly amended form⁹ [4]. Product claims that have no evident utility provide no technical contribution or solve no technical problem are obvious, too, even if this issue is more important in relation to the requirement that the invention must be capable of industrial application, to which we now turn¹⁰ [5].

22.4 Capable of Industrial Application

Thirdly, the invention must also be capable of industrial application. This requirement is traditionally somewhat undervalued, but potentially it can be crucial in areas such as patents for human stem cell-related inventions. In the United Kingdom, this potential was recently demonstrated in *Eli Lilly and Company v. Human Genome Sciences, Inc.*¹¹ [6]. The case concerned a request for the revocation of the HGS¹² patent for Neutrokine-a, the nucleotide and amino acid sequence of a novel member of anti-tumor ligand superfamily. The patent referred only to the protein sequences and was an early patent to protect the substance.

The relevant legal provisions set out the legal scene as follows.

Article 52 of the EPC¹³ provides, in relevant part:

(1) “European patents shall be granted for any inventions, in all fields of technology, provided that they are new, involve an inventive step and are susceptible of industrial application.”

Article 57 defines industrial application:

“An invention shall be considered as susceptible of industrial application if it can be made or used in any kind of industry, including agriculture.”

Essentially, this requirement is directed at ensuring that the invention has a real practical application.

⁷ United States Patent and Trademark Office

⁸ Wisconsin Alumni Research Foundation

⁹ See Knowles, Stem Cell Patents, see <http://www.stemcellnetwork.ca/uploads/File/whitepapers/Stem-Cell-Patents.pdf>, last visited 5th August 2009

¹⁰ UK House of Lords *Conor Medsystems Inc v. Angiotech Pharmaceuticals Inc* (2008) UKHL 49 per Lord Hoffmann

¹¹ *Eli Lilly and Company v. Human Genome Sciences, Inc.* High Court of Justice Chancery Division Patents Court (UK), 31 July 2008, (2008) EWHC 1903 (Pat), (2008) R.P.C. 29

¹² Human Genome Sciences Ltd

¹³ European Patent Convention

The European Biotech Directive¹⁴ [7] confirms that, in principle, biotechnology inventions are patentable. The Directive also addresses the question of industrial applicability. Recitals 23 and 24 read:

“23. Whereas a mere DNA sequence without indication of a function does not contain any technical information and is therefore not a patentable invention;

24. Whereas, in order to comply with the industrial application criterion it is necessary in cases where a sequence or partial sequence of a gene is used to produce a protein or part of a protein, to specify which protein or part of a protein is produced or what function it performs;”

And Article 5 of the Directive provides:

1. The human body, at the various stages of its formation and development, and the simple discovery of one of its elements, including the sequence or partial sequence of a gene, cannot constitute patentable inventions.
2. An element isolated from the human body or otherwise produced by means of a technical process, including the sequence or partial sequence of a gene, may constitute a patentable invention, even if the structure of that element is identical to that of a natural element.
3. The industrial application of a sequence or partial sequence of a gene must be disclosed in the patent application.

In *BDP1 Phosphatase/Max-Planck*¹⁵ [8] the EPO¹⁶ Board of Appeal provided guidance on the use and interpretation of the EPC provisions. The nature of Article 52 is that a practical application of the invention had to be disclosed:

“The requirement of Article 57 EPC that *the invention* “can be made or used” in at least one field of industrial activity emphasizes that a “practical” application of the invention has to be disclosed. Merely because a substance (here: a polypeptide) could be produced in some ways does not necessarily mean that this requirement is fulfilled, unless there is also some profitable use for which the substance can be employed.”¹⁷

and

“In cases where a substance, naturally occurring in the human body, is identified, and possibly also structurally characterized and made available through some method, but either its function is not known or it is complex and incompletely understood, and no disease or condition has yet been identified as being attributable to an excess or deficiency of the substance, and no other practical use is suggested for the substance, then industrial applicability cannot be acknowledged. While the jurisprudence has tended to be generous to applicants, there must be a borderline between what can be accepted, and what can only be categorized as an interesting research result which per se does not yet allow a practical industrial application to be identified. *Even though research results may be a scientific achievement of considerable merit, they are not necessarily an invention which can be applied industrially.*”¹⁸

¹⁴European Parliament and Council Directive 98/44/EC on the legal protection of biotechnological inventions (1998) OJ L 213/13

¹⁵*BDP1 Phosphatase/Max-Planck* (2005) T 0870/04 EPO

¹⁶The European Patent Office

¹⁷*BDP1 Phosphatase/Max-Planck* (2005) T 0870/04 EPO at paragraph [4]

¹⁸*Ibid.* at paragraph [6]

This was further elaborated upon in *Hematopoietic cytokine receptor/Zymogenetics*,¹⁹ where the EPO Board of Appeal held in Paragraphs 4 to 8 of its decision that:

4. “[...] patents being an incentive to innovation and economic success, the criterion of “industrial applicability” requires that a patent application describes its subject invention in sufficiently meaningful technical terms that it can be expected that the exclusive rights resulting from the grant of a patent will lead to some financial or other commercial benefit.”²⁰
5. “[...] the invention claimed must have such a sound and concrete technical basis that the skilled person can recognise that its contribution to the art could lead to practical exploitation in industry. It would be at odds with the purpose of the patent system to grant exclusive rights to prevent the commercial activities of others on the basis of a purely theoretical or speculative patent application. This would amount to granting a monopoly over an unexplored technical field.”²¹
6. “The board takes the view that, in the present context, the concept of “profit” should be seen in its wider sense of benefit instead of its narrower sense of financial reward. Accordingly, the expression “profitable use” should be understood more in the sense of “immediate concrete benefit.” This conveys, in the words “concrete benefit,” the need to disclose in definite technical terms the purpose of the invention and how it can be used in industrial practice to solve a given technical problem, this being the actual benefit or advantage of exploiting the invention. The essence of the requirement is that there must be at least a prospect of a real as opposed to a purely theoretical possibility of exploitation. Further, the use of the word “immediate” conveys the need for this to be derivable directly from the description, if it is not already obvious from the nature of the invention or from the background art. It should not be left to the skilled reader to find out how to exploit the invention by carrying out a research program. Not only is this the essence of the requirements of Rules 23e (3) and 27(1) (f) EPC, it also corresponds to the requirements of Articles 56 (the need to provide a non-obvious solution to a technical problem), 57 (the need to indicate how to exploit the invention), and 83 EPC (the need to provide a sufficient disclosure of the claimed invention). All those provisions reflect the basic principle of the patent system that exclusive rights can only be granted in exchange for a full disclosure of the invention.
7. Accordingly, a product whose structure is given (e.g., a nucleic acid sequence) but whose function is undetermined or obscure or only vaguely indicated might not fulfill the above criteria, in spite of the fact that the structure of the product per se can be reproduced (made) (cf. case of T 870/04, point 10 *infra*). If a patent is granted therefore, it might prevent further research in that area, and/or give the

¹⁹ *Hematopoietic cytokine receptor/Zymogenetics* (2006) T 0898/05 EPO Board of Appeal

²⁰ *Ibid.* relevant passage from paragraph 4

²¹ *Ibid.* relevant passage from paragraph 5

patentee unjustified control over others who are actively investigating in that area and who might eventually find actual ways to exploit it.

8. On the other hand, a product which is definitely described and plausibly shown to be usable, e.g., to cure a rare or orphan disease, might be considered to have a profitable use or concrete benefit, irrespective of whether it is actually intended for the pursuit of any trade at all. Thus, although no particular economic profit might be expected in the development of such products, nevertheless there is no doubt that it might be considered to display immediate concrete benefits.”

This analysis brought Kitchen J.²² to the following conclusion.

- The notion of industry must be construed broadly. It includes all manufacturing, extracting and processing activities of enterprises that are carried out continuously, independently and for commercial gain. However, it need not necessarily be conducted for profit, and a product that is shown to be useful to cure a rare or orphan disease may be considered capable of industrial application even if it is not intended for use in any trade at all. This aspect of the conclusion is rather favorable for human stem cell-related inventions of all categories. It will relatively easily be satisfied.
- The capability of industrial exploitation must be derivable by the skilled person from the description read with the benefit of the common general knowledge. Later information is not acceptable and the application must therefore provide sufficient guidance. This is probably not necessarily a problem for translational patent applications in relation to human stem cells, but the lower categories of inventions may be affected in case they are broad applications that essentially cover the stem cells without providing guidance on their specific use or utility (to use the American term²³).
- The description, so read, must disclose a practical way of exploiting the invention in at least one field of industrial activity. Again, the emphasis is on the required level of detail in the application itself. Broad theoretical information in its own right will not be sufficient. Again, lower earlier human stem cell-related patents are probably most vulnerable on this point.
- More recently, this has been re-formulated as an enquiry as to whether there is a sound and concrete basis for recognizing that the contribution could lead to practical application in industry. Nevertheless, there remains a need to disclose in definite technical terms the purpose of the invention and how it can be used to solve a given technical problem. Moreover, there must be a real prospect of exploitation that is derivable directly from the specification, if not already obvious from the nature of the invention or the background art. This conclusion

²² Mr Justice Kitchen was the judge who decided *Eli Lilly and Company v. Human Genome Sciences, Inc.* High Court of Justice Chancery Division Patents Court (UK), 31 July 2008, (2008) EWHC 1903 (Pat), (2008) R.P.C. 29. The conclusion is his conclusion in the case

²³ US Patent Law has a utility requirement, whereas European patent law requires capability of industrial application. According to the TRIPS Agreement there is no difference in substance

clearly puts a high threshold in place and one that can more easily be satisfied by the higher subcategories of applications.²⁴

- That means conversely, that the requirement will not be satisfied if what is described is merely an interesting research result that might yield a yet-to-be identified industrial application. A speculative indication of possible objectives that might or might not be achievable by carrying out research is not sufficient. Similarly, it should not be left to the skilled reader to find out how to exploit the invention by carrying out a research program.
- It follows that the purpose of granting a patent is not to reserve an unexplored field of research for the applicant nor to give the patentee unjustified control over others who are actively investigating in that area and who might eventually find ways actually to exploit it.
- If a substance is disclosed and its function is essential for human health, then the identification of the substance having that function will immediately suggest a practical application. If, on the other hand, the function of that substance is not known or is incompletely understood, and no disease has been identified which is attributable to an excess or a deficiency of it, and no other practical use is suggested for it, then the requirement of industrial applicability is not satisfied. This will be so even though the disclosure may be a scientific achievement of considerable merit. In stem cell terms, it must be understood how they operate and how any product based on them will work.
- Using the claimed invention to find out more about its own activities is not in itself an industrial application.
- Finally, it is no bar to patentability that the invention has been found by homology studies using bioinformatics techniques, although this may have a bearing on how the skilled person would understand the disclosure. If these techniques are or become applicable to human stem cells, nothing bars a successful patent application, but in their own right these techniques are normally not able to demonstrate that the invention is capable of industrial application.

On the facts, HGS therefore failed to demonstrate that its invention was capable of industrial application. A patent will not be granted for an idea that is mere speculation.²⁵ Whatever the merit of the discovery of Neutrokine-a, the specification contained no more than speculation about how it might be useful. It did not teach the person skilled in the art how to solve any technical problem and its teaching as to the range of applications of Neutrokine-a was implausible. Moreover, the claims to therapeutic and diagnostic products were insufficient in any event.

Also in relation to human stem cell-related inventions, such a thorough application of the requirement that the invention should be capable of industrial application

²⁴ One could think of patents on induced pluripotent stem cells that include the products of cellular reprogramming such as the Yamanaka patent of the Japanese patent office

²⁵ Product claims which have no evident utility provide no technical contribution or solve no technical problem are obvious, too; see UK House of Lords *Conor Medsystems Inc v. Angiotech Pharmaceuticals Inc* (2008) UKHL 49 per Lord Hoffmann

should stop unduly broad early patents that block further developments. And first steps-research tools should not easily be patentable as such,²⁶ which provides a strong filter and avoids the monopolization of a whole field of research on the back of a single patent that is too broad and non-specific. On this point, ethical concerns about monopolization run parallel to patent concerns.

22.5 Insufficiency

In US patent law, the patent specification must be complete enough so that a person of “ordinary skill in the art” of the invention can make and use the invention without “undue experimentation.” Canadian patent law turns this into the requirement for there to be an ability to fully reveal or explain the invention in a manner sufficient to allow others to reproduce the patented subject matter. And in Europe the same idea is found in Article 83 EPC²⁷ [9], and Article 100 EPC makes disrespect of the rule, i.e., insufficiency, a ground for the revocation of the patent. Insufficiency should stop the patent from being granted and if granted in error it provides grounds for revocation. One should note, however, that it is only the specification and its shortcomings that give rise to this threat; there is no comparable provision to ensure the clarity of the claims made for an invention. Insufficiency is therefore a serious risk to a patent applicant who is naturally inclined to disclose as little as possible, making the issue of sufficiency a key issue in many patent cases, especially as a counterattack argument in many infringement cases²⁸ [10].

This requirement begs several questions, not least of which is what degree of detail is in fact going to be necessary. Obviously this will ultimately depend on the facts of each case, but some guidance is given by the old UK case *No-Fume Ltd v Frank Pitchford & Co Ltd*.²⁹ An ashtray designed to retain the smoke inside itself was the subject of this dispute. Romer LJ in the Court of Appeal upheld the validity of the patent even though the application, in describing the various features of the ashtray, failed to indicate the size and relative proportions of the various parts of the ashtray. He stated³⁰ that it was not necessary that the specification have all the detail in it that might be expected in the detailed specification given to a workman in order to make an article. Rather, it was sufficient if the workman could reach the desired result through a combination of the information in the specification and the common knowledge of his trade using trial and error if necessary to achieve the desired

²⁶ See the problems raised by Bergman and Graff, “The Global Stem Cell Patent Landscape: Implications for efficient technology transfer and commercial development,” (2007) 25 *Nature Biotechnology* 419

²⁷ See *Philips* (2007) T 1191/04 EPO Board of Appeal

²⁸ See, e.g., *Kirin-Amgen Inc v Transkaryotic Therapies Inc* (2003) RPC 31 (United Kingdom, Court of Appeal), (2005) 1 All ER 667, (2005) RPC 9 (United Kingdom, House of Lords)

²⁹ (1935) 52 RPC 231

³⁰ *Ibid* at 243 and 245

result. Or, in more modern EPC language taken from the original Rule 27, the description “shall disclose the invention, as claimed, in such terms that the technical problem...and its solution can be understood.”³¹ That implies that the hypothetical addressee is not a person of exceptional skill and knowledge...not to be expected to exercise any invention nor any prolonged research, inquiry or experiment, but “prepared to display a reasonable degree of skill and common knowledge in making trials and to correct *obvious* errors in the specification if a means of correcting them can readily be found”³² [11].

Or, as the Technical Board of Appeal of the EPO put it recently, the requirements of sufficiency of disclosure will be satisfied only if the invention can be performed by a person skilled in the art using common general knowledge and having regard to further information given in the patent³³ [12]. While the skilled person can put in some work on the back of his common knowledge and engage in trial-and-error experimentation, the amount of work to be put in cannot be unreasonable under the circumstances. The skilled person will try to make the invention work. Insufficiency will therefore not follow, e.g., if he realizes that one method would work and another would fail if the claim is broad enough to include both methods³⁴ [13], but he cannot engage in inventive activity to fill gaps in the common general knowledge he possesses, augmented by the information given in the patent³⁵ [14]. Despite all this, the question of whether or not the specification was sufficient remains highly sensitive to the nature of the invention. It is therefore important to identify the invention in a first stage and to decide what it claims to enable the skilled man to do. That simplifies the next question as to whether the specification enables the skilled man to do that³⁶ [15].

However, the specification must enable the invention to be performed and this requirement covers not just a single embodiment. The United Kingdom case *Biogen v Medeva*³⁷ [16] teaches that the specification must enable the invention to be performed to the full extent of the monopoly claimed. This may well be crucial in relation to human stem cell-related patents. Many of the patents, especially in the first two sub-categories, will have many embodiments. The patent must meet the requirement of sufficiency for each of these embodiments, i.e., for the full extent of the monopoly claimed. Speculative claims to future developments and uses will therefore fail the sufficiency test. Translational inventions on the other hand are almost by definition more specific and should face fewer problems related to the sufficiency requirement.

³¹ See the judgment of the UK Court of Appeal in *Mentor Corp'n v Hollister Inc* (1993) RPC 7

³² See the UK case *Valensi v British Radio Corp'n* (1973) RPC 337 at 377 (Court of Appeal)

³³ Case T-1121/03 *Union Carbide/Indicator Ligands* (2006) EPOR 49

³⁴ *Kirin-Amgen Inc v Transkaryotic Therapies Inc* (2005) 1 All ER 667, (2005) RPC 9 (United Kingdom, House of Lords)

³⁵ *Halliburton Energy Services Inc v Smith International (North Sea) Ltd* (2007) 30 (2) IPD 30009 (United Kingdom, Court of Appeal)

³⁶ *Biogen v Medeva* (1997) RPC 1 (United Kingdom, House of Lords)

³⁷ (1997) RPC 1 (House of Lords, per Lord Hoffmann)

22.6 Excluded Subject Matter and Morality

Particularly in Europe,³⁸ not all inventions that have passed the requirements of novelty, inventive step and capability of industrial application will be patented. Certain subject matter is excluded, mainly on the grounds of morality. On the other hand, US patent law does not include a specific morality clause, and Chinese law has adopted a permissive and flexible approach, too. Coming back to Europe, it would lead too far to go into detail here, but the main concepts are reflected in articles 5 and 6 of the Biotech Directive³⁹ [17]. The EPO has in turn translated these exclusions on grounds of morality into its own rules. The main problem with this approach is that it contains no definition of the appropriate moral standards.

22.6.1 No Common Standard of Morality

There is an (unwarranted) assumption that such a common European standard of morality exists and that patent law can simply use it. Different practices in different member states and national patent offices prove otherwise, and it is relatively easy to demonstrate that such a single European concept of ethics and morality does not exist. And one should note that the Opposition Division seems to agree in its decision in the Edinburgh case. From an EU law point of view, in such cases where there is no common or commonly defined concept, EU law has used the concept of a referral back to national law and standards in order to give meaning to a term used in EU legal provisions. It is submitted that this may also be the way forward when it comes to the concept of morality when one needs to apply it to the issue of the patentability of stem cell related inventions. There is case law from the Court of Justice of the European Communities that supports such a solution. Suffice it to quote from *R v Henn and Derby*:

“Under the terms of Article 30 of the Treaty the provisions relating to the free movement of goods within the Community are not to preclude prohibitions on imports which are justified *inter alia* ‘on grounds of public morality.’ *In principle it is for each Member State to determine in accordance with its own scale of values and in the form selected by it the requirements of public morality in its territory*”⁴⁰ [18].

And in case one would doubt the relevance of these statements in relation to the Biotech Directive, the Court of Justice ruled as follows in *The Netherlands v Parliament and Council*:

³⁸ See Xi Jin, Lin Zheng, Ruo-heng Zheng & You-ming Li, “China’s policies on stem cell research: an opportunity for international collaborations,” available online at <http://www.stemcellcommunity.org/metadot/index.pl?id=3051> (last accessed 9th August 2009)

³⁹ See A. Plomer, P. Torremans, B. Knoppers, C. Denning, J. Sinden, and M. Levin (2006) *Stem Cell Patents: European Patent Law and Ethics Reports*, Report for the European Commission, available online at www.nottingham.ac.uk/law/StemCellProject/project.report.pdf

⁴⁰ Case 34/79 *R v Henn and Derby* (1979) ECR 3795, paragraph 15 (emphasis added)

“As regards, first, Article 6 of the Directive, which rules out the patentability of inventions whose commercial exploitation would be contrary to *ordre public* or morality, it is common ground that this provision allows the administrative authorities and courts of the Member State a wide scope for manoeuvre in applying this exclusion.”

“However that scope for manoeuvre is necessary to take account of the particular difficulties to which the use of certain patents may give rise in the social and cultural context of each Member State, a context which the national legislative, administrative and court authorities are better placed to understand than the Community authorities” ⁴¹ [19].

The EPO nevertheless has to apply these broad exclusions to individual applications, irrespective of a common definition or concept of morality. In that respect, it has a number of options open to it at a structural level.

22.6.2 *Extreme Approaches*

Since such uniform concept of morality does not exist, the EPO can apply a minimum test. This would mean that the morality clause would only block the grant of a patent for a biotechnological invention if the exploitation of the invention would offend against the morality or *ordre public* of all member states of the European Patent Convention. This also fits in with the idea that morality clauses must refer to the moral principles of the whole population, i.e., here all the member states. Straus has defended this position by arguing that it shows respect for sovereignty of the contracting states to uphold their own morality concepts. In his view, the acceptability of an invention in even one contracting country would constitute evidence of absence of a European wide morality and *ordre public*. On this ground he considers that such acceptability should eventually lead to at least dismissing objections on grounds of morality⁴² [20]. Alternatively, one could adhere to the view that the EPO should apply a maximalist test. An invention the exploitation of which is morally unacceptable in one member state should therefore not be patented. This view is based on the idea that any other approach would lead to the grant of a patent as part of the bundle of national patents that is in breach of the moral principles to which the country concerned adheres. In this view, the EPO should not assist in the creation of such a situation.

22.6.3 *A Preferred, but Still Extreme, Solution*

It is submitted that the EPO should adopt the minimalist approach if it has to choose between the two extremes. In the end a bundle of national patents is granted and it

⁴¹ Case C-377/98 *The Netherlands v Parliament and Council*, paragraphs 37 and 38 (emphasis added)

⁴² Straus, “Ethische, rechtliche und wirtschaftliche Probleme des Patent - und Sortenschutzes für die biotechnologische Tierzüchtung und Tierproduktion,” (1990) GRUR Int., 913

would be unfair to the applicant if its application for a patent in a number of member states were to be turned down on the basis of morality concerns that do not apply to that member state. And the revocation procedure can provide an adequate solution if a patent is granted in respect of a member state where morality concerns apply. Such a patent becomes the equivalent of a national patent upon grant and can therefore be the subject of national revocation procedures. Revocation can then take place on the grounds that the invention was not patentable in the first place because its exploitation would infringe the morality clause. This may seem a weird result, whereby different concepts of morality apply at different stages, but it is submitted that this is the inevitable result of the fact that upon grant the nature of a European patent changes back to a national patent.

The WARF application in relation to human embryonic stem cell-related inventions is a case in point. The patent was granted, for example, in Sweden as Swedish patent number SE526490 (Applicant: Wisconsin Alumni Research Foundation, Priority: US 1999-11-08 09/435578). This patent concerns a method for differentiation of human embryonic stem cells into hematopoietic cells. When granting this patent the subject whether such claims complied with Swedish Patents Act. Section 1.c.3. (e.g., the implementation of directive 98/44/EC article 6.2.c.) was considered. The conclusion was that it did, for the following reason: "To produce human embryonic stem cells, human embryos are required. However, the present method does not require that the stem cells need to be produced from embryos as a consequence of the invention, since the method can be performed using already existing (deposited) stem cells." The Swedish view was that the commercial exploitation of this method does not need the use of a human embryo – the stem cells may have been isolated – for example, for legitimate research purposes – long before the invention was made. The object of the provisions in, for example, Art. 6.2.c was to avoid a repetitive use of the humans or parts of humans such as embryos, thus leading to an instrumentation of humans/embryos. This invention is not directly linked to the use of an embryo and moreover does not repeatedly need human embryos. Accordingly, the Swedish concept of morality did not hinder the grant of the patent, whereas the EPO has many more problems with it.

On that basis, a minimalist approach should lead to the grant of the patent by the EPO, as not all member states object to it. Revocation proceedings in those member states that have problems with it can then follow and one could of course expect a rational applicant not to pursue the application in respect of those member states where revocation can be expected with a reasonable degree of certainty. The EPO could even advise the applicant in that sense and steer towards such a conclusion.⁴³ In Dr. Ulrich Schatz's view, if an invention is contrary to *ordre public* or morality prevailing in only one or a part of the designated states, the applicant may, on his own initiative or following a corresponding ruling of the examination division, withdraw the designation of the states in question while maintaining it for the

⁴³ See Schatz, in: Singer/Stauder, *The European Patent Convention. A Commentary*, Volume 1 (Carl Heimanns Verlag: Cologne, 2003), Section 22, at 91

others. In this way he acquires protection in the other states. Dr. Schatz then veers to the maximalist approach if the applicant does not make use of that opportunity and argues that the application must then be refused as a whole. This conclusion does, however, not logically follow.

Perhaps more importantly, the EPC system is not just a national system of its equivalent and that in the presence of a cross-border element, the rules of private international law must be applied. Apart from producing desirable results in substantive patent law, the minimalist approach is also in conformity with the principles of private international law that were set out above, whereas a maximalist approach goes against them. A maximalist approach in a sense pretends that the EPC system is a single jurisdiction with no external influence or links, which the very provisions of the EPC turn into a conclusion that cannot be upheld.

A minimalist approach may therefore be the way forward rather than the maximalist approach. But all this will nevertheless result in a refusal to grant certain patents that could have been granted on a national basis. The negative impact of this barely acceptable point at first sight is limited in practice. Applicants will indeed still have the opportunity to apply for national patents and even on the basis of the current⁴⁴ EPC for a European Patent for those countries that would accept the grant of the patent. One should add, though, that national applications are by no means the preferred route of the researchers and the industry in this field. This is another argument against a generalized application of the maximalist approach.

22.6.4 The Ideal Solution – Pushing It One Bit Further

It may, however, be possible for the EPO to go beyond a minimalist approach. Indeed the minimalist approach could be turned into a distributive application approach. Since in terms of morality there is a referral back to national concepts of morality, the EPO could test each application under the national standards of morality in each of the member states in which the applicant wants to be granted a patent for the invention. The patent would then only be granted for those member states with whose concepts of morality the application complies. Going back to the example of the WARF application, this would, for example, be granted for Sweden, but not for other member states whose morality concept rules out patents for embryonic stem cell-related patents altogether. This would of course require the EPO to compile information on morality standards in the member states, but this burden has to be assumed by it in any case, even if it applies a maximalist approach. National research ethics committees can be a useful source of information in this respect, as they will have to authorize the research that leads to the patent application. It is, however, clearly understood that the invention and its exploitation may lead to additional ethical and moral concerns when compared to the research that led to the invention. It is an

⁴⁴ Without a need for amendment *de lege ferenda*

inevitable consequence of Article 53 EPC in its current form whatever approach one prefers. The mere absence of a substantively defined concept of morality makes this exercise unavoidable, however difficult or impossible the EPO may think it is. Such a distributive application approach would avoid the revocation option that goes with a pure minimalist approach and the potential political difficulties that go with it.

But such a distributive approach is not only desirable from a policy point of view, it also fits in with substantive patent law and with the rules on private international law that were set out above. The EPC system results in the grant of a bundle of patents, one per member state applied for, and the consistent application to each of these patents of the local concepts and rules on morality is therefore fully in line with the choice of law rules in private international and the concept of public policy in that respect⁴⁵ [21].

22.6.5 The Test Case: WARF Before the Enlarged Board of Appeal

Unfortunately, this suggested approach was not taken up by the EPO in the WARF case. No doubt also due to procedural issues, the Enlarged Board⁴⁶ saw the case as a mere technical case on the interpretation of the EPO Rules. The crucial element in the Enlarged Board of Appeal's decision in the WARF case is in its own view the interpretation of Rule 23d(c). It is worth looking at the arguments that are advanced by the Enlarged Board to support its view that the WARF application does involve "uses of human embryos for industrial or commercial purposes." At the start of its analysis, the Enlarged Board also states that the EU legislator must have wanted to rule out any commodification of the human embryo,⁴⁷ but it fails to pick up on the fact that commodification as a concept refers, in all almost cases, to repeated use.

22.6.5.1 The Meaning of Use and Research

The discussion then turns to the concept of "uses" of the human embryo. Further on in the decision, the Enlarged Board will effectively fill in the concept of "use," but at this stage it is glossed over. The fact that the use needs to be for industrial or commercial purposes and that it has to be use of a human embryo take center stage. But via the

⁴⁵ See A. Plomer and P. Torremans, *Embryonic Stem Cell Patents: European Patent Law and Ethics*, OUP (2009), Ch10

⁴⁶ The Enlarged Board of Appeal handles questions where the law needs to be interpreted or explained, e.g., because a new set of facts has arisen, or because the Boards of Appeal have given different interpretations

⁴⁷ Case G 0002/06 *Wisconsin Alumni Research Foundation*, decision of 25th November 2008, at para 18

back door, a discussion on the relevance of research funding raises the fundamental question of whether “uses” equals “all uses” or rather “certain uses” enters the debate. The applicant raised the point that the EU funds certain types of stem cell research and that that includes research based on embryonic stem cells that were at an earlier stage extracted from a human embryo. In the applicant’s view, this point is relevant, even if the funding does not extend to the extraction part and is therefore selective in nature. In the view of the Enlarged Board, this does not offer support for the position of the applicant.⁴⁸ By brushing this argument to one side, the Enlarged Board seems to suggest that the word “uses” must cover “any use.” But surely the argument does not offer support for the Enlarged Board’s view that any use that starts with embryonic material is by definition covered by the concept of “uses” of the human embryo for industrial or commercial purposes. Such a view presupposes on top of that a moral consensus at the European level that holds that any use of embryonic material outside a strict pro-creational context is immoral that was then reflected in the provisions of the Directive. That would also mean that that consensus, for example, now bars the non-implantation of super-numerous embryos in the course of IVF treatment, as the alternative would be that they are disposed of, i.e., effectively involving their destruction. That is clearly not the case and never was the impact of the Directive. The fact that a number of member states allows the use of super-numerous embryos for research purposes, which may involve the extraction of human embryonic stem cells and the fact that the EU funds the research that follows must therefore at least mean that there is no moral consensus that can be read into the Directive in support of the Enlarged Board’s view that all uses of the human embryo are covered by the exclusion. The Enlarged Board also skirts around the fact that the Common Position that led to the Directive specifically stipulates that only “certain uses” are morally objectionable and therefore excluded⁴⁹ [22]. Some uses of the human embryo are therefore clearly acceptable, but the Enlarged Board fails to take this into account in an adequate way. It only refers to permitted uses when it points out that inventions involving uses for therapeutic and diagnostic purposes that are applied to the human embryo and are useful to it are not covered by the exclusion. The case where there is a benefit to the embryo itself is clearly an example of a scenario where a patent is not excluded,⁵⁰ but it is not a complete answer to the issues that are raised and which the Enlarged Board fails to address.

22.6.5.2 The Meaning of the Term “Embryo”

Next the decision turns to the specific meaning of the term “embryo.” The absence of a definition in either the Directive or the Rules creates a problem of interpretation. The

⁴⁸ Ibid

⁴⁹ Common Position (EC) No. 19/98 adopted by the Council on 26 February 1998 with a view to adopting Directive 98/44/EC of the European Parliament and of the Council on the legal protection of biotechnological inventions. (1998) OJ C 110/17 (8.4.1998)

⁵⁰ Case G 0002/06 *Wisconsin Alumni Research Foundation*, decision of 25th November 2008, at paras 26–27

Enlarged Board takes exception to the suggestion by the applicant that one should effectively restrict the exclusion to embryos of 14 days or older, in accordance with usage in the medical field. In its view, giving such a restrictive meaning to the term “embryo” is problematic. That may well be the case in the absence of a political consensus in the Directive, but the fact that the Enlarged Board solves the matter by effectively putting in place an extremely extensive definition that starts as it were from the fertilized egg-cell stage is equally problematic. If the matter is in the words of the Enlarged Board “a question of fact in the context of any particular patent application” one was at least entitled to a substantive argument as to why the legislative intent of the European legislature had to be interpreted in this way in this particular case. No such argument is offered and no explanation given for the extremely extensive definition that the Enlarged Board effectively applies in this case.⁵¹

One could of course argue that the Enlarged Board does not define the term “embryo” at all. From that perspective, the Board simply refused to adopt the one restrictive definition that was put to it and proceeded to leave the term undefined. But by rejecting the one restriction that was proposed, the Board implicitly adopts a wide interpretation. The Board itself refers to national definitions that are more restrictive. The question how to define the term “embryo” is therefore a very real live question in Europe. If one then considers that the EPO itself has argued that the exception concerned is a specific technical exception, as seen above, one has no option but to conclude that a definition of the term is required. Leaving it undefined was not an option that was really open to the Board. By rejecting any restriction that was proposed it effectively applies an extensive definition, without properly defined borders.⁵²

22.6.5.3 The Definition of “Use of the Embryo”

Leaving the concept of an “embryo” behind the discussion moves on to consider the use of the embryo and specifically whether the use of the embryo must be claimed to be covered by the exclusion. Whether the exclusion only applies to the content of the claims of the application is therefore the first question that arises. The Enlarged Board correctly points out that the exclusion does not use the term “claim,” but instead talks about the “invention.” What are the implications though of the use of such terminology? It is clear that the invention is broader than the claims and also involves the technical teaching that underpins them. One could say that everything that is needed to satisfy the sufficiency requirement in patent law is included in the “invention.” But the Enlarged Board then goes on to say that “before human embryonic stem cell cultures can be used, they have to be made.” And since that backward-looking exercise eventually leads to the point where a stem cell that is later put in culture is extracted from an embryo that is therefore destroyed, the invention is covered by the exclusion and by definition immoral. This conclusion does not follow. The EPO has never looked at

⁵¹ Ibid, at paras 19 and 20

⁵² Ibid, at para 20

the origin of raw material before and the origin of the biological (and eventually human) material that eventually led to an invention in respect of which a patent is applied for has never been the object of enquiries. Surely, material that originates in organs or genetic material that has been removed without the proper consent of the patient, or that has been obtained in breach of the Convention on Biodiversity and the national measures that flowed from it, must also lead to moral complications, but that has never stood in the way of the granting of a European patent.

The Enlarged Board argues in support of its unduly extensive view that:

“To restrict the application of Rule [...] 23d(c) [...] EPC to what an applicant chooses explicitly to put in his claim would have the undesirable consequence of making avoidance of the patenting prohibition merely a matter of clever and skillful drafting of such claim.”⁵³

This argument does not hold water. It applies in as far as it is in essence a sufficiency argument. The claims are neither here nor there. The whole invention is to be taken into account in as far as the sufficiency threshold needs to be met. But it is simply not correct to argue that skillful drafting of a claim will on its own allow one to get away with circumventing the exclusion. It is, on the other hand, the right of the applicant to restrict its application to an invention that consists of a further development of existing technology and that does not cover the gathering or creation of the required raw materials in a laboratory or elsewhere. Applying the exclusion from patentability to such cases where the problem lies upstream effectively means that patent offices go beyond their role in society and start to police overall morality standards in public life. That is not the role that has been given to them and it usurps the role of other instruments and institutions in society. Patent offices should restrict themselves to the inventions for which an application is made. Whether or not it is morally acceptable that human embryonic stem cells be “available” for non-procreative purposes is arguably a decision that goes beyond a mere patent focus and it better left to others outside the patent office.

The Enlarged Board tries to overcome that point by stressing the fact that the only route to obtain the human embryonic stem cells necessarily involved the destruction of the embryo and that at the relevant time, no alternative route was known or available.⁵⁴ While this makes the case more compelling, it does not convince, as it does not overcome the objections set out above. It is also seriously arguable that such an approach would effectively be in breach of article 27(2) of the TRIPS Agreement, a point to which we will return later.

22.6.5.4 Industrial or Commercial Purposes

The fundamental issue here is how to define the concept of industrial or commercial purposes in the context of use of the embryo for these purposes. Unfortunately, the Enlarged Board of Appeal does not engage the major arguments in that debate.

⁵³ Ibid, at para 22

⁵⁴ Ibid, at para 23

It suggests that the only permitted purposes, i.e., the ones that are not industrial or commercial, are those that are beneficial to the embryo. Diagnosis or treatment as applied to an embryo surely is not excluded, but it is also hardly relevant in this context. A treatment (or diagnosis) that is beneficial to the embryo will hardly ever involve “use” of the embryo and cannot therefore be the criterion to distinguish industrial or commercial use from permitted use. No attempt is made to properly define industrial or commercial use of an embryo and fundamental questions such as the one whether these terms necessarily involve repeated use of the embryo are not even addressed. Instead the argument is raised that making the product is a crucial point in the commercial exploitation of the invention and that for the invention concerned, one needs to have an extracted stem cell at some early stage of making the product. Exploitation of the invention is an element in relation to Article 53, but that is not the point under discussion. The Board deals explicitly with Rule 23 d(c) and the use of the embryo for industrial or commercial purposes. The latter concept needs to be defined and the Board fails to do so. Its only argument is that somewhere at the basis of the invention is an extracted human embryonic stem cell and that that brings whatever follows within the exclusion. We have argued above that this looking back is not admissible and even so, it does not define the concept under discussion. Such a definition is required to apply Rule 23 d(c) correctly and it is particularly regrettable that the Enlarged Board fails to properly address the issue.⁵⁵ Instead, one finds itself stuck in a backward looking capable of industrial application logic that is beside the point.

22.7 Conclusion

It is hard to escape the impression that the WARF decision is above all a narrow, inward-looking decision that fails to engage the wider fundamental points and arguments that are equally of crucial importance in this area. That does not detract, though, from the fact that this chapter demonstrated that human stem cell-related patents can exist, but that they require, first of all, a rigorous application of the requirements of patentability. Special emphasis was placed on sufficiency and the often-underestimated requirement of capability of industrial application. Excluded subject matter and morality also play an important role, especially so in Europe. The chapter suggested a way forward for the EPO to deal from a procedural point of view with the absence of a common concept of morality. Coming back to the WARF test case it then had to accept that the EPO has not taken up the decision and has instead gone for a narrow technical approach.

In terms of practical guidance, one can with reasonable certainty derive from the case that in terms of human stem cells non-embryonic stem cells, or at least inventions relating to them are patentable. But since the focus is on the destruction of the

⁵⁵ Ibid, paras 26–27

embryo, it seems also likely that inventions relating to human embryonic stem cells will be patentable if the new technology to extract the stem cells that are put in culture allows for the survival of the embryo. This kind of technology has been announced on a couple of occasions in recent months and if it turns into a practical reality it would arguably overcome the objections raised in the WARF case. On the other hand, inventions, the development and exploitation of which involve the repeated uses of new human embryonic stem cells, are clearly not patentable. The same seems to apply to inventions that are based on a stem cell line, the origin of which lies in a stem cell extracted from an embryo that was destroyed in the process, no matter how remote the stem cell is from the actual invention and its exploitation. It is submitted that the exclusion should not apply if the invention itself and its exploitation do not involve the destruction of a human embryo in as far as the invention and the raw material used for it are based on a legitimately sourced stem cell, i.e., from an authorized stem cell line. But the WARF decision may still catch that kind of case. That remains uncertain, however, and shows the lack of clarity and predictability in the WARF decision. Despite that uncertainty the bottom line must be that human stem cell-related inventions, and especially translational ones, will be patentable as long as embryo destruction was avoided. That opens a way forward even if harmful uncertainties remain.

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Chapter 23

Patenting of Human Stem Cell-Based Inventions: Can There be Technological Solutions to a Moral Dilemma?

Aliki Nichogiannopoulou

Abstract Patents are a means of securing returns – if any – from one’s intellectual property. In the evolving intellectual property landscape it has become increasingly clear that biotechnology is not just another field of technology. Especially the field of stem cell biotechnology, as a prime example of translational research, has been pressing this very point. Legislation evolves at a slower pace than the emergence of new technologies in this field, rendering the task of keeping abreast equally challenging for legislators, scientists, innovators and the lay public. The fact that a human embryo is the primordial source of human embryonic stem cells, renders the ethical, legal, philosophical and scientific evaluation of the framework within which we manoeuvre, full of challenges. Given the political consequences of such considerations, the scientific community has attempted to overcome some of the points raised by technical innovations that have themselves become the subject of patent applications. The European Patent Office – the patent granting authority for Europe with a mission to support innovation, competitiveness and economic growth for the benefit of the citizens of Europe – is faced with the challenge of deciding which of these overcome the issues and which do not. Several questions remain unanswered while technology advances posing new ones. The debate is ongoing, as is the synergy between science and society in the quest for universally ethical or ethically universal human embryonic stem cells.

Keywords Stem cells • Patents • European Patent Office • Moral dilemma • Case law

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23.1 Introduction

The patenting of innovations in all fields of technology is a well-established practice. Although challenged for its continuous validity in light of the societal and economic changes that have accompanied the shift of the millennium, it is still a predominant driving force for continued innovation and development. Understanding the patent landscape is particularly essential in all the processes regulating translational research due to the latter's *sui generis* status in the transition from bench to bedside. Among the many fields of translational research, few have involved the attention and scrutiny of the public as much as stem cell research. The derivation and use of human embryonic stem (ES) cells raise considerable ethical, religious, legal and political concerns despite their potential therapeutic usefulness. Human ES cells are pluripotent cells derived from the early stages of the human embryo, the best established techniques to date involving the destruction of the embryo. Stem cells obtained this way possess the highest possible plasticity, i.e., the ability to differentiate into specialized cell types, and may be induced to differentiate to all cell types of the body.

In an attempt to bridge the division between ethical questions and potential scientific and medical benefits, considerable efforts have been devoted to the search for alternative technologies for human ES cell derivation. Methods have been described in the scientific and patent literature, whereby the embryo remains viable after the procurement procedure. Yet further alternatives involve the use of non-viable developmental entities as a source for human ES cells. Examples of the latter case include – but are not limited to – embryos carrying a lethal mutation as a result of genetic manipulation, parthenogenetically activated developmental entities and triploid or otherwise aneuploid embryos. Each of these presents scientific challenges and raises ethical and legal questions. This chapter will attempt to analyze whether and to what extent the origin of the stem cells and the subsequent fate of the utilized developmental entities – embryo or otherwise – affect their status under the current European Patent Convention (EPC) legal framework. In addition to ethical objections to the methods, patentability of human ES cells derived by these methods would have to be carefully assessed and evaluated. This assessment is to be undertaken in light of the ensuing case law of the Boards of Appeal of the European Patent Office (EPO) and the currently very actively evolving consensus on the ethical values and the moral code of conduct as these are rooted in the European culture.

23.2 The Legal Framework for Patentability

In its Article 52(1), the EPC sets the stage with a positive presumption in favor of patentability. This article states that European patents will be granted for any inventions, in all fields of technology, provided that they are new, involve an inventive

step, and are susceptible of industrial application. This presumption notwithstanding, patents may be refused in Europe on ethical grounds. As a matter of fact, conformity with ethical principles might be seen as a legal norm of higher ranking than the provisions of Article 52(1) EPC, the latter having to yield in case of conflict [1]. Whereas in the past, this issue has arisen only infrequently, recent developments in human ES cell research have given rise to conflicting opinions in Europe as to the approach that should be adopted in relation to patents. Under Article 53(a) of the EPC, it is not possible to patent inventions, the commercial exploitation of which would be contrary to “*ordre public*” or morality; such exploitation shall not be deemed to be so contrary merely because it is prohibited by law or regulation in some or all of the contracting states. Specific guidance on the exact meaning of this in respect of biotechnological inventions is provided under Rule 28 EPC. In particular, Rule 28(c) EPC states that inventions that concern uses of human embryos for industrial or commercial purposes will not be patentable. It is this explicit exclusion from patentability of uses of human embryos that has created the controversy surrounding human ES cells and their patentability on the grounds of morality. This exception to patentability has been transposed from Article 6.2(c) of Directive 98/44/EC of the European Parliament and of the Council of July 1998 on the legal protection of biotechnological inventions (Directive) [2].

The Directive was proposed by the European Commission in 1988 and had a 10-year history of debates within the European democratic system. It has been incorporated in the EPC by decision of the Administrative Council of 16.06.1999, which took effect on 01.09.1999 [3]. Since human ES cells are derived from human embryos, issues surrounding their patentability inescapably fall under the provisions of said Article. On a timeline, the Directive was adopted in July 1998 while the first report on the isolation and cultivation of human ES cells by James Thomson of the University of Wisconsin was published in November of the same year [4]. It follows that during the long debating history of the Directive, and even at the time it was finally passed, the question of how Article 6.2(c) is to be applied to human ES cell-related technologies had not been contemplated. On a side note, embryonic germ (EG) cells, which have a potency comparable to that of ES cells, are typically derived from the gonadal ridges at 8–11 weeks of gestation and are thus of fetal rather than embryonic origin. Embryonic carcinoma (EC) cells, induced pluripotent stem (iPS) cells and further ES-like cells, are of postnatal origin and thus do not fall under the scope of the above prohibitive regulations. The European Patent Office (EPO) announced in November 2008 that it will not be issuing patents for stem cells that have been obtained through the destruction of human embryos [5]. Although this ruling expressly rejects destruction of the human embryo, some room for maneuver remains for patent applicants and examiners alike. Faced with the moral dilemma inherent to human ES cell research, politicians and scientists have been trying to evade the issue by technological advances. These techniques provoke the consideration whether viability of a developmental entity and its potential to develop through gestation to birth is both a necessary and sufficient condition for its classification as embryo under the provisions of Rule 28(c) EPC, i.e., Article 6.2(c) of the Directive.

23.3 The Definition of the Term “Human Embryo” and Its Implications for Patentability

The definition of the term “embryo” has been posing a significant challenge to scientists and legal scholars alike, especially in light of recent technological advances. This discussion is of cardinal importance due to the fact that the way an entity is categorized is pivotal to whichever set of legal dynamics it is placed within. Linguistically from the Greek “εν” and “βρῦω,” the word means “that which is plentiful and grows.” A definition of the human embryo, from its biological, legal and moral standpoint, would be inconsistent if it did not take into account emerging technologies and trends in reproductive science. The potential or potentiality of a developmental entity plays a key role in the debate. It should be furthermore borne in mind that the scientific community is still pondering the issues of pluripotency versus totipotency. The blurring of the distinction between the legal categories “pluripotent” and “totipotent” is one example of how the biological understanding that the law is based on is changing, thus providing a need for conceptual reflection or new legal categories. The lesson taught to us by Dolly, the cloned sheep [6], is that each and every terminally differentiated mammalian cell can be induced to generate an entire organism and produce a new individual when and if placed in an appropriately conducive environment (here: an enucleated oocyte). The allegation that it is the nucleus that is reprogrammed by the egg cytoplasm, and not the cell, which has the potential to develop into a new organism, is invalid in the light of the fact that it is ultimately always the nuclear genes that dictate the developmental fate of a cell. The fact remains that a fully differentiated cell can be reprogrammed to give rise to a new individual. Somatic cell nuclear transfer is, however, a highly artificial process that pushes the cells beyond their original developmental limits. This awakening of a dormant developmental potential is the result of an advanced biotechnical process and a technique that nature is incapable of accomplishing by itself. Should we envisage all these highly artificially awakened potentials when addressing the issue of the potentiality of an entity? Not if we adopt the view that possibility and probability are necessary conditions for potentiality. According to this view, the totipotent cells should have a real physical possibility of developing into a mature human being and this development must be probable. If, on the other hand, we adopted the view that the potentiality of an entity refers to its intrinsic qualities and inherent capacities, then we would be faced with the oxymoron of applying moral consideration and even protection to cellular entities hitherto unsuspected of possessing such merits. When considering what defines an embryo in the light of recent technological advances, it is important that the definition not become so wide as to encompass human cells or cellular structures that traditionally have not been considered to be embryos.

For the purposes of this discussion, the focus will remain on the biological definition reflecting the multi-step process of development that involves recognition of observed events with potential for further development. What has to be borne in mind during an attempt at defining a human embryo is that fertilization, development and potential for further development are not static processes, but rather components of a multifactorial process and status that is ongoing from conception to birth and beyond.

The start of this plastic process can be set either at the first mitotic division after fertilization of a human oocyte from a human sperm is complete or, alternatively, at the start of any process that initiates organized development of a biological entity with a human nuclear genome that has the potential to develop up to, or beyond, the stage at which the primitive streak appears [7]. Of importance already at this stage of the discussion is that the proposed definition also includes biological entities with an altered human nuclear genome. The primitive streak is a structure that forms during the early stages of embryonic development. Its formation is one of the first signs of gastrulation, i.e., the phase in development during which all three germ layers of the embryo (endoderm, mesoderm and ectoderm) are created. Gastrulation is followed by organogenesis, when individual organs develop within the newly formed germ layers. In humans, this is at 8 weeks of development when embryonic development gives way to fetal development. It is striking that there is no definition of a human embryo in the EPC. Similarly, there is no definition of the term in the Directive, which under Rule 26(1) EPC shall be used as a supplementary means of interpretation of the relevant provisions of the EPC. In its decision G5/83, the Enlarged Board of Appeal of the European Patent Office (EPO) being the competent body to decide on points of law referred to it, has decided that terms in the EPC should be construed according to their normal meaning. It is such a normal meaning of the term human embryo that is lacking in any unified European legislation. As an example, the German Embryo Protection Act of 1990 [8] in paragraph 8(1) defines as an embryo the fertilized human oocyte, capable of development, from the moment of pronuclear fusion. Paragraph 8(2) states that a fertilized human oocyte is capable of development, unless it can be established within 24 h after pronuclear fusion that it cannot develop past the one-cell stage. On the other hand, the British Human Fertilisation and Embryology Act of 1990 [9], Section 1(1), defines as a human embryo a live human embryo where fertilization is complete. It becomes apparent with the example of two different European legislations, that no consensus has been achieved on the requirements that have to be fulfilled by an entity to qualify as an embryo. A key point in the above discussion is whether the potential to produce a viable developmental entity culminating in a live birth is a key element for the definition of human embryo. If this were the case, then some of the emerging technologies discussed below would not be considered to be techniques producing a human embryo and the restrictions imposed by Rule 28(c) EPC would not apply.

In its decision G2/06, the Enlarged Board of Appeal of the EPO has deliberately and explicitly refrained from defining the term embryo, stating that a precise definition of the term has not been given by the legislator intentionally in order to prevent any restrictive interpretation of Rule 28(c) EPC. The Board stated that neither the EU nor the EPC legislator has chosen to define the term, although they were presumably aware of the conflicting definitions used in national laws, in order to maximize protection of human dignity and prevent the commercialization of embryos. In any case, the Board concluded, what an embryo or an embryonic cell is within the context of any particular patent application is to be considered as a question of fact, irrespective of the vocabulary used (G2/06, Reasons 20). Consistent with an unrestricted approach, the embryonic period would begin with fertilization; however, neither the lower nor the upper limit of the embryonic period has been confirmed in legal terms by the EPO case law.

23.4 The European Patent Office's Position So Far

European patent application EP 0 770 125, filed by the Wisconsin Alumni Research Foundation (WARF) in 1995, was based on research carried out by James Thomson of the University of Wisconsin in the late 1990s on the derivation of human ES cell lines. The application was initially rejected by the competent examining division of the EPO in 2004. The reason was that the method disclosed in the application for obtaining ES cells used as the starting material a primate (including human) embryo, which was destroyed in the process. WARF appealed this decision to a Technical Board of Appeal, which in turn referred questions of law relevant to patentability of human ES cell-related inventions to the Enlarged Board of Appeal, the supreme judicial body of the EPO, responsible for uniform application of the EPC [10]. In November 2008, the Enlarged Board of Appeal announced its laconic decision G2/06, which ruled as unpatentable under Rule 28(c) EPC inventions concerning human ES cell cultures, which can only be obtained by the use (involving their destruction) of human embryos. The Board expressly stated that Decision G2/06 is not concerned with the patentability in general of inventions relating to human stem cells or human stem cell cultures (see G2/06, Reasons 35).

23.5 Technological Alternatives for the Procurement of Human ES Cells

A number of European patent applications have been filed encompassing the procurement of embryonic stem cells from *in vitro* artifacts or entities that do not fully conform with the consensus understanding of the term embryo. Further methods have been developed that do not involve the destruction of a viable human embryo. Such methods seek to provide cells with features similar to those of conventionally derived human ES cells bypassing ethical, religious or political concerns and, most importantly in this context, patentability prohibitions. In other words, they seek to provide a technological solution to a moral dilemma.

23.5.1 *Alternatives Involving Non-viable Entities*

23.5.1.1 Triploid Embryos

Several scientists have focused their efforts toward alternative techniques to derive human ES cells without destruction of viable human embryos. Toward this end, stem cells were obtained from dispermic triploid zygotes that arise as a by-product of *in vitro* fertilization (IVF). This method comprises the microsurgical removal of one excessive male pronucleus to create a diploid heteroparental zygote, followed

by culture to the blastocyst stage. The inner cell mass (ICM) is then removed and cultured to provide ES cells [11]. It should, however, be noted that polyspermy can be corrected through microsurgical extraction of the excess male pronucleus [12]. A clinical case study has been reported whereby a normal healthy baby boy was born at 38 weeks and 4 days' gestation, after removal of the extra male pronucleus in a triploid dispermic zygote [13]. It follows that although triploid dispermic zygotes appear embryologically non-viable in the unmanipulated state, they can be rescued with assisted reproduction technologies, implant, and result in live births. When assessing patentability of a process for deriving human ES cells from a triploid embryo, attention is drawn to the fact that there is no legal basis for assuming that the term "embryo" in Rule 28(c) EPC is meant to embrace euploid human embryos only. Accordingly, an assessment to the effect that triploid embryos should not be covered by the same provisions as euploid embryos cannot take place.

23.5.1.2 Arrested Embryos

Following a similar rationale, stem cells have been derived from arrested human embryos that were unable to develop past the blastocyst stage [14–16]. Such embryos do not continue their cleavage even after 24 h of observation and are thus labeled as dead. However, due to mosaicism based on postzygotic nondisjunction, i.e., defective segregation of chromosomes in a single cell after the first cell division, normal blastomeres can be present in such dead embryos. In such a case, the ethical and legal framework currently used for obtaining essential organs for transplantation from deceased adults could be extended to cover obtaining stem cells from dead human embryos. From a scientific point of view this approach faces the insurmountable hurdle of how and when we can pronounce an embryo to be dead. Whereas the question of when life begins is still a matter of debate, a consensus on when it ends has been reached that requires the diagnosis of brain death [17]. It becomes immediately apparent that this criterion is useless in the definition of death in an embryo. Criteria for determining death of a developing embryo before the onset of neural development have not been formulated. It can reasonably be argued, that the defining capacity of an early (4- or 8-cell) human embryo is continued and integrated cellular division, growth and differentiation [16]. An embryo that has lost this capacity could be considered organismically dead, even if its individual cells are alive. For it to be useful in the derivation of stem cells, the embryo must contain some viable cells. However, clinical experience has indicated that embryos with grave morphological impairments and only a few surviving blastomeres can occasionally proceed to normal development. In any event, viability of an embryo or irreversible loss thereof can only be established with hindsight at a time point by which it is too late for research purposes. Either because the embryo has developed into a viable organism or else, because apoptotic, i.e., programmed, cell death has already progressed. The objective diagnosis of embryonic death would require universally accepted and straightforward criteria for determining irreversible developmental arrest. It is in particular the criteria for

determining irreversibility that are lacking. In the absence of such consensus criteria at the time point of cell procurement, even an apparently arrested embryo must be seen as falling under the provisions of Rule 28(c) EPC and processes related thereto would thus be deemed unpatentable.

23.5.1.3 Nuclear Transfer Embryos Incapable of Implantation

A further technique resulting in non-viable embryos is known as altered nuclear transfer (ANT) and was proposed in 2006 [18]. Briefly, the donor cells were genetically altered to disrupt the expression of a gene that is essential for the formation of a functional trophoblast. The resultant entities formed inner cell masses, from which embryonic stem cells could be derived. This technique is a practical demonstration of William Hurlbut's idea of altered nuclear transfer to generate a non-implantable clonal artifact [19]. In July 2005, William Hurlbut, professor at Stanford University and member of President Bush's Council on Bioethics, recommended the support of research for the creation of "embryo-like, non-implantable entities" that have been engineered to lack the capacity to develop into humans, a process he termed "altered nuclear transfer." Such entities would then lack the moral status of human embryos and could be used for research with fewer ethical objections. A gene in the nuclear donor's cell is switched off before the nucleus is transferred into a fertilized cell. The resulting egg grows into a blastocyst but lacks the ability to implant in a uterus and develop into a living organism. Inner cell mass cells are then extracted from these non-viable blastocysts in the classical way. The embryos are thereby destroyed, but since they could not implant anyway, their destruction is not the destruction of a potential life. Because the genetic modification would be undertaken before the creation of the cloned embryo, Hurlbut argued that the resulting entity would have "...no inherent principle of unity, no coherent drive in the direction of the mature human form, and no claim on the moral status due to a developing human life" [20]. The process can be compared to organ donation from brain-dead patients. On the other hand, it can be reasonably argued that this technique is tantamount to the deliberate creation of defective human embryos in order to justify their destruction. A researcher is cited to have said that the altered nuclear transfer technique "is an abuse of cloning technology. It will be a sad day when scientists use genetic manipulation to deliberately create crippled embryos to please the Church."

Be that as it may, patent applications claiming this technology and human ES cells thus obtained have to be examined in all their aspects under the terms of Article 53(a) and Rule 28 EPC. From a technical point of view, transfer of a somatic nucleus in an enucleated oocyte is the first step in any process for cloning by nuclear transfer – whether therapeutic or reproductive. Rule 28(a) EPC specifically and explicitly rules that under Article 53(a), EPC processes for cloning human beings are not patentable. Although no distinction is made in the text of this rule between reproductive and therapeutic cloning, the general consensus within the EPO is that the *ratio legis* of legislator when drafting this provision was to exclude

from patentability any process for cloning humans, irrespective of the intended purpose of the process. This interpretation is corroborated by national and international declarations banning reproductive and therapeutic cloning alike [21]. An attempt to obtain patent protection for a technology involving transfer of a human nucleus into an enucleated oocyte would thus fail on this principle alone. Furthermore, in the absence of a consensus definition that would set such an entity outside the realm of the term “embryo,” as intended by the European legislator, the activated nuclear transfer unit is to be regarded as an embryo in the sense of Rule 28(c) EPC, unless case law of the EPO establishes the contrary. A further attempt to overcome the ethical hurdles is the use of oocytes from other species as hosts for an altered human nucleus. In such a case, the nuclear genome of the resulting entity would be human, whereas the mitochondrial genome would originate from the other species. The technical feasibility of such an approach is questionable, since the resulting mitochondrial heteroplasmy might cause developmental problems. This issue notwithstanding, such an approach would also fail a stringent patentability assessment for the following reason. Under Rule 26(1) EPC, the Directive shall be used as a supplementary means of interpretation of the relevant provisions of the EPC. Recital 38 of the Directive explicitly excludes from patentability processes, the use of which offend against human dignity, such as processes to produce chimeras from germ cells or totipotent cells of human and animals. Patent application claims embracing processes directed to the potential creation of a human/non-human chimera are as such unpatentable under the provisions of the EPC.

23.5.1.4 Parthenotes

Artificial parthenogenesis as a way to create entities eluding the patenting prohibition of Rule 28(c) EPC has been repeatedly contemplated and claimed in European patent applications. Parthenogenesis is the development of a uniparental embryo directly from an oocyte without fertilization by sperm. The resulting parthenote is capable of occasional implantation but lacks the full developmental capacity of a normal embryo [22–24]. Parthenogenetic embryos with potential to develop into viable individuals have been reported in mice, albeit after substantial genetic manipulation [25]. In macaques, parthenogenetic preimplantation embryos can develop to the blastocyst stage and are amenable to the generation of embryonic stem cells [26]. Homozygous stem cells derived from parthenotes will have half as many problems of tissue rejection as stem cells derived from eggs fertilized by sperm, which will have the same tissue compatibility problems as transplanted organs. With respect to ethical issues, such an application might argue that parthenotes do not fall under the definition of “embryo” because they do not result from a fertilization process and/or because they are not viable. It follows that their destruction is not destruction of potential human life. There is indeed a tendency to create novel names for novel, hitherto non-existing *in vitro* artifacts. Thus the term “ovasome” has been proposed to linguistically more accurately describe the product of a parthenogenetically activated oocyte [27]. However, in the absence of a

consensus on the definition of an embryo, such an interpretation might seem unsupported and ultimately not convincing. The inclusion of fertilization and syngamy as necessary elements in a definition of human embryo remains at the moment arbitrary, and some would argue even inappropriate [7]. Such an inclusion might eliminate from the definition entities produced by emerging technologies with the – theoretical – potential to produce a new individual. The parthenotes' lack of viability and their uncoupling from fertilization events are thus not sufficient criteria to set them outside the intention of the legislator. Consequently, parthenotes, gynogenetically or androgenetically activated germ cells, and other entities designed to mimic embryonic developmental stages *in vitro* will have to be considered to be “embryos” in the sense of Rule 28(c) EPC until and unless case law is established to the contrary. For the time being, it is noted that the Enlarged Board of Appeal of the EPO has ruled in decision G2/06 that what is an “embryo” is a question of fact in the context of any particular application (G2/06, Reasons 20). Since neither the EPC nor the Directive provides an explicit definition of the term “embryo,” the Board warns against giving this term any restrictive meaning, on grounds that such meaning would undermine the intention of the legislator to protect human dignity and prevent commercialization of embryos.

23.5.2 Alternatives Not Involving the Destruction of the Human Embryo

Human embryos generated by assisted reproduction technologies in the framework of an *in vitro* fertilization (IVF) program can be tested for genetic defects very early in their development. For this pre-implantation diagnosis (PID), a single blastomere is being microsurgically removed from an 8-cell-stage morula. The procedure does not impair the embryo's developmental capacity, and biopsied embryos develop reportedly normally after removal of this single blastomere. This admittedly inefficient procedure requires improvements and skillful micromanipulation of early human embryos, with sometimes adverse effects. This single-blastomere biopsy (SBB) process has been employed for the procurement of pluripotent stem cell lines from early stage embryos [28, 29]. In the SBB procedure, a single blastomere is removed microsurgically from an eight-cell-stage embryo and ES cells are subsequently grown from it. ES cells are thus derived from a mammalian embryo without destroying it. The moral objections to this technology are the same as the ones used by PID critics. For patentability purposes, it should be borne in mind that the removed single blastomere might be totipotent, in which case using it would amount to using – and even destroying – a human embryo. Totipotency is thus far attributed to the fertilized oocyte and to all the cells of the embryo up to the 8-cell stage, after which they are reported to acquire a restricted, specialized fate. In that case, the use and subsequent culture of the single blastomere might fall under the prohibitive terms of Rule 28(c) EPC. Concerning the argument that the SBB process does not destroy the biopsied embryo and thus cannot be considered as

falling under the exclusion of Rule 28(c) EPC, it is to be noted that Rule 28(c) EPC excludes from patentability “the use of human embryos” irrespective of whether this use results in the destruction of the embryo or not. It is therefore of little relevance, for patentability issues, that the embryo remains viable. Clarification of whether “use” under the terms of Rule 28(c) EPC is restricted to consumptive use or includes any and all uses lies outside the room for maneuver left to the examining divisions of the EPO and would have to be clarified at a higher level. At the moment, the examining divisions interpret this exclusion to cover processes for removing cells from an embryo, even in the event that this removal does not result in the destruction of the embryo. In the current opinion of the EPO, the legislator’s intention when drafting Rule 28(c) was to safeguard embryos from commercialization to an extent that goes beyond Rule 29(1) which protects the embryo itself as a stage of formation and development of the human body. The availability of embryos in a Petri dish and their relative straightforward experimental accessibility as compared to the fetus or the adult was, in the EPO’s current opinion, the *ratio legis* of Rule 28(c) EPC. In conferring absolute protection to the embryo, Rule 28(c) would override Rule 29(2), which allows the patentability of isolated elements of the human body, and it is as such that the examining divisions currently interpret it. In accordance with decision G2/06 of the Enlarged Board of Appeal of the EPO, a cell line obtained by culturing said isolated blastomere is also excluded from patentability under Rule 28 (c) EPC. On a final note, it should also be borne in mind that the SBB might qualify as a method of cloning the embryo by embryo splitting. Recital 41 of the Directive, in combination with Recital 40, unequivocally excludes from patentability processes for cloning human beings by *inter alia* embryo splitting. On the other hand, Recital 42 of the Directive does not exclude from patentability therapeutic or diagnostic methods practiced on the embryo that are useful to it. A patent application may then argue that the derived human ES cell line might be somehow useful to the embryo from which the single blastomere was obtained and thus try to evade the prohibition of Rule 28(c) by evoking application of Recital 42 of the Directive. For such an argument to be accepted, the burden of proof lies with the applicant to show that the produced cell line or its derivatives are therapeutically useful to the embryo from which they were obtained. The technical details of such an application should be considered carefully.

23.6 Outlook and Conclusions

From the above analysis, it becomes apparent that several issues surrounding intellectual property rights on human ES cell-based technologies remain unresolved. The EPO is bound in its practice by the requirements set forth in the European Patent Convention, the Implementing Regulations and the ensuing case law from its Boards of Appeal. In interpreting Rule 28(c) EPC, the first and second instances of the EPO interpret a provision of European Community Law, i.e., Article 6(2)(c) of the Directive. Ideally, a judiciary community body, such as the European

Court of Justice (ECJ), should be called upon to interpret the *ratio legis* of a legislative community body. Article 234 of the EC Treaty states that “The Court of Justice shall have jurisdiction to give preliminary rulings concerning: [...] the validity and interpretation of acts of the institutions of the Community [...]. Where such a question is raised before any court or tribunal of a Member State, that court or tribunal may, [...] request the Court of Justice to give a ruling thereon. Where any such question is raised in a case pending before a court or tribunal of a Member State against whose decisions there is no judicial remedy under national law, that court or tribunal shall bring the matter before the Court of Justice.” When asked by the appellant to refer the interpretation of Rule 28(c) EPC to the ECJ for guidance, the competent Enlarged Board of Appeal in case EP 0 770 125 rejected the request for lack of an institutional link between the EPO appeal boards and the EU. In interpreting the provision themselves, the Enlarged Board of Appeal ruled that the question of what is an embryo is a question of fact to be assessed in the context of any particular application (G2/06, Reasons 20). The Board explicitly warns against giving this term any restrictive meaning, on the ground that such meaning would undermine the intention of the legislator to protect human dignity and prevent commercialization (commodification) of human embryos. The interpretation of this provision was, however, referred to the ECJ after all by the German Federal Supreme Court (Bundesgerichtshof), which being a court or tribunal of an EU member state under the terms of Article 234 of the EC Treaty has the discretion to do so. In a dispute between a German inventor and a non-governmental organization about German patent DE 19756864 the German federal supreme court has decided in November 2009 to refer to the ECJ questions regarding the interpretation of Article 6 of the Directive [30]. According to the German Federal Supreme Court, Article 6 of the Directive is not unequivocal, in that the term “human embryo” is undefined in the text of the Directive. A definition of the term from the ECJ is thus eagerly awaited. Furthermore, the question remains whether use of human embryos under the terms of Article 6 of the Directive is restricted to consumptive use and whether a use for research or therapeutic purposes is a use for commercial purposes under its provisions. The ruling of the ECJ will not be binding for the EPO due to the lack of institutional links between the two institutions. However, the EPO has in the past adopted community legislative provisions without being legally bound by them, for the purpose of harmonizing practice among its member states. It remains to be seen how the patent landscape will evolve around human ES cells in light of ensuing judicial decisions. In the meantime, the quest for universally ethical or ethically universal human ES cells [31] will continue.

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Chapter 24

Patenting of Human Stem Cell-Based Inventions: Ethical Issues Including and Beyond the Morality Clause

Göran Hermerén

Abstract Using the goals of patent law as a starting point, controversies over patent applications are put in a wider ethical and societal context. I argue that ethical aspects are relevant in three phases: (1) before the patent application is granted, when the conditions of patentability are applied to concrete cases; (2) in the patent law, especially in interpretation and application of the so-called "morality clause" in the European Patent Convention; (3) but also after patents have been granted. Differences between different patent systems are mentioned briefly, and the choice between more liberal and more restrictive policies is obviously not ethically neutral.

After a brief discussion of the relations between patents, economy and politics, some current criticisms of patent and patent law are discussed. Internal criticism, more or less accepting the premises of the system and trying to improve it, is distinguished from more radical, external criticism, which challenges one or several of the premises of the current patent system. This paves the way for a discussion of future relations between ethics and patent law. Some problems of definition and interpretation, again highlighting the role of values in the controversies over patents, are indicated, and the chapter concludes with a discussion of the present and future relations between EGE (European Group on Ethics) and EPO (European Patent Office).

Keywords Ethics • Patents • Stem cells • Morality clause • Biotech directive

24.1 Introduction

Important aspects of patent law have been clarified by Paul Torremans in his contribution to this book. A generally accepted point of departure of some importance for several issues discussed below can be stated as follows: The patent does not give

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the patent holder a right to manufacture the product or method patented; it is up to national legislations to decide about this. But it does give the patent holder a right to prevent others from doing so, unless they pay a license fee. Provided that the conditions of patentability are met, a patent will be granted. In exchange for openness, the patent holder is granted a monopoly.

Under the TRIPs agreement¹ – part of the WTO treaty – inventors of new medicines must be granted 20-year monopoly patents in all WTO member states. Patents and criteria of patentability are not ethically neutral since they represent an attempt to strike a balance between different interests; that is why it is important to highlight ethical issues raised by patent applications, including and beyond the morality clause. It is essential to clarify the underlying goals and values in the controversies over patents on SC-based (stem cell-based) methods and products.

The goals of patent law is a useful starting point for this discussion. They include striking a balance between the interests of the inventor (fair reward), the industry (protection), the users (access at reasonable cost) and society at large (trust, economic competitiveness and development). These interests may clash; when they do, we are confronted with a problem and have to make a decision. Legal systems may not only be different, they sometimes clash – and occasionally there are underlying moral conflicts.

24.2 Ethics: Preliminaries

To begin with, some clarification of the notion of ethics may be useful, since “ethics” and “ethical aspects” are used in several senses. Normative ethics is concerned with rights, liberties, duties and their foundation or basis, whereas descriptive ethics essentially describes and analyzes practices, values and norms, in different societies at different times.

A perhaps even more important distinction in this context is the difference between practical and theoretical ethics. The purpose of the former is to make or propose (and argue for) decisions on controversial ethical issues, while the latter is mainly concerned with clarification, exploring logical and semantic relations, promoting understanding of the key concepts used and the relations between arguments used for and against various contested positions. Theoretical ethics in this sense requires specialist philosophical knowledge and training.

All major ethical traditions raise theoretical issues. In consequentialism, some key theoretical problems include the interpretation of “alternatives” and “consequences,” analysis of concepts of harm and the possibility of interpersonal comparisons. For deontologists, central theoretical problems include definitions of rationality,

¹The Agreement on Trade Related Aspects of Intellectual Property (TRIP) is administered by the World Trade Organization (WTO)

interpretations of different kinds of rights, obligations and liberties, conflicts between duties and the indivisibility of rights. Fundamental theoretical problems in virtue ethics include interpretation of, and relation between, concepts like virtue, practical wisdom and eudaimonia (sometimes translated as “flourishing”) and analysis of the extent to which virtue ethics can be used as a basis for action.

Theoretical ethics in the sense characterized above can be separated from practical ethics, of which two forms can be distinguished. Contrary to theoretical ethics, both forms have in common that (1) a certain normative framework is explicitly chosen as a point of departure, and that (2) the objective is to reach or recommend a decision as to what to do. One of these forms is monodisciplinary and philosophical, the other is interdisciplinary and requires cooperation of different experts.

The typical monodisciplinary form of practical ethics is carried out by philosophers, choosing a certain ethical theory as a point of departure, such as Kantianism or utilitarianism. The theory, or certain aspects of it, are presented and then applied to a particular problem, such as whether certain forms of cloning, embryo research, prenatal diagnostics, terminal sedation, genetic testing, or biobanks are ethically acceptable, and if so, under what conditions. This is more or less the approach used by philosophers like Peter Singer, John Harris, Torbjörn Tännsjö, and Marcus Duwell, to mention only a few.

The typical interdisciplinary form of practical ethics is carried out by a group of people with different research backgrounds, ideally also with different experiences and cultural backgrounds. In addition to specialist philosophical knowledge and training, evidence from many different areas, such as law, behavioral and social sciences, as well as economics, is required to reach a robust decision. An explicit framework is chosen, usually some international set of documents on which political agreement has been reached, such as international codes and conventions by the UN, Council of Europe and the EU Charter of Fundamental Rights. The principles and values outlined in these documents are then applied to the problem at hand. This is the way the EGE, the Nuffield Council and similar groups have worked.

The distinction may not be sharp, but it is nevertheless important. While theoretical ethics focuses on clarifying the meaning and implications of concepts and assumptions, making conditions and consequences explicit, it is necessary for those working in practical ethics to choose certain theoretical and normative points of departure, such as a particular ethical theory or international codes and conventions by the UN, Council of Europe and/or EU.

Of course, no philosophical positions, and no codes and conventions are above criticism. But the international codes and conventions by the UN, Council of Europe and the EU Charter of Fundamental Rights have the advantage, from a democratic point of view, of being agreed on politically even if they sometimes contain vague clauses, which may be open to several interpretations. Even so, the documents provide instruments that can be used as starting points for discussion and deliberation.

Comparing the two forms of practical ethics, it seems clear that the advantage of the monodisciplinary approach is that to philosophical purists it may be more interesting in terms of philosophical rigor than the other. The disadvantage is that its

political effect is often insignificant. Politicians are sensitive to what their voters think, since they want to be re-elected, and voters rarely think as consistent utilitarians or Kantians.

The strong and weak points of the interdisciplinary approach are different and opposite. From a purely philosophical point of view, it may be less interesting than the monodisciplinary form of practical ethics. But since it bases its deliberations on documents that have been politically agreed on, some of which are the result of compromises and negotiations, the political impact of its recommendations may be considerable.

In the analysis of practical ethical problems, “choice” and “value conflicts” are key notions. The structure of an ethical problem of the sort that will be in focus in what follows can be outlined as follows:

First, there is a choice between alternatives. If there is no choice between alternatives, we are not confronted with an ethical problem. This choice involves parties with different interests, values or rights, all of which cannot be satisfied at the same time. If this is not the case, there is no ethical issue. In other words, a choice has to be made, creating winners and losers – and occasionally only losers. Power and vulnerability are important and sometimes neglected aspects of this situation.

In the previous contributions to this section of this book, some contested cases are referred to. Should the Edinburgh patent application have been approved and the WARF (Wisconsin Alumni Research Foundation) patent application been rejected? Which interests, and whose interests, would then have been promoted? If the former had been approved, certain interests would have been favored; if the latter had been approved, other interests would have been promoted.

24.3 Ethics and Patents

I will argue that we should avoid a narrow legalistic view of controversies over patent applications, but place them in a wider ethical context, indicated by questions such as: Does the present patent system contribute to the common good? Is it compatible with human rights? Does it strike a fair balance between the interests of the inventor, the industry and society at large, including users? Does it widen the gap between developed and developing countries?

Different answers to these questions are possible due to, among other things (1) which version of the patent system (US, EU) we have in mind, (2) which collaborative licensing models (patent pools, clearing houses, open source models and liability regimes) we include, and finally (3) which ethical points of departure are used (utilitarianism, human rights, human dignity etc.).

Some of these questions concern the ethical aspects of both means and ends, as indicated by the following questions: (a) Which goals are to be achieved by patent legislation; what does the legislator want to achieve – and to avoid? (b) What is a possible way or method to achieve these goals (regulation, economic incentives and sanctions, information, etc.) ? (c) What criteria (e.g., time, cost, effectiveness, ethical

acceptance) can or should be used in comparing the methods used to achieve the goals?

The relations between ethics and patents and patent law are complex. Ethical aspects are relevant and enter into the picture before the patent is granted, in the patent law, and after a patent has been granted, in particular when patents on stem cell-based inventions are discussed.

24.3.1 Ethics Before the Application Is Granted

The standard conditions required for a patent to be granted are: novelty, inventive step and industrial application. The question facing patent examiners is then, of course, does a particular application meet these conditions? But the question is not always easy to answer – for several reasons. First, the patent application may be incomplete or obscure. Moreover these conditions can be interpreted and implemented in different ways, and the relation between utility and industrial application can be understood in more ways than one. The choice between interpretations is again not ethically neutral, as it will create winners and losers.

There are two underlying distinctions taken for granted in these controversies – between invention and discovery, and between product and method. They are reasonably easy to understand when we discuss screwdrivers and mechanical inventions. But they create problems when applied to biotechnology. The liberal praxis – some would say “excesses” – of the USPTO (US Patent and Trademark Office) in granting patents have helped to blur some of these distinctions.

Also, in the regulatory literature, this distinction is not always maintained clearly. For instance, according to US Code 35, 101: “Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefore, subject to the conditions and requirements of this title.”

Thus the distinction both in theory and in practice is not as clear as it might appear to be at first sight. Moreover, consider Article 5.1 and 5.2 of the Biotech directive [1]:

The human body, at the various stages of its formation and development, and the single discovery of one of its elements, including the sequence or partial sequence of a gene, cannot constitute a patentable invention (Article 5(1)).

An element isolated from the human body, or otherwise produced by means of a technical process, including the sequence or partial sequence of a gene, may constitute a patentable invention, even if the structure of that element is identical to that of a natural element (Article 5(2)).

Accordingly, it is possible to patent something that has been isolated from the human body, including a (partial) gene sequence, even if its structure is identical with the one occurring in nature. But a “simple discovery” of some of the elements of the human body cannot be patented. Is the borderline crystal clear? How is the notion of “isolation” to be understood more precisely here?

The point of these comments is not to criticize patent law, but to illustrate that ethical aspects play a role in interpreting and implementing clauses and conditions of the law.

24.3.2 *Ethics in Patent Law: The Morality Clause*

Let us now move on to ethics in the law. A peculiar and much-discussed feature of patent law is that in this law there are explicit ethical clauses. According to the celebrated Article 53 a EPC:

European Patents shall not be granted in respect of: inventions the publication or exploitation of which would be contrary to “*ordre public*” or morality; such exploitation shall not be deemed to be so contrary merely because it is prohibited by law or regulation in some or all of the Contracting States.

This has been applied to patent applications concerning human embryonic stem cell (hESC)- based methods or products, since according to current technology, an hESC line is created by harvesting the inner cell mass and thereby destroying the blastocyst, which means the destruction of a potential human life. Those, including the Vatican, who think this is morally unacceptable, take a backward-looking perspective; they look at the origin of the stem cell line, rather than – as is suggested by the clause above “the publication or exploitation of which” – taking a forward-looking perspective.

Anyway, the problem facing the examiners is obviously: what is contrary to “*ordre public*” or morality, and how do we find out? A starting point is a well-known list of exceptions, giving examples of what could not be patented for moral reasons: processes for cloning human beings, processes for modifying the germ line genetic identity of human beings, uses of human embryos for industrial or commercial purposes, and processes for modifying the genetic identity of animals that are likely to cause them suffering without any substantial benefit to man or animal, and also animals resulting from such processes.

The problem with the list is that it is explicitly said not to be complete, and that the reasons behind the list are not stated. Moreover, notions likely to figure in possible reasons for the list, including “identity,” “human dignity” and “integrity” can be interpreted in several ways. More could be said about this, and I will later return to some problems of definition and interpretation.

24.3.3 *Ethics After Patents Have Been Granted*

When a patent has been granted, which are the consequences, intended or not, desired or not, anticipated or not? If patent rights have been granted, how can, may, or should these rights be used?

Perhaps this is not a problem patent examiners and patent offices are obliged to deal with. But it needs to be discussed in view of the recent controversies over, for instance, how the patent rights granted to Myriad Genetics have been exercised [2].

24.4 Patents, Economy and Politics

For lack of space, I will not discuss here the intricate relationships between ethics, politics and economy. I will just underline the obvious – that politics has to do with the exercise of influence and power, and that some of the political visions and goals are anchored in ethical ideas.

The patent system has an important regulatory function in an increasingly knowledge-based economy. Market integration and efficiency are important objectives in that context. But the diversity of political and economic interests among EU member states, and indeed globally, makes implementation, harmonization and reform of the system difficult. The reform process has become politicized, as several commentators have pointed out [3].

If patent offices are self-funding agencies, there is always a danger that those who benefit most from the patent system (big pharmaceutical companies and biotech industries in the developed parts of the world) will be able to exercise a strong influence on the development and reform of the system. We should not be surprised if the users of the system have vested interests. This is an empirical question that could be investigated; if that is done, data would be available to assess this risk.

Of course, the challenge is to balance agency autonomy with political control, and this is not an ethically neutral task; no matter which way the balance is struck, winners and losers are created.

The debate over patents on SC-based methods and inventions illustrates that politicians sometimes have goals that may clash internally and with certain ethical and legal goals. They may want to keep their party together, to get re-elected, to form coalitions with other parties, to stay in power, to make citizens feel secure, confident and safe, and so forth.

This paves the way for a discussion of some current criticisms of the patent system.

24.5 Some Current Criticisms of Patents and Patent Law

The patent system has been criticized on several grounds, not only for lacking in transparency and democratic accountability. We may begin with criticism accepting on the whole the premises of the system, including criticism of particular decisions taken by the European Patent Office (EPO), USPTO, such as the fate of

the applications by Brüstle, Smith and WARF. This should be distinguished from criticism of the system of a more general nature, challenging the premises more or less radically.

For example, to illustrate criticism of the first kind, it may be argued that the Enlarged Board of Appeal (EBoA) decision g2/06 is of limited scope. It is not concerned in general with patentability of inventions relating to human stem cells or human stem cell cultures. Future or already pending inventions in the field of hESC cells do not automatically fall under the patenting prohibition of Rule 28(c) of the European Patent Convention, EPC. For each relevant invention, it is to be assessed whether there is a misuse of the human embryo in the sense of a commodification. Thus, the decision gives little guidance for how to deal with future cases; in that sense it is open.

Moreover, there has been criticism of the present patent system from ethical and societal points of view. For instance, it has been argued that it favors the big players in the arena (big pharma etc.) and that it increases the costs, also of health care (for instance by “royalty stacking”), that it delays access to inventions, widens the gaps between developed and developing countries, and so forth.

Some of these allegations are controversial and the dispute is not easy to settle, as is indicated in a recent controversy over whether patents keep drugs out of the reach of many: Do arsenic patents keep drugs for rare cancer out of the reach of many... or is it rather the case that the patent got the drug into the hands of many quickly [4, 5].

Some technical solutions have been developed within the system to help deal with some of these problems, and to improve the patent system, including collaborative licensing models such as patent pools, clearing houses, open source models and liability regimes. They are useful in certain situations A, B, C,... and under some conditions X, Y, Z... [6]. But they do not solve all the problems the critics have pointed to, and they are not intended to do so.

A different sort of criticism, which would deserve a more lengthy discussion, focuses on the relations between the current patent system and global justice. Thomas Pogge has argued in several works [7, 8] that the current patent system is valuable but insufficient to provide incentives to develop and distribute drugs that will achieve major global health impacts.

Concerning access to medicines: the problems are that the most innovative pharmaceutical companies find it difficult to make money on treatments focused on the needs of the developing countries. Charity donations are insufficient and lead to the problem of parallel imports.

Pogge’s proposed solution, very briefly, is to create a global “Health Impact Fund,” paying innovator companies based on the global health impact of their new product or treatment. The more QALYs (quality-adjusted life years) that are saved by a new treatment, the more money a company makes. The intention of this reward scheme is to focus the innovators on developing and distributing treatments that will have the greatest global impact on health.

Pogge’s proposal raises many questions – for example, about the reasons to believe that governments would be willing to invest the very considerable amount

of money required yearly in the health impact fund. And how is one to make sure, with so many vested interests and confounding variables (non-compliance, etc.), that the measurements of the health impact achieved by various drugs are accurate and fair?

But this is not the place to pursue such critical remarks; what is important in this context is that Pogge is one of those who are dissatisfied with the present patent system and has suggested a way of reforming it without abandoning it completely.

24.6 Future Relations Between Ethics and Patent Law

These criticisms, and the earlier discussion of the political and ethical context of patents, raise the issue of the future relations between ethics and patent law. If, when, and how should there be a marriage of ethics and patent law? Many patent examiners and patent lawyers resist the idea – hence, the “if.” But I hope to have shown that ethical aspects are integrated in the interpretation and implementation of patent law. This raises the following questions: Should ethics be integrated in examination of patent applications? Should ethical aspects rather be examined before or after the legal examination? In the latter case, how, and by whom?

The two main options are (1) the same group checks legal and ethical dimensions. This will also require training in ethical analysis of this group, not only in patent law, and (2) one group checks the legal dimensions, another the ethical aspects. The problem and point of departure is that patent examiners rarely have any education in ethics. Moreover, ethicists rarely have any education in patent law. If integration is to be successful, what is needed are people with backgrounds both in ethics and patent law – as well as in the area of research relevant to the patent application.

The views on many moral issues are divided in Europe, so there are problems with both avenues. To see a possible way out, we have to take a somewhat closer look at ethics again.

24.7 A Closer Look at Ethics

Hardly surprising, arguments used in practical ethics are action-guiding. Ethical analysis requires interdisciplinary efforts; it based on, and makes explicit, several types of premises. Some are knowledge-oriented: What do we know? What is the current state of the art in science plus regulations and... Other premises are value-oriented: What do we want to achieve, avoid and why: What do we owe each other (norms, values, goals)?

Knowledge gaps, risks and uncertainties are particularly important in rapidly developing research areas, a point well made by Nils-Eric Sahlin in his contribution to the present book. Knowledge gaps can pave the way for unduly raising expectations, for avoiding beneficial options, for creating harm, for funding projects that should not be funded, or vice versa.

Four main types of starting points will be relevant in the present context. I will describe them briefly, indicating their main focus, their strengths and weaknesses. They can all be argued for, and there is no arbitrariness in disputes over what contributes to human good. These starting points may in many cases lead to very similar conclusions, but not always. If and when they do, the reasons are likely to be different. I want to stress that the chosen order of presentation does not imply a value judgment – it is not a hidden order of importance.

The first ones are utilitarian traditions: stressing the obligation to do as much good for as many as possible. The test of a controversial patent application according to a utilitarian, or more generally a consequentialist approach, is to check and value the (actual or likely) consequences of approving or disapproving the application. There are several versions, depending on whether “good” is interpreted in terms of “happiness” (classical hedonism, [9]) or in terms of “interest satisfaction” (preference utilitarianism, [10]).

The strength of this approach is that it provides a method for dealing with conflicting obligations. But the problems include the difficulties of identifying alternatives, anticipating future consequences, and managing interpersonal comparisons in a non-arbitrary way – that is, addition, subtraction and weighing of harms, benefits, utilities, etc.

Another point of departure is the human rights traditions. The test of a controversial patent application according to this approach is to check whether it would be a violation of human rights to grant or to refuse the application. There are several versions, depending on what is taken to be the basis of the rights and whether the approach is philosophical [11, 12], theological or not.

The strength of this approach is that it is in line with widespread moral intuitions, which also are reflected in many international documents: moral life needs more than one concept and one principle. The problems raised by the approach include identifying the basis of the rights, the grounds for identification of new rights, the scope of the rights, how to solve possible conflicts between rights and freedoms, and so on.

The third kind of approaches to be discussed here can be labeled human dignity approaches [13, 14]. The test of a controversial patent application according to this approach is to check whether granting or refusing the application would undermine or respect human dignity. There are several versions, depending on whether human dignity is understood in a Kantian or in other, more fundamentalist, terms.

The strength of this approach is that human dignity is a basic concept in many internationally agreed-upon declarations. It is the key concept in Article 1 of the Oviedo Convention [15], for instance. But the problems include the vagueness and ambiguity of the notion of “human dignity.” A wide interpretation can block new advances in medicine. What violates human dignity is clearer: eugenics,

slavery, discrimination, stigmatization, commercialization, reproductive cloning, and degrading treatment, including trafficking and instrumentalization of human beings.

A fourth perspective is offered by virtue ethics, a tradition going back to Aristotle, emphasizing the role of virtues or moral character in normative ethics [16, 17]. The basic questions for moral philosophers are “What sort of person should I be?” and “How should I live?” Virtue ethics theorists differ from deontologists and consequentialists who aim primarily to identify universal principles – such as maximize utility, do your duty – which can guide actions in any situation.

There are different varieties of virtue ethics that cannot be discussed here, and there is a debate concerning to what extent virtue ethics can be action-guiding. It is sometimes not quite clear what a virtuous person would do, for example, when confronted with a controversial patent application. The attempt to act like a virtuous person is not always action-guiding in the sense that it tells us what to do.

These starting points have several implications and differences when applied to controversies of patents on human SC-based methods and inventions. A basic question is: Can new knowledge and medical benefits for patients outweigh violations of basic human rights? of human dignity? be consistent with certain virtues? Under certain conditions, the answer to a controversial patent application might be “yes,” according to utilitarianism, but “no” according to other options – or “don’t know” according to still others.

If patents on biotechnological inventions based on induced pluripotent stem cell (iPSC)- research would not violate human dignity or any human rights, and the publication or exploitation of these inventions would promote the public good, then such a patent application should be less controversial than a patent application of hESC-based methods or inventions.

So far, there seems to be an advantage for patent applications on methods or products based on iPSC research – no human rights are violated and it is hard to see how respect for human dignity could be undermined. But as other contributions to this book indicate, iPSC research and applications raise other ethical issues. Some old problems are solved, and others enter into the picture. Recent discoveries have also indicated that there are important differences between hES cells and iPSC cells [18], so at present it appears premature to conclude that one cell type can be replaced by another.

Moreover, if hES cells deserve a special moral status because they can give rise to embryos, and iPSC cells could also theoretically give rise to an embryo, it would seem that for the sake of consistency, either the moral status of the iPSC cells would have to be upgraded or the moral status of the hES cells would have to be diminished. If these theoretical possibilities were to come true, it is difficult to predict how this would influence the future practice of the patent offices.

Furthermore, are there other relevant differences, in terms of, for instance, basic biological knowledge or clinical applicability that are relevant for the ethical evaluation of a patent application – such as tumorigenicity? stability? plasticity? epigenetics? If strange things happen, is it possible to deal with them successfully, to reverse the process?

How should one then proceed to elucidate a particular patent application from an ethical point of view? The idea is simple: identify the ethical issues raised by the application, and check them in light of different ethical starting points. If the result of all of them points in the same direction, it will be possible to arrive at robust conclusions. If not, time is needed, and the strength of the arguments will decide the issue.

Some room has to be left for disagreement over tenability, relevance, and missing arguments. But in this respect, there is little difference between ethical and scientific controversies; also in the latter case, there may be disagreement over tenability, relevance, and missing arguments.

Analogously, the interpretation of the scientific information available and the uncertainties can be varied. If the variations do not affect the conclusion, it will be robust. The tentative conclusions can be checked in both these ways. New technological solutions may change the ethical landscape, and provide new ways out of a moral dilemma, as indicated by the contribution of Aliki Nichogiannopoulou to this book.

This raises epistemic risk problems, essential also to patent problems. How unstable can the evidence be on which the decision is taken? And in what regards may the evidence be unstable? It is possible to conceive of a technique for transplanting cells to the brain, which is successful in the sense that the graft survives and the patient improves. But long-term consequences are not studied, for instance, epigenetic effects or the migration of the transplanted cells to other parts of the brain.

24.8 Definition and Interpretation Problems

There are not only choices between different ethical starting points, but also choices between different interpretations of assumptions and definitions of key terms, which are not unrelated to ethical considerations. There are interpretation and definition problems that should not be hidden, and that is evident from controversies in the literature.

The key term “human embryo” is defined in somewhat different ways in different legislations, and many courts in several countries are now waiting for the European Court of Justice to settle this issue and come up with a definition. The point I want to make here again is that the choice of definition – whether the court comes up with something or decides to leave it to the member states – is not ethically neutral. It will favor certain interests at the expense of others. Masquerading as legal, ethical decisions are taken that will have consequences for researchers, patients, patient organizations, industry, and so forth. Moreover, this shows that the distinction between ethics and law in practice is not as clear as it is sometimes perceived to be.

This is also illustrated by related problems of interpretation. Can, for instance, genetically modified animals like the “oncomouse” be patented? Views have differed on the last issue. In 1988, the United States Patent and Trademark Office (USPTO) granted a US patent on the oncomouse to Harvard University [19]. The

European Patent Office initially refused the patent, then later granted it, a decision that was opposed by several parties and later revoked and maintained in restricted form [20]. But the Canadian Supreme Court, in a divided judgment, rejected the patent, arguing that higher life form is not patentable because it is not a “manufacture” or “composition of matter” within the meaning of “invention” [21].

This illustrates again that the choice of definitions of key terms such as “higher life forms,” “manufacture,” “composition of matter,” and “invention” are not ethically neutral. Accordingly, the underlying ethical premises ought to be made explicit.

24.9 EPO and EGE

To begin with, the EGE wrote several years ago in an opinion on hESC patentability [22]:

“Only stem cell lines which have been modified by in vitro treatments or genetically modified so that they have acquired characteristics for specific industrial application, fulfill the legal requirements for patentability.”

Let us now return to the ethical and political context of patent controversies. The political features, including EPO’s self-regulation and political autonomy, raise problems of legitimacy and democratic accountability. Voices asking for more public input into the patent system are getting stronger.

As perhaps a small way to deal with that problem would be to discuss how Article 7 of the earlier-mentioned patent directive can be implemented. If it is implemented, voices not only of patent examiners and those who benefit most economically from the current patent system, including patent lawyers, will be heard.

According to this article in Directive 98/44/EC: “The Commission’s European Group on Ethics in Science and New Technologies evaluates all ethical aspects of biotechnology.” This article has not been implemented. This suggests an option, which will be outlined below.

24.9.1 *A Proposal*

EPO asks the EGE, in certain cases, to be specified further below – not for a “yes” or “no” to a patent application under consideration, but for an analysis of what ethical issues are raised by this particular application. This is especially important if a class of inventions that ought not to be directly exploited commercially has to be defined. This information is used, along with other information, as a starting point or basis when the EPO reaches its decision.

In other words, the idea is simply that the EPO could send a request for an ethical evaluation to the EGE, phrased as “What ethical issues are raised by applications

of this or that kind?” The idea is not that the EGE is to provide a green or red light – the EPO still makes the decisions – but the EGE would identify and describe the ethical issues raised, along with the concerns, present a position or some positions and the arguments for and against these positions – which is a background evaluation that the EPO could take in along with other information the EPO bases its decisions on, including scientific, regulatory, social, and economic information.

24.9.2 *Different Levels*

There are different levels to be separated in the work of the EPO. The strategy proposed above can be adjusted to the distinction between examiner level, operational level (e.g., technical fields), the Board of Appeal or the Enlarged Board of Appeal (EBoA). I can see the possibility of useful interactions – somewhat different – on several levels. For the EBoA level, I would imagine a more qualified interaction than on other levels. But as the EBoA decisions sometimes take a long time, I see no serious obstacle here.

Moreover, in view of the current workload of the EGE, the focus should anyway be on types of problematic or controversial applications, so that one could learn something and generalize from these cases – rather than that the EGE is asked to provide comments on each individual file.

Whether there are the formal links necessary between EGE and EPO, and whether this proposal would be compatible with the remit of the EGE will have to be left to the legal services of the EPO and EGO to decide. But in view of the independence, the multicultural and interdisciplinary composition of the EGE, it would at least provide a possible step in the direction of implementing Article 7 of the patent directive.

An alternative might be that the EPO in other ways widened its circle of consultation – as it has by the recent addition of an economist, a political scientist and a representative of the generics industry to its Standing Advisory Committee. In a way, my proposal is in line with the thinking underlying this broadening approach. Thus, this is good in my view, but I want to focus on the ethical aspects, which is something else and is what Article 7 explicitly addresses.

24.9.3 *Possible Objections*

There are several objections to this proposal, which obviously need to be discussed more thoroughly than is possible here. They include legal, political, and economic aspects of the proposal, such as:

It is inconsistent with the current EGE remit, since the formal links between EGE and EPO are not strong enough. Clearly, the legal aspects of the proposal will have to be left to the legal services of both institutions. But I have discussed these

objections in talks given at the EPO both in Munich and the Haag, and I have not been convinced that they are lethal to the proposal. Besides, a formal institutional link is apparently not always necessary, according to Aliko Nichogiannopoulou's contribution to this book. The EPO is free to decide to consult with the experts they think relevant.

If accepted, the proposal will create more legal uncertainty. Moreover, it will prolong the time before a controversial patent application is either granted or rejected. But there have already been surprises in the current patent system, so there is no guarantee of legal certainty; besides, some of the controversial applications have taken years before they have been finally decided by the EBoA, so I don't think this is a fatal objection.

24.9.4 *Some Advantages*

If this works out, it would be good for both the EGE and the EPO. The EGE bases its decisions primarily on European values, enshrined in declarations and conventions such as the Oviedo Convention, the Charter of Fundamental Rights and the European Convention for the Protection of Human Rights and similar documents. These declarations and conventions have been agreed on politically. Thus in a sense they can be said to represent a public voice, even if this voice is sometimes somewhat vague and ambiguous. For the EPO, it would show the rest of the world that they have made an attempt to include ethical aspects, which is important, since many people take the ethical concerns raised by certain patent applications seriously. This might eventually persuade member states to implement Article 7.

24.10 Concluding Remark

In this paper I have tried to show that ethical issues are involved in granting or rejecting patent applications. This has implications both for the evaluation of patent applications and for the training of patent examiners. It is therefore essential for transparency as well as trust that the ethical issues are not hidden but are identified, reviewed and taken into account. Some proposals as to how these problems could be dealt with have been suggested, against the background of some criticisms of the current patent system and the fact that Article 7 of the patent directive has not yet been implemented.²

²I am grateful to Nils-Eric Sahlin for helpful comments on an earlier version of this chapter

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Part VII
From General Public to Researchers,
and Vice Versa: Communication Issues
in Translational Stem Cell Research

Chapter 25

Ethical, Legal and Social Implications of Translational Stem Cell Research: Effects of Commercialization on Public Opinion and Trust of Stem Cell Research

Ubaka Ogbogu and Amy Zarzeczny

Abstract We conduct a systematic review of studies of public opinion regarding stem cell research in Canada, US and the UK, and analyze the implications of findings for research governance. In particular, we examine and analyze available data on public trust of efforts aimed at promoting and commercializing stem cell research, and suggest strategies for managing public expectations, concerns, and attitudes.

Keywords Stem cells • Public opinion • Public trust • Commercialization • Patents

25.1 Introduction

There are a great deal of studies of public perceptions of stem cell research (SCR) and associated issues in relevant literature [1–19]. Most of these studies are directed at early, controversial or sensational forms of stem cell research, such as human embryonic SCR and human cloning for derivation of stem cells. Still, there is scant but recent data on how various publics regard emerging and less controversial advances in SCR [6, 11]. While there is significant variation in the data, especially among national jurisdictions and between various forms of SCR, the studies suggest aggregate public support for SCR. In general, certain elements of this public response to stem cell technologies have been fairly homogeneous and consistent, and are likely to remain so even as less ethically complex and less publicly contentious approaches to SCR emerge. In this chapter, we examine one such element of public perception, namely the linkages between research commercialization and public trust as an aspect of the public response to stem cell technologies. Specifically, we explore the public response to SCR commercialization (encompassing such

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factors as industry involvement in research, patenting, commercialization pressure and optimistic portrayal of future [economic] benefits of the research), and the linkages between such response and the degree of public trust in SCR.

We begin the chapter by providing a brief overview of, first, the current state of stem cell research, and second, associated public opinion data. Since excellent reviews of public opinion studies already exist in the literature [11–19], our focus in the latter case will be to highlight a number of current or interesting trends emerging from the studies, particularly as relates to perceptions of pivotal research methods, knowledge of SCR, regulatory activity, and public trust. The discussion then shifts to the challenges associated with commercialization in the SCR context, and their real and probable effects on public trust. Our aim is to show that these challenges and their effects on public trust are likely to endure even as less ethically contentious stem cell technologies emerge, or associated perception and policy issues recede. We conclude by briefly reflecting on governance strategies for curbing commercialization excesses and maintaining public trust in SCR.

25.2 A Primer on Stem Cell Research

Stem cells are unique unspecialized body cells that are capable of self-renewal through cell division and differentiation into specific specialized cell types. In humans and animals, stem cells act as progenitors of different cell types, and as an internal repair system for expended or damaged tissues and cells. Two main types of stem cells exist in humans and animals, namely somatic (a.k.a. adult) and embryonic stem cells. Somatic stem cells possess both unique qualities of stem cells, but the potential for differentiation has, until recently,¹ been known and shown to be limited to cells of the same type as the organ or tissue from which they originated. Embryonic stem cells, which exist naturally in early stage human embryos, are known to develop into many cell types. This ability to transform from an unspecialized state into a variety of specialized cell types – otherwise known as pluripotency – sets embryonic stem cells apart from somatic counterparts. Research on stem cells seeks to harness their unique potential for a variety of purposes, including use in cell-based regenerative therapies for diseases associated with cell damage or demise, increased understanding of the anomalous effects of abnormal cell division and differentiation, and use as controls for screening new drugs prior to clinical testing and use.

To accomplish these research goals, scientific researchers seek to derive stem cells from a variety of sources, including human and animal adult tissues and cells, gametes, and early stage embryos. Of these, stem cell derivation from embryonic sources is of immense interest to researchers, chiefly due to the increased differentiation and developmental potential of embryonic stem cells. Actual and proposed methods of deriving embryonic stem cell lines for research purposes include derivation from supernumerary live or frozen embryos created for assisted

¹See the discussion on induced pluripotent stem (iPS) cells below.

reproduction (e.g., *in vitro* fertilization) procedures, from embryos created specifically for stem cell procurement (through a variety of processes such as *in vitro* mixing of gametes, or from cells taken from an existing embryo), and somatic cell nuclear transfer (SCNT), a form of cloning involving the creation of “research-specific” embryos by inserting the nucleus of human somatic cell into an enucleated human or animal ova [20–26].

Its promise and potential notwithstanding, stem cell derivation from embryonic sources has also attracted considerable and perennial social controversy. The main issues of controversy have centered on the ethical and moral propriety of conducting research on human embryos, which are destroyed in the process of research [27], and in the case of SCNT, the threat posed to human individuality and the integrity of our species by research that invites the possibility of unconventional human reproduction. The social controversy is intensified by the fact that to date, SCR in general has yielded little or no routine applications of its anticipated benefits. Research remains largely in the basic laboratory stage, and clinical translation is in the very early stages.² In addition, a host of scientific questions and obstacles remain, which in turn raise a variety of very specific research ethics concerns [20, 27, 30].

Nonetheless, relying on knowledge gained from existing research methods, and working with intent to address the scientific and ethical challenges associated with such methods, scientists have made and continue to make substantial strides in understanding and harnessing the potential of stem cells [20]. For example, researchers in Japan and the United States recently discovered ways to coax somatic cells back to a pluripotent state by inserting a number of defined genes into the cells [31, 32]. The resulting cells, termed induced pluripotent stem cells or iPS cells, have opened up a new frontier in SCR, and there have been recent successful attempts aimed at improving the efficiency and effectiveness of the method [33, 34].

Attempts to address the scientific, ethical and social challenges associated with SCR have led to significant regulatory activity all over the world. Much, if not all of the regulatory activity has been directed at the controversial forms of SCR, namely cloning, research on embryonic sources, and research using human/animal gametes. Regulatory policies fall into two main categories: a public ordering approach involving direct state intervention (mainly through legislation) or state-led regulatory initiatives, and a private ordering approach based on self-regulation through adherence to professional or institutional research guidelines [35]. Public ordering regimes vary widely in form and focus among jurisdictions, reflecting a plurality of views, values, and attitudes toward the deeply divisive and intractable issues raised by embryonic stem cell and cloning technologies. Commenting on the nature of policy variation associated with SCR regulation, an interdisciplinary group of stem cell law and policy scholars noted recently that

hopes regarding the therapeutic promise of [SCR] are countered by social concerns associated largely with the sources of stem cells and their uses. This interplay between

² The United States Food and Drug Administration (FDA) recently approved the first known human clinical trials for medical treatment of spinal cord injuries derived from embryonic stem cells [28]. However, the trials have been put on hold for safety reasons [29].

promise and controversy has contributed to the enormous variation that exists among the environments in which stem cell research is conducted in different jurisdictions around the world. Such variation is layered upon intra-jurisdictional policies that are also often complex and in flux. The resulting multifaceted and, at times, overlapping and discordant regimes present both within and between different jurisdictions constitute what we term a “patchwork of patchworks” [36, p. 83].

In general, three regulatory regimes exist among jurisdictions that rely on a public ordering approach: restrictive regimes, which prohibit all forms of embryonic stem cell and cloning research, often with criminal sanctions, intermediate regimes, which strictly regulate some procedures while restricting others, and liberal regimes, which generally allow many or all research procedures – subject to strict regulations [35]. Canada’s governing legislation, the Assisted Human Reproduction Act (AHRA), for example, is considered intermediate as it permits, subject to strict regulatory scrutiny, research on supernumerary IVF embryos and interspecies SCNT (embryos created by inserting the nucleus of a human somatic cell into enucleated animal ova), but prohibits SCNT and embryo creation for research purposes. By contrast, the equivalent UK legislation, the Human Fertilisation and Embryology Act, is considered permissive as all major forms of embryonic SCR and cloning for research are permitted, subject to strict regulatory controls.

Public opinion has played a somewhat visible role in stem cell research regulation in many jurisdictions. In Canada, for example, consultations with members of the public (including targeted consultations with groups and persons with special interests in SCR) were part of the policy and legislative process leading up to the enactment of the AHRA [37].³ Similarly, the UK’s embryo research and assisted reproductive technologies regulator, the Human Fertilisation and Embryology Authority (HFEA), has widely sought public opinion on a number of SCR-related regulatory issues such as cloning, creation of hybrids and chimeras for research, and gamete, embryo and tissue donation for research [6–8]. The HFEA public opinion gathering process, which often includes public education and public dialog components, has been an excellent model of public engagement in SCR regulation. There is also some indication from published reports that results of the HFEA public consultation process have been influential to policy outcomes [6]. Perhaps the most striking example yet of public involvement in SCR regulation is the ballot proposition, a referendum-style form of direct democracy employed by US states such as California and Florida. In California, for example, voters approved a ballot proposition in 2004 to render it a constitutional right to conduct SCR, and to establish a government agency to regulate and fund such research [19]. The voters also approved a ban on human reproductive cloning research.⁴

While it is tempting to conclude that public opinion is largely ignored where policy outcomes contradict available public opinion data – indeed, in some cases it

³ Negative public opinion was cited as justification for the ban in the AHRA on certain aspects of SCR, despite the existence of public opinion data showing the contrary.

⁴ A similar Florida initiative did not make the ballot. See <http://election.dos.state.fl.us/initiatives/initdetail.asp?account=41859&sequum=1>.

is difficult to measure the relevance or actual impact of public opinion to policy outcomes – the latter two examples suggest that public opinion sometimes achieves noteworthy policy traction. Public opinion data is also often used as tools for legitimizing policy proposals, and as a means of seeking further public support for “preferred policy outcomes” [13].

25.3 Trends in Public Opinion

Available public opinion data on stem cell research issues has emerged from a broad variety of constituencies, including academic researchers, professional public opinion research firms, media organizations, and government agencies [1–19]. The mode of engagement with the public also varies widely, ranging from polls and focus groups to public meetings and the use of new media forms such as online consultations. As noted in the preceding section, engagement strategies sometimes include a public education component whereby potential respondents are primed on the relevant facts and issues prior to soliciting opinions (see, e.g., [6]). The diversity of sources and methods employed, taken together with sustained societal interest in and controversy surrounding some forms of SCR, have resulted in a great deal of public opinion research activity. As one social scientist puts it, “[f]ew scientific and technology-related issues have sparked as much survey attention as the public controversy over human embryonic stem cell research and therapeutic cloning” [13, p. 131].

Putting aside for a moment the source, methods, and volume of research, available public opinion data also reveal a theme of variation consistent with the social and regulatory response to SCR. It seems accurate to say that public opinion is a distinct “patchwork” in the larger “patchwork” SCR landscape. Public perceptions of SCR issues differ and fluctuate in temporal and spatial terms, and are continually shaped and molded by factors such as the framing of research questions, pivotal research events, politics, religion, media coverage, targeted framing of the issues by “interested” groups, and even popular culture, among other factors [13, 37]. As far as public opinion data goes, variation is likely the norm. At a minimum, it appears safe to assume so. In the US, for example, spikes in public awareness of and response to stem cell research issues have been linked to political milestones such as President Bush’s speech in 2001 outlining his executive decision to restrict research funding for various forms of SCR, and to key research events like the cloning of Dolly the sheep [13]. It has also been noted that deliberate framing of the embryonic stem cell issue by patient advocates in the US using ideas of “social progress” and “economic competitiveness...helped to drive up public support for funding between 2001 and 2005” [38]. Nisbet also points out that “evidence indicates that question-wording in surveys can have strong effects on the public’s stated response to these volatile [SCR] issues” ([13], p. 138). Of course, the “opinion shapers” present in these examples may not occur at all, in the same manner, or to the same degree in other jurisdictions, and vice versa.

The moral of the variation story is that in presenting trends in public opinion of SCR, one should avoid drawing broad generalizations or transcendent conclusions from the data. It would also not be very helpful to merely roll out statistics from a few or diverse but disparate research studies. Rather, a better approach would be to constrain each step of the analysis to specific data points, taking care to clearly delineate the source, method, and jurisdiction from which the trend in question emerged. In keeping with this rough statement of methodology, our approach here is to highlight trends emerging from two kinds of public opinion studies: (1) tracking surveys where available; and (2) recent individual opinion surveys that either comprehensively revisit information provided by past public opinion, or are specifically targeted at areas for which there is scant or no available previous data. The tracking surveys are more likely to reveal trends over time; the former kind of individual survey will serve as an update on fairly established trends, while the latter kind will provide original information on less researched or emerging areas. For our purposes here, it will also suffice to limit the analysis, for the most part, to research data from the US, UK, and Canada. Lastly, our analysis will be constrained to the following trends, which we think will suffice to provide the necessary context for the main discussion on trends in public trust: support for or opposition to SCR (including specific research methods and applications, and the factors influencing support or opposition), public awareness of SCR, and perceptions of the regulatory environment.

25.3.1 Public Support of or Opposition to SCR

A review of public opinion data within the scope of our analysis confirms that public attitude to SCR is somewhat specific to jurisdiction and to the context of each opinion survey. Nonetheless, the data generally reveal that the public is *relatively* supportive of SCR in most jurisdictions, provided it is tightly regulated [1–19]. This observation, though indicative of a *support* trend, is perhaps more accurately understood in the negative, which is that despite the social controversy surrounding SCR, there is neither overwhelming nor noteworthy public disapproval or condemnation of the research. Succinctly stated, public opinion urges caution and regulation, not disapproval. The following discussion of public opinion data from the jurisdictions included in our analysis illustrates this point.

In Canada, results of an 8-year (1999–2004) tracking research study on public attitudes about biotechnology and related public policy confirm this picture of public perceptions of SCR [1–5]. The research study, commissioned by the Canadian Biotechnology Secretariat, consists of 12 public opinion surveys, two cross-national comparative surveys of attitudes to biotechnology in Canada and the USA, and over 80 focus group surveys. Although the study is directed at biotechnology in general, several waves of the study have included targeted inquiries into particular research areas such as genetically modified foods, nanotechnology, and SCR [1–5]. Significantly, a summary report on the entire study notes that results from all areas

“have been remarkably consistent since the inception of the research program” [39]. According to the study results, Canadians’ overall attitude can best be described as cautiously supportive. While a vast majority of Canadians support the research because of the belief and expectation that it will yield health and medical benefits, this support is largely contingent on disassociating SCR from human cloning. To avoid the latter possibility, most supporters of the research indicate a need for strict regulatory controls. Indeed, study results indicate that “the only situation where ethics trump other considerations, and where Canadians are prepared to accept a ban of an application on ethical grounds, is in the case of cloning human beings” [39]. There is also a noticeable upward shift in support when the research relies less on embryos created specifically for research, and more on non-embryonic sources (such as umbilical cord blood) or supernumerary embryos created for IVF purposes.

Recent public opinion data from the US similarly indicates cautious support for SCR. A July 2009 Pew Research Center public opinion study (conducted in collaboration with the American Association for the Advancement of Science), which comprehensively examined attitudes toward a variety of science-related issues and norms, found that a slight majority of the US public (58%) supports federal funding for embryonic stem cell research [9]. While this result specifically addresses the issue of funding for only one form of SCR, it is highly suggestive of a support trend for two reasons. First, the issue of research funding is intimately connected to stem cell research regulation in the US. Funding restrictions, rather than direct legislation, have been employed by successive US Federal and state governments as a means of controlling what forms of SCR are undertaken by scientists [40]. Under President Bush, federal funds could not be used for various forms of SCR, with the exception of adult SCR and research on approved stem cell lines derived before a 2001 cut-off date [41]. Upon assuming office, President Obama reversed the Bush directive, but did not significantly expand the scope of federal funding for SCR. Obama’s policy allows federal funding for embryonic stem cell research using supernumerary IVF embryos, but not for targeted creation of embryos for research, the creation of human-animal embryos, or SCNT [42, 43]. The point here is that funding controls are a *highly visible* means of judging the propriety of certain forms of SCR in the US. For members of the US public, support for federal funding for research is very likely to be an important measure of support for the research itself. Second, embryonic stem cell research (including SCNT) has proved to be the most controversial and socially divisive form of SCR in many jurisdictions, including the US. It follows therefore that support for the controversial kind of research should be indicative of similar or even higher levels of support for less ethically objectionable research methods.

But the preceding points need not be overemphasized. The results of the Pew study confirm the preexisting state of public opinion about SCR in the US. Other prominent studies have noted a relative level of support for SCR in the US, even in its most controversial form [4, 13]. Also, support becomes “broader and deeper” where the research does not involve the destruction of embryos, or relies on

supernumerary IVF embryos [13]. Much like Canadians, the US public also emphasizes the need for tight regulation of this field of inquiry [4].

Similar levels of support are observable in UK data. A 2006 HFEA opinion poll, which is consistent with prior UK data, reveals that over half of the UK population is supportive of human embryo research in general (56%) [6]. More than half also supports the use of supernumerary IVF embryos for research, and more people support targeted creation of embryos for research than those who oppose it. In the HFEA poll, support increased dramatically when the research was tied to a beneficial rationale, such as understanding disease. However, the reverse was the case with the creation of cytoplasmic hybrid embryos (human embryos with a small amount of animal genetic material, created through interspecies SCNT) and true hybrid embryos (embryos that contain half human, half animal genetic material). A majority of respondents did not favor the creation of either form of hybrid embryo for research, although support for cytoplasmic hybrids nearly doubled when linked to future relevance to understanding disease conditions. Similarly, support was higher among those who have prior knowledge of the “possibility” of creating both kinds of hybrid embryos. The latter group was also less likely to have concerns about the procedures [6].

A contemporaneous written survey conducted by the HFEA as part of the public consultation process described earlier (which also included the poll discussed in the preceding paragraph, public meetings, and deliberative workshops) casts some doubt on the trends reported in the polling data [6]. The survey revealed a public that is highly skeptical of any type of embryo research, and of the creation of animal–human hybrids for research. In that survey, a majority (65%) of written responses expressed the view that no research using human embryos (cloned, created for research, or obtained through IVF donations) is acceptable (810 responses were received, mainly from individuals). An overwhelming majority of respondents were also opposed to hybrid embryo research. Reasons advanced for objections to both forms of research include the belief that life is sacred, the potentiality of the embryo, the view that embryo research amounts to “playing God,” intuitive disgust, human dignity, and slippery slope arguments. Interestingly, when asked to state whether limits should be placed on human embryo research, a small minority of respondents (53 out of 810) felt that current regulations (which are among the most liberal in the world) were sufficient [6]. Since the HFEA poll and written survey are the first and possibly only instance of UK public opinion research on interspecies SCR, the results of hybrid scenarios reported here constitute the prevailing trend of public opinion on the matter.

What, then, are the reasons for the disparities between the poll and written survey results? One possible reason noted in a report on the consultation process is that the written consultation was designed to solicit the opinions of those with a “specific interest in the issues,” and findings were not intended to be representative as the participants were self-selecting [6]. Nonetheless, the UK data reinforces the need to exercise caution when drawing conclusions or trends from public opinion data.

Aside from the UK data on hybrid embryo research, there are very few existing studies elsewhere that examine public opinion about emerging SCR technologies such as interspecies SCNT, iPS cell research, and embryo creation through parthenogenesis and single blastomere extraction (for a lay explanation of the various emerging techniques, see [20]). The only other study we found on point is a recent Canadian focus group study by Einsiedel and colleagues, which reported high levels of support among the Canadian public for interspecies SCNT, but with the *caveat* that it be undertaken only under strict regulatory scrutiny [11].

Lastly, it is evident from the discussion in this section that public opinion about SCR has not been static. In particular, different forms of SCR have elicited varying degrees of public opinion in various jurisdictions. Relatedly, shifts toward supportive or favorable public opinion tend to correlate with shifts from ethically problematic to less ethically challenging SCR methods.

25.3.2 *Awareness or Knowledge of SCR*

Our foregoing discussion of the 2006 HFEA poll data indicates that level of awareness or knowledge of SCR impacts public views of the research [6]. In general, higher levels of awareness or prior knowledge is linked with increased support for various forms of SCR. However, this positive association is somewhat mitigated by low levels of general awareness about SCR and related issues among the UK public. According to the HFEA data, less than one in ten persons reports knowing a lot about stem cells and the use of human embryos in research. A lesser proportion of persons (1 in 20) has a working knowledge of the possibility of creating hybrid embryos for research. The majority of the UK public report merely having heard of or knowing a little bit about stem cells, and embryo/hybrid research, mainly through news media sources [6].

Contrary to the UK situation, available data suggests that there is widespread public awareness of SCR and related issues in the US and Canada [4, 15]. A 2005 Canadian government public opinion tracking research report notes, for example, that “in general public groups (where there is generally less understanding of new technologies), participants knew an incredible amount about the technology...its potential...and some of its side effects and implications” [4]. The report also reveals that familiarity with SCR and related issues is higher in the US, most likely due to the fact that in the US, SCR is considered the “poster child” of biotechnology and attracts higher levels of media and political attention. The 2009 Pew study referred to above, which found that a majority of Americans (52%) know that what distinguishes stem cells from other cells is that they can develop into many different kinds of cells, confirms this awareness trend [9]. Most strikingly, the Canadian report concludes that “[u]nlike other areas of technology...the general public tend to hold similar levels of knowledge and interest in stem cell research as found among [i]nvolved Canadians/Americans” [4].

A review of pre-2004 US public opinion data points to a much more nuanced story about the state of US public awareness or knowledge of SCR [13]. Matthew Nisbet, the author of the review, concludes that although the American public reports having a good basic knowledge of SCR and associated issues, such knowledge is sorely lacking in specifics, especially as regards the “science and the policy driving the [SCR] controversy” [13, p. 138]. He also notes a tendency toward spikes in public attention to SCR around pivotal events, often aided by media attention. For example, public attention to SCR rose sharply following President Bush’s 2001 nationally televised address on the scope of federal funding for SCR, but soon waned with a drop in media attention to the issue.

Nonetheless, the recent trend toward deeper public engagement with SCR can be viewed as an example of the upward mobility of knowledge inspired by sustained controversy and media attention. As the Canadian government report observes, “in the right media environment, the general population’s knowledge and understanding of an issue will “catch up” to where involved Canadians/Americans are” [4]. That said, one should be careful about generalizing from one data point to another, and it is perhaps best to interpret the data as presenting a flavor of trends about the state of public awareness or knowledge of SCR.

Lastly, the correlation between knowledge and support observed in the UK data is missing in the US and Canadian context. The dominant conclusion drawn from existing public opinion studies is that familiarity with SCR is not “strongly correlated” with support, and “perceptions of moral issues have been found to be the strongest determinants of individual support for SCR” [15, p. 71]. Nisbet found, for example, that people still expressed strong reservations to certain forms of SCR and cloning research despite very limited knowledge of the science and relevant policy [13].

25.3.3 Perceptions of the Regulatory Environment

As we have noted throughout this chapter, various public opinion polls have found that approval of SCR is contingent upon the existence of strict or at least adequate regulatory structures. Polls that have directly explored this link reveal that members of the US public are generally satisfied with usual levels of government control, while members of the Canadian and UK public insist on tighter regulations [15]. Two inferences can be drawn from this duality. First, the data might be indicative of trust and confidence in existing regulatory structures for research governance in general. It is conceivable that the US public holds a favorable opinion of highly visible, politically independent, and generally well-regarded regulatory institutions such as the US National Institutes of Health (NIH) and the institutional review board (IRB) system of research ethics governance, which are both responsible for governance and oversight of broad areas of research, including SCR and embryo research. Indeed, public opinion data has shown that there is a very high level of public confidence in research ethics boards [4] and in regulatory institutions

perceived to be independent of politics and governmental influence [4, 44]. Conversely, the data might also suggest a critical view of existing regulatory structures, especially those considered to be overcome with gaps, ineffectual, inappropriately biased, or barely visible. For example, calls for stricter regulation from members of the US and Canadian public have been linked to a general lack of knowledge about SCR governance systems, and about how rigorous or stringent they are [4].

Second, the data highlights a possible link between public perception and “policy exceptionalism,” by which we mean the idea that certain areas of research require special regulatory policies above and beyond those applicable to similar research areas. Claims of stem cell policy exceptionalism, though a relatively unexplored territory in relevant literature, have had some traction in jurisdictions like Canada, particularly as regards informed consent issues [45] and the regulation of conflicts of interest in the SCR context [46]. In any event, this data should be interpreted with caution in view of the earlier finding that the public in some jurisdictions is not intimately knowledgeable about stem cell policy issues and regimes [6, 13, 47].

In Canada, confidence in the regulatory system for SCR also appears to be undermined by concerns about regulatory lag, i.e., the perception that regulators “do not necessarily have the skills or resources to adequately “keep up” with rapid advances in technology in areas like stem cell research” [4]. Public perception of SCR regulatory systems is also affected by concerns about the influence of corporate interests on research governance, and by high profile cases of research misconduct (such as in the case of fabricated studies in support of pharmaceutical products like Celebrex and Vioxx) [4].

We could find only one study that has explored public preference for existing SCR regulatory policy options [11]. A majority of participants in the focus group study indicated a preference for the permissive model highlighted in our earlier discussion of public ordering regimes. The permissive model, which is characterized by the UK policy regime, permits most, if not all forms of SCR, and research governance is typically provided by a regulatory agency with powers to extend the scope of governing legislation, monitor the area and craft regulations, or provide de facto or case by case approvals of novel or emergent research methods.

25.4 Public Trust and Research Commercialization in the SCR Context

Very few public opinion studies have explored matters pertaining to public trust in science and/or biotechnology. Fewer studies have focused specifically on the SCR context. Such specific focus is probably not necessary or warranted, as there are no obvious or compelling reasons to believe that public trust issues pertaining to SCR are radically specific, or different from other areas of science or biotechnology. In fact, as we shall soon see, much of the theoretical and practical assumptions about

threats to public trust and confidence in biotechnology or scientific research are not unique to SCR, and have primarily emerged from challenges posed to public trust in other areas of medical or scientific research. Therefore, our analysis here would rely, to a considerable extent, on studies of public trust concerns parallel to those presented by the SCR context.

But first things first. Since our intention in this chapter is to examine the influence of commercialization on public trust in SCR, a proper starting point for this analysis is to explore first, the nature and scope of commercialization in or applicable to the SCR context, and second, discuss theoretical and observed effects of commercialization strategies on public trust. We then conclude the section by presenting and discussing available public opinion data on the latter effects as it relates to SCR specifically (where available) or to parallel issues in related areas for which opinion data exists.

25.4.1 Stem Cell Research and Commercialization

It is now common knowledge among observers that scientific research is currently immersed in an era of aggressive commercialization.⁵ No longer the exclusive preserve of private or industry-sponsored research, the commercial exploitation of scientific research has become a fundamental aspect of (public) research planning, funding, execution, translation, and regulation. Many jurisdictions have implemented measures to facilitate a shift in research ethos – from an environment where scientific knowledge is primarily valued “for its own sake” – to one that emphasizes commercial exploitation and practices commonly associated with industry involvement in research [48, 49]. Scientific researchers supported by public funds (the main source of research funding in many jurisdictions) are now encouraged, or even required to orient their research protocols toward commercial goals and strategies, such as building links with industry, translating research outcomes into commercial products, and pursuing avenues for commercial exploitation [46, 50, 51].

Numerous instances of the push toward commercialization of biotechnology research have been discussed in relevant literature [46, 50–52] and do not warrant repetition here. However, at least one example is apropos to the preceding remarks. Since the late 1980s, the Canadian government has increasingly oriented the nation’s scientific research funding mandate to emphasize the main pillars of the commercialization ethos (see discussion of these pillars below). The most striking example of this new direction is arguably the Networks of Centers of Excellence (NCE) program, which consists of distinct multi-institutional, multi-disciplinary

⁵ The term “commercialization” is used widely in the literature, oftentimes in a manner that hints at a negative connotation of the term, namely an emphasis on procuring financial gain or profit at the expense of value or quality. Here, the term more properly connotes any activity or process that “transforms knowledge and technology into new goods, processes or services to satisfy market demands” [55].

and multi-sector research networks funded by public and private sector contributions [48, 53]. The Networks have been described as a “national system of innovation” aimed at linking scientific research with industrial know-how and commercial exploitation, and one of their primary objectives is “narrowing the gap between research and commercialization and producing research that is applicable to Canadians” [53]. Each Network is formed by a pool of researchers with mutually related research interests, and each operates as a semi-autonomous entity under the auspices of Canada’s three major research funding agencies. To successfully obtain funding, Networks are required to provide, *inter alia*, a comprehensive plan for “knowledge and technology exchange and exploitation,” comprising elements such as “new products, processes or services to be commercialized by firms operating in Canada” ([54], p. 9), collaboration with public and private sector partners, effective intellectual property management and protection, and the ability to help develop receptors to exploit research breakthroughs [54]. In 2008, the NCE program was expanded to include centers dedicated to commercialization of research [55].

Canadian stem cell research has benefitted from the NCE program through the creation in 2001 of an SCR-specific NCE called the Stem Cell Network (SCN). SCN has actively pursued the commercialization mandate of the NCE program [46], most notably by means of a research program designed to align its activities toward “three of the most understood routes to the clinic/market: cellular therapies, drug discovery, and tools, reagents and diagnostics” [56, p. B14]. Strategies used by SCN to meet this goal include support for IP and venture capital development, requiring co-funding from private sector sources for funded research projects, and the creation of a biotechnology receptor company to seek commercial exploitation of IP emerging from funded research [46].

The NCE and SCN models have been established or adopted to varying degrees in other jurisdictions. A review of law and policy-related instruments in various jurisdictions, including several US states (CA, NY, MA, IL, NJ), the United Kingdom, South Korea, Japan & Singapore, reveal a similar pattern of support for and promotion of research commercialization goals. For the most part, the main objective of the commercialization drive, which is to build and increase the translation capacity of knowledge gained from scientific research for economic and social benefit, has either been met or is in the process of implementation.

Nonetheless, questions have been raised about the “price” of such enthusiastic focus on commercialization, and its likely effects on at least three “moral” pillars of publicly funded university research: research integrity, academic freedom, and public support. Occurring in *pari passu* with increased commercialization, a number of facts, events, and speculative assumptions have led to questions about the real economic and social value of biotechnology commercialization, and especially its implications for the aforementioned research pillars [46, 57–60]. These questions have in turn been explored through various research studies focused on themes commonly associated with commercial exploitation of research, such as conflicts of interest (the relevant “interest” being that between pure research goals and commercial exploitation goals), “inordinate” and inappropriate pressure on

researchers to seek early introduction of technologies into the marketplace (in a manner that raises questions about readiness), hype or exaggerated promotion of the benefits of emerging and emergent technologies, and the use of patents as a means of protecting commercial interests to the detriment of knowledge diffusion and innovation [60].

All of these themes and the empirical research studies exploring their connections to research commercialization have received considerable treatment and discussion in relevant literature [60]. As such, and because it would require a dedicated chapter to fully engage the topic, we avoid repeating the discussion here. However, it will be useful and sufficient to offer some general comments about trends and outcomes that have emerged from the empirical studies on some of the themes. Starting with the theme that has received the most attention, namely the patent issue, the claim that patents have a blocking effect on knowledge dispersal and innovation (commonly referred to as the anti-commons effect) [61] – mainly by increasing transaction costs and hindering access to the free flow of information needed for further research – has been fairly discredited by available empirical research. For instance, a series of studies by John Walsh and colleagues (in the US) [62–64] and Caulfield and colleagues (in Canada) [65] establish that even with significant commercial activity, patents do not commonly prevent access to information and materials needed for research. Similarly, an international report by the American Association for the Advancement of Science concludes that there is scant or no evidence to support the anti-commons claim [66]. Despite the lack of evidence, and perhaps due to its intuitive appeal, the anti-commons claim has generated considerable policy activity, and in some cases, regulatory responses seeking to prevent or remedy its “virtually non-existent” effect [60]. Such remedial measures may also mask some level of public frustration with an intuitively held belief that commercial imperatives are often likely to engender practices that restrict free and open access to research outcomes. Also, if one assumes that public policy reflects public opinion (a highly debatable position, in our opinion), then the policy response to the anti-commons claim would serve to illustrate a possible gap between public perception and evidence. This in turn should render suspect any reliance on public perception as a component of the policymaking process.

Focused empirical research on the other themes mentioned above is not as robust. However, available studies and certain controversial events linked to the respective themes suggest that there is reason to be concerned about the linkages between commercialization and the issues of market readiness, inordinate pressure to commercialize, conflicts of interest, and hype [60]. Numerous studies have shown, for example, that industry involvement in research impacts adversely on research publication practices, the training environment, and public trust and confidence in scientists [60]. Researchers involved with industry are likely to be less collaborative, and to engage in data-withholding practices (although other factors, such as academic competition, may also play a role). The issue of market readiness has recently been shown to be a legitimate concern in the SCR context, where a “black” market for stem cell therapies has developed despite the fact that the technology remains unproven or validated scientifically [60, 67]. Relatedly,

studies also show that some areas of biotechnology, including SCR, have delivered more on promise and optimistic portrayals than on outcome or benefits (economic or otherwise) [60].

Lastly, and more germane to our discussion here, we offer a few comments on the association drawn between the above themes and public perception of or support for science in general, and SCR in particular. At a most abstract level, the primary claim made about the effects of commercialization on scientific research is that rigorous and sustained commercial exploitation will negatively impact on a “normative public view” of how publicly funded research should be conducted. Stated simply, this claim often assumes the following form: the public is generally more favorably disposed to the view that scientific research should be primarily concerned with the pursuit of and improvement of scientific knowledge, and the translation of such knowledge into socially useful products and services; while the objective of commercial exploitation is allied to this primary objective, it is merely a secondary objective that should be tolerated only when pursued in a manner that maintains the integrity of the primary objective; and finally, the vigorous pursuit of the secondary objective – even without actual impropriety or attempts to undermine the primary objective – results in a negative public perception of scientific research in general, and commercialization in particular. Of course, in some instances, negative public perception could be fostered by actual cases of misconduct arising from or linked to commercialization motives or imperatives. Indeed, several notorious cases of research ethics breaches have been linked to or motivated by factors attributable to commercial imperatives and interests, including the famous Olivieri and Healy incidents in Canada (involving interference with academic and research freedoms and interests) [57, 68], and the Hwang Woo Suk affair (involving the manipulation and fabrication of data presented in two seminal but bogus papers on the isolation of human embryonic stem cells) [69, 70].

25.4.2 Public Opinion regarding Commercialization and Impact on Public Trust

On an intuitive level, it is not hard to identify reasons that public trust in science is important, and this issue has received significant attention in the literature. As Caulfield points out:

Without public support, the ability to do biomedical research will be greatly disadvantaged. Indeed, the public plays a critical role in all aspects of knowledge creation and knowledge translation. Public support is necessary to gain the political backing for research funding, for the recruitment of research participants for clinical trials, and for market acceptance of emerging drugs, technologies, and therapies [71, p. 56].

As discussed above, commercialization is an increasingly prominent force in science generally, and in various areas of emerging biotechnology – including SCR – in particular. Indeed, one researcher notes that “financial relationships among industry, scientific investigators, and academic institutions are pervasive.

About one-fourth of biomedical investigators at academic institutions receive research funding from industry” [72, p. 463]. In addition to the complex and numerous issues associated with commercialization of science generally, such as negative impacts on research collaborations, publication practices and training, addressed earlier, concern also exists regarding the potential impact the commercialization trend has on public trust in and, by extension, support for science [60]. For example, one prevalent concern is that real or perceived conflicts of interest associated with the increasingly blurred boundaries between academic and corporate research will erode public trust in implicated researchers and in the science they produce. Another worry is that “[i]ntegration of academic research into the market, however innovative, demands a price on the role and credibility of scientists,” and that as a result, “[s]cientists are no longer perceived exclusively as guardians of objective truth, but also as smart promoters of their own interests in a media-driven marketplace” [73].

Such impacts are particularly important given that public trust is essential to the research enterprise, especially as regards controversial areas such as human embryonic SCR [74]. Indeed, aside from the many ethical, legal and social issues associated with commercialization, without public trust it will also be difficult for stakeholders to even effectively pursue the commercialization agenda. More so, key aspects, such as public uptake of a product or service and support for the development of a market around a technology, will likely be impeded [51, 75].

A review of relevant evidence confirms general suspicions that commercialization does have an impact on public trust in science, although the nature and degree of this impact may vary. Public opinion research consistently shows that Canadians tend to have a high degree of trust in publicly funded university researchers, particularly as compared to researchers working for industry [4]. However, the introduction of commercial forces to the academic realm is also viewed as being important and relevant to public trust, as university researchers who receive funding from industry are often viewed as being less credible than those who do not. For example, when rating credibility on a scale of 1 to 5, 53% of Canadian respondents gave university scientists funded by government a score of 4 or 5, while only 23% came to the same conclusion for researchers funded by industry [4, 74]. These results support the data obtained in an earlier study, which similarly found that in assessing the credibility of various actors in the research environment, Canadians consider the source of funding, and the degree of independence from funding/commercial influence as critical determinative factors [76].

Data from other jurisdictions suggests that Canadians are not alone in their suspicions regarding privately funded research. For instance, in the UK, survey data consistently shows that public trust is lacking in respect of science associated with government or industry [77]. This lack of trust is further associated with increasing skepticism regarding scientists’ pronouncements about any science-related policy issues [77]. Interestingly, the survey data discussed in this research also shows that “independent scientists” and those “working for environmental groups” tend to score well on issues of trust, while governmental and industrial scientists do not. These findings appear closely tied to commercialization issues,

as “it is widely perceived that the beneficiaries of much new science are the large multinational corporations and their managers, while the public is left to carry the risk” [77, p. 2.44].

It can, however, be challenging to compare and contrast how public trust across numerous different jurisdictions is affected by the issues of commercialization, particularly in the absence of more standardized research on point. However, available data from various jurisdictions suggest an association between trust and the degree of independence from funding sources or commercial motives or influence, as is observed in Canadian public opinion studies. In the US, for example, government-funded scientists are placed at or near the top of a list of trusted sources, while privately owned companies and biotechnology company executives are ranked near the bottom [4, 78, p. 7-37]. In Europe, the story is slightly different – public trust in both university and industry scientists has increased since 1999, and in general, university and industry researchers enjoy very high overall levels of trust (80%+) across the EU [10]. Recent research out of Australia, focusing specifically on SCR, also suggests that public trust in SCR is closely linked to the context in which the research is conducted [79, 80]. The Australian studies found that the public is more likely to approve of SCR that is conducted within a public university than in a private company, and that this approval is closely associated with the level of trust accorded to the researchers [79, 80].

This latter data is particularly significant for our analysis, as the results provide the first direct empirical evidence of a link between public opinion about SCR and activities associated with the commercialization of scientific research (in this case, privatization). There is no reason to doubt that this association exists in other jurisdictions, especially since links between industry and the scientific academy and the pressure for commercializable research results are unlikely to wane in the near future.

25.5 Conclusion

Despite the prominence of optimistic timelines for groundbreaking clinical applications, many of which have since gone unmet, it remains clear that there is immense promise associated with SCR, both in terms of advancing general scientific knowledge and improving healthcare. However, in order for the field to achieve this highly anticipated and desired success, it requires continued support on various levels. Two key levels of support include the policy level, so that the research is permitted in the relevant jurisdiction, and the funding level, so that researchers have the means to conduct the research. Public support is vital for both objectives. Hence, when there are factors that impact public support for a technology, such as SCR, it is important to examine the nature of that impact so that negative effects can be mitigated, where possible and appropriate.

The growing prominence of commercialization in scientific research generally, and in the realm of SCR, is one such factor that, as discussed above, has been found

to have a significant impact on public trust in the science and its researchers. Indeed, research shows that the public is largely suspicious of commercialization of scientific research and that elements such as source of funding, conflicts of interest, and perceived motives are key factors influencing public trust and confidence in researchers. Given the likelihood that commercial forces are here to stay in the world of research, at least for the foreseeable future, the importance of devising strategies to mitigate their detrimental effects on public trust and research integrity is increasingly recognized and merits continued attention and focused efforts. As noted by Caulfield and colleagues,

governance strategies are needed to ensure that researchers are, as much as is practical, perceived to be distanced from industry influences and are seen to be working in the public's interest. This seems particularly important in controversial areas of research that receive substantial government support. For these areas of research, public trust could be facilitated by the creation of an independent governance entity that has the authority to oversee commercialization [74, p. 1353].

Various governance strategies and policy recommendations have been proposed to respond to concerns associated with commercialization of research, generally with the long-term goal of improving public trust in scientists and in the institutions directing their work. A commonly suggested approach is to focus on improving financial transparency and accountability by, for instance, the adoption of robust conflict of interest policies. The National Institutes of Health Conflict of Interest Guidelines are one example of such an approach, "designed to demonstrate that universities are now working together to make sure that their institutions are paying attention to all aspects of COI, including consulting, stock ownership, intellectual property rights, and COI within institutional review boards" [81, p. 1560]. Such measures may be accompanied by additional measures, such as oversight mechanisms that monitor the nature and extent of relationships between faculty and funding agencies, including industry investors and sponsors, policies limiting the amount of time that university researchers can devote to non-academic activities, or the amount of money they can make from consulting fees, and sanctions [81–83]. It must be noted that transparency and conflict of interest disclosure are increasingly viewed as being important not only for scientists, but also for commentators, bioethicists and, arguably, for regulators as well [46, 75, 84].

Other approaches have also been devised to address specific facets of the commercialization issue. For example, patent pools, which bring together patent holders in related areas so as to encourage sharing of technology in support of more efficient technology development, have been widely recommended [74]. Patent pools are generally governed by an independent body or board, and the mandate of such an entity could be structured to include promoting public good as a key objective [74]. Of course, patent pools are but one example of a specific initiative designed to address a particular problem – that of the "patent thicket." They likely would not be appropriate for all realms of research, but may have real possibility for streams of research that rely on government funds.

It must also be acknowledged that commercialization and its associated conflicts of interest, of various shapes and sizes, may largely be inevitable in our current

time. Accordingly, it has been suggested that the focus of the foregoing efforts should be on managing these forces effectively, as opposed to attempting to eliminate them [83]. We propose that regardless of the favored form of governance strategy adopted in any particular realm or jurisdiction, it should be preceded or, at the very least, accompanied by funding for current empirical research examining the actual impact of commercialization on research outputs, transmission of knowledge and the diffusion of technology, among other key elements of research innovation [60]. This call for further empirical research is necessary in order to improve our understanding of the genuine extent of the commercialization issue, so that any resulting policy measures can be informed by relevant data rather than relying on speculation or perception. The issues associated with commercialization of science and their potential ramifications are of sufficient importance to warrant considered, cautious and informed policy making.

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Chapter 26

Patients' Organizations and Their Opinions: How Much Have They Been Taken into Consideration When Regulating Stem Cell Research?

Mary Baker and Philip Watson

Abstract As advances in stem cell research have accelerated, a growing chorus of concern has emerged worldwide, that traditional values have been put aside in the quest for scientific progress. Almost uniquely within biomedical research, stem cell research offers the potential to improve the health of patients living with a wide range of medical conditions. At the same time, such research also poses deep ethical and moral dilemmas that may impede the progression of the development of new medicines or therapies, that aim to improve the lives and suffering of patients living with a variety of complex disorders. Medical and religious ethics have been drawn into a complex social and political debate around future directions of stem cell research and how this should be funded within diverse national and European systems. The development of the patient advocacy movement, within diverse international health care systems has now developed into a profoundly influential force that, for the first time, is now engaged in the debate around the allocation of European research funding and the future directions of translational stem cell research. Patients with a variety of conditions have expressed views that their suffering is real now and is often profound. This suffering needs to be considered in the context of a complex moral and ethical debate, particularly where this is driven politically active groups, whose views or beliefs are not shared by patients. How do we, as a diverse society, pursue research in order to alleviate long term suffering and endeavour to progress research sensitively, all within a pluralistic society? In this context, religious ethics also have to be considered within a multi faith framework and progress needs to be made, while trying to reconcile the diversity of viewpoints. Future research directions must be pursued with stringent and enforceable rules, regulations, and public oversight, anchored both in law and reverence. With the further extension of influence of the patient advocacy into the realm of health care research and perhaps, the allocation of health care funding, we must make a plea for patient responsibility and awareness of the needs of all constituencies within an increasingly complex international health care environment.

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Keywords Informed consumers • Patient and health advocacy • Societal perspective
• Translational stem cell research

26.1 Introduction

As advances in stem cell research have accelerated, a growing chorus of concern has emerged worldwide that traditional values have been put aside in the quest for scientific progress. Indeed, almost uniquely within biomedical research, stem cell research offers the potential to improve the health of patients living with a wide range of medical conditions while at the same time posing deep ethical and moral dilemmas that may impede the progression of the development of new medicines or therapies. Echoing themes raised by Mary Shelley in her famous book of 1818, fears that a Dr. Frankenstein will use this new technology to create a monster, has caused many observers to ask again how far science can go before it offends the laws of nature and of God. Now that we can clone a dog and extract stem cells from human embryos, will nuclear transfer from embryonic stem cells be used to save lives and relieve suffering in ways that most people find too frightening to contemplate? This debate is of enormous public health concern, bearing in mind the interests of millions of people worldwide with Parkinson's and Alzheimer's diseases, type 1 diabetes, and some forms of cancer, among a myriad of other disorders, who believe that they also have a stake in the translation of stem cell research to the clinical care environment.

Over the past two decades, the patient advocacy movement has evolved rapidly, through successes relating to state provision of a breast cancer biopharmaceutical in the United Kingdom, to patient involvement in prospective health care technology assessment and citizen participation in healthcare governance and now extending still further, to an emerging science reflecting greater patient participation in health care research. The latter has involved the inclusion of general and disease-related quality of life (QOL) measures as outcome measures in clinical studies and, more recently, to the development of patient preference models that can be used to inform objective benefit risk assessments. To what extent though, can patients and patient advocacy organizations be influential in the direction that health care research may take or to the extent and direction that health care *research* budgets are allocated?

As scientists, ethicists, and theologians weigh in with opinions, their often disparate views seem to grow further apart even as the rhetoric ratchets up irreversibly. Oddly, the opinions of patients, families, and their caretakers have received less attention in the debate around future directions of stem cell research. However, consistent with emerging trends in an increasingly patient-centric health care environment, it is reasonable to suggest that the collective judgment of people living with diabetes, hematological, neurological, and many other organ system disorders, should be considered by policymakers.

To this end, the EU Commission has recognized that much information relating to stem cell research has been considered by European institutions, including the

European Parliament. However, the importance of involving patients, their caretakers and representatives in a process of dialogue on issues relating to stem cell research, has also been considered of central importance in making the work of the European institutions more accessible to its citizens. In order to address this situation, the European Federation of Neurological Associations (EFNA), convened a Patient Conference on Stem Cell Research in Brussels, funded by the European Commission, and attended by patients invited from the 34 countries constituting the European Research Area. The intent of the conference was to initiate and promote a meaningful dialogue on stem cell research between science and society and to involve patients, for the first time through a unique event, that was to allow patients and their representatives to engage actively with scientists, physicians, religious and political representatives, as well as the media, in order to contribute to an informed, balanced debate.

For success, many Conference participants agreed that translational stem cell research should proceed with stringent and enforceable rules, regulations, and public oversight, anchored both in law and reverence. Like health care itself, the handling of human tissues is an intensely moral enterprise, no matter what the scientific goals and objectives. The dilemma is somewhat analogous to that of law enforcement. We validate the sanctity of human life, yet each society authorizes police and others to use deadly force under specified situations, and we ensure through public scrutiny that all workers are held accountable for their behavior.

26.2 The Patient Advocacy Movement: A Brief History and Successes to Date

Until recently, in the doctor-patient encounter, the physician carried sole responsibility for medical knowledge, whereas the patient was only accountable for his or her own preferences. The use of information has greatly facilitated established trends and now, by more easily obtaining medical information prior to seeing their doctors, patients have a different role in the decision making process, possessing both preferences and knowledge prior to any physician contact. Indeed, patient advocacy groups have developed a key role in directing patients toward trusted sources of information [1]. While there are significant differences in the delivery of health care throughout the world, there is one constant – the patient. Patients' needs vary greatly on the basis of socioeconomic and geographic issues, expected prognosis, family dynamics, health literacy, and many other factors. In the past 20 years, the role of patient advocacy organizations has added an important new dynamic to patient care [2].

In areas relating to policymaking, patient advocates increasingly work for positive change in international health care systems that has lead to improved access to quality care, protection and enhancement of patients' rights from positions in government agencies, disease-specific voluntary associations, national health policy organizations and the media. In the USA, patient advocacy has emerged from its

beginnings in the patient rights movement of the 1970s and following incorporation of the National Welfare Rights list of patients' rights into hospital accreditation standards, the Patient Bill of Rights was adopted by the American Hospital Association in 1972 [3]. The structure of international health care systems have also been influential in the development of patient and health advocacy, which has now developed with professional association, credentials and education programs to include a hospital-based practice. More recently, the President's Advisory Commission on Consumer Protection and Quality in the Health Care Industry has stated that "consumers have the right to fully participate in all decisions related to their health care" [4]. Elsewhere, for example in Europe, where health care systems tend to be more centralized, patient advocacy has developed into specific disease-related patient advocacy groups and further as umbrella organizations such as the European Federation of Neurological Associations and the European Federation of Crohn's and Colitis Association (EFFCA). The latter organization typically represents 23 countries; each country represented has a different political health care system, for which EFCCA organizes a General Assembly once a year in one of the member countries, which is hosted by one of the national associations. National and Europe-wide patient organizations now exist for a large number of diseases, such as AIDS, cancer, heart disease, and diabetes as well as rare diseases.

From an international perspective, there are well-documented success stories about patients and medical professionals working together to improve patient care [2]. Many have found that education is the cornerstone to success, and when patient advocacy organizations have strong medical advisory components, they are highly effective. These collaborations have created informed consumers who increasingly seek a more active role in the quality of care and research decisions [5]. With this level of health care activism, much of which may be politically directed, there is a clear need for increased responsibility and this is of central importance in involving patients and their advocates in direct decision making about their health and medical care. The need for responsibility is of particular concern when patients and their families are faced with serious and potentially life threatening illness. Consider the case of Herceptin in the UK. Herceptin is one of a new generation of (expensive) drugs that has revolutionized the treatment of breast cancer [6]. The availability of clinical trial data suggesting benefit in earlier, as-then unlicensed populations (already licensed and available on the National Health Service for women with advanced breast cancer but not for the treatment for early breast cancer) lead to threats of legal action with the subsequent intervention of the Secretary of State for Health, prompting health authorities to complain about political arm-twisting to the Department of Health.

From individual hospital-based advocacy in the USA, to national and regional lobbying in the European Union, for specific disease and groups of diseases, the patient advocacy movement has rapidly progressed. Continued trends are evident with increasing focus on information sources and evidence-based medicine and involvement in medical decision making. What, then, about the current and future trends of patient advocacy, particularly with respect to the regulation of translational stem cell research and the allocation of finite research funding?

26.3 The Role of Patient Advocacy in Translational Stem Cell Research

Given the widely diverse constituencies involved in the debates involving stem cell research, the EU Commission sought to provide an environment where patients, their caretakers and representatives are in a process of dialogue on issues relating to stem cell research. Many of these issues were behind the organization of a unique European event that was held in Brussels on December 15–16, 2005. For the first time, a European conference was held to focus on the patients' views about stem cell research and therapy. This conference was conducted as a patient-oriented event that set out to initiate and promote a meaningful dialogue on stem cell research between science and society. For the first time, it was to involve patients, through a unique event in a process that may have implications for the health of many patients throughout Europe, as well as elsewhere in the world. Patients and their representatives were asked to actively engage with scientists, physicians, religious and political representatives, as well as the media, in order to contribute to an informed, balanced debate [7]. Furthermore, the importance of an environment for patients and their representatives to become involved in the debate around stem cell research was recognized, through which accurate and responsible information, in an understandable form, could be provided to those patients and patients' organizations with an interest in stem cell research.

It was intended that this event would be as inclusive and diverse as possible and that patients from throughout the European Research Area would be as strongly represented as possible. To this end, invitations were widely sent to patient advocacy organizations and medical societies throughout Europe, and the conference web site attracted wide attention around the world. The invitation was sent openly with no prior selection regarding the possible views of participants and it was hoped that while the numbers were limited, the process of invitation was broad and open enough so that the views expressed would be representative of the broader European patient perspective.

Utilizing a novel interactive keypad voting tool, the conference participants were asked: "Are patients or patient organizations sufficiently involved in the debate on stem cell research?" 85% of the conference participants answered "no" to this question, which provided strong endorsement for the rationale behind the conference. A pre-conference patient survey results were also available as preliminary results and these were referred to with the keypad voting throughout the conference. Further questioning revealed strong support for stem cell research in general, with 89% of participants being generally supportive. When asked about research on embryonic stem cell research, 71% of participants revealed their support, with 11% against and 18% undecided. Each session was preceded by televised background, provided to inform delegates and stimulate debate, in a conference that was simultaneously translated into seven languages. Appropriate context was also provided so that all participants were able to understand the historical context as well as some helpful description of some unavoidable technical aspects, such as the precise

nature of the blastocyst and human embryonic stem cells. Early on, it was apparent that those opposed to human embryonic stem cell research suggested that EU funds should not be allocated to support human embryonic stem cell research on the basis of a lack of European consensus and that not all countries had an agreed position. However, 91% of patients present were in favor of embryonic stem cell research. At the same time, the conference consensus was that research should be actively pursued in all stem cell populations, including embryonic, umbilical and adult, as there are pros and cons associated with each. Other issues raised included:

- Many people who themselves were not in a state of need but sought to influence the future of patients who are suffering today, expressed views about stem cell research;
- The level of natural waste of fertilized human eggs as part of natural human conception is very high. Many patients felt that research should not be hampered by relatively abstract ethical debates that might divert interest from research into therapies that might benefit patients in the future;
- Many supported the need for synergy between different areas of medical research, including stem cells, gene therapy and pharmaceutical research;
- Differential approach to development may include approval of human embryonic stem cell research-based treatments in the future. Many wondered how countries, where such therapies were not allowed, would react to health tourism, when their citizens might travel to other countries allowing such therapies. Concerns were expressed that this may result in further health inequity in Europe that would further discriminate against the poor and disadvantaged.

A further session, which focused specifically on "The Patient's Perspective," heard a number of powerful and moving stories from patients who were living with a number of long-term conditions. Testimonials from patients living with stroke, caring for a child with rare neurological illness, and living with cancer and the effects of chemotherapy, all showed an understanding and sensitivity to the complexity of the issues involved. It was apparent that the attitude of patients toward all forms of stem cell research is not the same but importantly, there was broad awareness of the issues relating to human embryonic stem cell research and sensitivity towards those who object to such progress for moral or ethical reasons. The personal stories and views of a patient table and auditorium speakers (blindness, heart disease, multiple sclerosis, type I diabetes) provided further powerful support to the importance of the views of patients in the direction and funding of stem cell research. A number of patients expressed the view that the nature of long-term suffering should not be underestimated and that society should make every endeavor to progress research sensitively within a pluralistic society. The suffering of patients and caretakers is real now and is often profound. Some patients felt that this suffering had to be considered in the context of a complex moral and ethical debate, that some patients felt was relatively detached from their own suffering, particularly when compared to the actual deep suffering experienced by many. This was particularly evident as patients felt that future research was being influenced by politically active groups, whose views or beliefs they did not share.

Broader societal perspectives were also explored in a session entitled "Stem Cell Research and Society" and which widened the debate and sought to cover perspectives informed by culture, religion, ethics and law. It was suggested that an engaged and informed public should be encouraged to participate in the debate around stem cell research and at the same time, scientists should also be encouraged to be more "public-literate." How do we balance the real needs of patients who are alive now, living and suffering with diverse medical conditions with the theoretical and abstract rights of an early embryo that will never develop into a mature human being? The use of spare embryos from in vitro fertilization is considered by many to be entirely justifiable as these embryos would likely be otherwise destroyed, and surely it is better to positively use such embryos to further research.

Conference consensus suggested that society needs scientific and technological innovation and there is a need for broad debate leading to a positive societal framework for future research, that must involve an informed and engaged public. Patient advocacy and other trends are leading to the development of a health-literate and science-literate public, but there is also a need to take steps to develop a public-literate scientific community that should be encouraged to communicate their work in an understandable manner to society as a whole so that patients, in particular, can become involved in the setting of the research agenda. There is thus a need to broaden the debate and educate all relevant constituencies, including the media.

Religious ethics were also considered within a multi-faith framework, and it was evident that there needs to be a way to reconcile the diversity of viewpoints. It was felt that some of the debates around embryonic stem cell research, in particular, can be so highly emotive that medical ethics can be confused with religious ethics. Religious ethics are clearly important but there are other issues to consider. Representative views of a number of religions, including Judaism, Islam and the Roman Catholic and Protestant Churches, were heard. Top table speakers suggested that both Judaism and Islam favored existent life and that the early embryo did not necessarily have the status of full human life. The conference further heard that life in the eyes of Islam begins at conception but human life does not begin until much later. Both Judaism and Islam favor actual or existing life over a potential life and this principle can be applied to a number of different areas of medical research. Within the Protestant Churches, there exist a recognized range of viewpoints, from a highly liberal, progressive stance to the viewpoint taken by the Roman Catholic Church. However, the dominant view within the Roman Catholic Church is that the life of the embryo begins at the time of conception and this strict, conservative viewpoint is widely held within that Church, as well as in Eastern Orthodox Churches. Furthermore, the Roman Catholic Church is against the instrumentalization of the embryo, and this is further argument against the use of spare embryos.

Asked whether there was disconnection between the views of some churches and the views of ordinary citizens, 81% of the participants felt that there was indeed a profound difference between the views of the hierarchy, e.g., of the Roman Catholic Church, and the views of patients and citizens living within

predominantly Catholic countries. Many issues relating to Europe, its diversity of culture and history, informing contemporary national views in the context of religion, political and the media environment were all discussed in earlier sessions. Particular issues were raised relating to stem cell research and to its funding at a European level. In this context, the conference was asked to consider the role of Europe, rather than just national funding and regulation. This unique event brought people together at the extremes of the ethical divide and sought to provide an environment for meaningful debate involving all those with an interest in this exciting research area. While only a beginning, all those present began to see the benefits of engaging, through a process of dialogue, with those with a different, but equally valid opinion.

Important issues in agreeing to a way forward included:

- The need for national and EU regulation for stem cell research. A regulated framework can offer an ethical, scientifically robust, and carefully monitored framework for further research;
- The issue of EU funding for human embryonic stem cell research using funds indirectly provided by countries who are against such research remains an issue and needs further discussion;
- Recognition of the need to pursue all relevant research areas in the fight against disease. This includes pharmaceutical products, vaccines, gene therapy and cell-based approaches. The latter should include research involving adult, umbilical and embryonic stem cells;
- The need to build bridges between the patient advocacy movement, the scientific and clinical community, politics and the media;
- The need for continued attention to involve the views of patients through patient associations and organizations and to maintain multidisciplinary discussion;
- The ongoing need to respect and care for different national views;
- The need for realism and honesty in the way research is represented in the media;
- The need for greater engagement of religious leaders with the ordinary people in order to understand the views of patients;
- Consideration of how patients, scientists, religious leaders, politicians and the media can be brought together as a community and connect this effort to the work of members of the European Parliament.

26.4 Conclusions

The patient advocacy movement, while still early in its development, has grown into a sophisticated, organized and informed cross-national force. Successes include the development of hospital-based advocacy in the USA to political lobbying in the allocation of scarce health-care resources in the European Union. More recently, this has extended to the engagement of patients with scientists, ethicists, politicians, religious leaders and the media, in a far-reaching societal debate addressing issues

relating to translational stem cell research. After considerable debate, many patients saw a need to create mutual understanding among disparate perspectives to forge acceptable bridges. Traditionally, science, theology, and philosophy have been regarded as three complementary approaches in the quest for truth and wisdom. Yet among a minority of Conference participants, science seems to be feared as a substitute for faith and as a competitor for the minds and souls of man.

For success, the majority of Conference participants agreed that such work must be undertaken with stringent and enforceable rules, regulations, and public oversight, anchored both in law and reverence. Like health care itself, the handling of human tissues is an intensely moral enterprise, no matter what the scientific goals and objectives. Our dilemma is somewhat analogous to that of law enforcement. We validate the sanctity of human life, yet each society authorizes police and others to use deadly force under specified situations, and we ensure, through public scrutiny, that all workers are held accountable for their behavior.

With the further extension of influence of patient advocacy into the realm of health care research and, perhaps, the allocation of health-care funding, we must make a final plea for patient responsibility and awareness of the needs of all constituencies within an increasingly complex international health care environment.

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Chapter 27

Communicating Translational Stem Cell Research to the General Public: Challenges and Suggestions

Sébastien Duprat

Abstract This chapter aims to define the wide societal context in which the stem cell research was born and is developing, and to guide the establishment of dissemination methods that correlates with the reality of modern society. An important component of success is to secure quality contributions from academics, by providing them with increased experience, meaningful acknowledgement and proper support that we would expect from a member of our own working team. The conduct of outreach programs should fully adopt the democratic ideal: Citizen's freedom of choice needs to be reinforced by scientific knowledge, with respect for diversity of opinions and beliefs. This construction places scientific reality – which is in essence fully neutral – above personal views in order to feed them. The scientist's duty to report to society complements the citizen's duty to make informed choices. Finally, a long term plan in dissemination programmes allows the building of networks of science communicators and relationships of trust that are crucial for the continuity of science developments, especially in research fields that are not unanimously supported.

Keywords Controversy • Citizen • Dissemination • Society • Outreach

27.1 Introduction

Scientific research is a central activity of modern societies and its developments are considered as a major component of the evolution of our civilizations. However, there are always types of research that are not acceptable to some people, regardless of its aim; the simple goal of an increase in knowledge cannot override any moral or ethical questioning of the research. It is crucial to set up boundaries that allow maximum benefit without compromising society's values. The controversy arises

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when sub-parts of society disagree on aspects of the research clashing with their moral values.

Society needs and promotes scientific research, but research requires public support for its longevity – and with uncertainty on this aspect, lobbying and communication strategies are needed for short-term survival.

The vast majority of scientists are not trained or even prepared to advocate their cause. If there is no absolute need for scientists to lead this activity, their contribution is nonetheless necessary. Public support might be improved by funding communication experts and/or initiating lobbying activities. This is often the case for large-scale coordinated campaigns. Most of the time, small-scale activities are organized around universities and research centers with experts having discussions in one way or another with members of the lay society.

This chapter aims at providing a contextual analysis of what the stem cell field is facing, and proposes tools and approaches to academics wishing to secure wider support for their research.

27.2 Background of Science–Society Interactions

In western societies, the scientific community and the general public went through a wide spectrum of interactions, from full integration to extreme suspicion. I will very briefly describe the historical background – with no intention of mentioning all major contributions and in order to define the context in which we are evolving.

The tradition of science originates in ancient Greece, from which we developed the scientific method. According to Aristotle, “Science is for wisdom to increase in men according to what is before them” [1]. Science was accepted as a central aspect of civilization’s development and was meant to benefit the community in any possible way, including in times of war (i.e., the defense of Syracuse with the help of Archimedes [2]).

In Euro-Mediterranean history, and specifically during the Middle Ages, much suspicion developed between science and the Roman Catholic Church. The Inquisition fought every “alternative truth,” and science contradicted some interpretations made upon the holy texts at this period of history. Alchemy was occasionally accepted to be compatible with Christianity and through its practice a limited amount of scientific research remained. The vast majority of scientific developments came however from other faiths (Muslim and Hebraic) and typically made in “non-Christian” territories. Architecture and war technologies in Europe became the field of engineers. Scientific or medical research was done in secrecy. Later, the Reformation proposed an “alternative” truth that could not be silenced by Catholic authorities and scientific developments started to expand in parallel. Several major contributions favored this movement, either by providing tools (printing presses, microscopes, etc.) or supporting the comparison of data (i.e., Lavoisier’s chemical nomenclature [3]). This developed towards what I would call a “romantic” interpretation of science, in the late nineteenth and early twentieth century: science was to be done exclusively for the benefit of humanity – as illustrated by Alfred Nobel’s

testament [4]. The First World War would result in a turning point in the use of science. The great European powers, rapidly running out of resources and trapped in a devastating conflict, looked for any inventions that could turn the odds in their favor. Fritz Haber, a German chemist, was generously rewarded by the Kaiser for developing the first chemical weapon in history (based on chlorine gas) – this also resulted in the suicide of his wife, Clara Immerwahr [5] (also a chemist) who felt that this was an inhuman application of science. Fritz Haber received the Nobel Prize in Chemistry in 1918, leading to science and technology becoming an increasingly determining component of war ever since. Another consequence of both World Wars is that the ethics of science slowly escaped the control of scientists themselves. In 1945, German scientists were shared among victorious allies as war trophies and tools for the ideological cold war to come. The political power was to set the extent and direction of scientific investigations, sometimes (and increasingly in recent years) with advice from philosophers, theologians, lawyers and/or industry leaders.

The second half of the twentieth century showed the emergence of a new generation of powerful lobbies springing from an increasing societal concern on ecology and related scientific issue (nuclear technology, oil spills or other ecological disasters, species extinction or animal tests, etc.). Large and long-lasting confrontations placed activists opposing their political power, with scientists being blamed alongside politicians. The boom in biotechnologies initiated in the early 1990s happened in this context of suspicion. The population had difficulties following the pace of scientific development, politics had a careful approach, and successes in their activities left a few major lobbies short of causes to fight for. However, efforts in communication, relative emancipation of the scientific community and major health-related improvements started to produce more change in science/society relationships. Science is now organizing itself as a pressure group, often able to oppose politicians with the support of the population. In France in 2003, a group called “Sauvons la Recherche” (“Save Research”) [6] initiated a petition signed by about 74,000 researchers (it represents about two thirds of the overall scientific workforce) and 230,000 citizens. Possibly as one of the consequences was that in 2007, during the French presidential elections, a journalist named Nicolas Hulot surrounded himself by top scientists and based his presidential candidacy upon an “ecological pact,” later adopted by all other major candidates. The European Union also acknowledged the renewed interest of citizens in science and calls for ever-more involvement of academics to fulfill this curiosity [7, 8]. Those might be early signs of a new era in which society re-adopts the scientific community and secures its support to fight common causes.

27.3 A Controversial Field

Talk of controversial science can only be done with a specific cultural, historical and sometimes religious context. Today’s astronomers fuel our dreams and ideas of romance, and activists opposing their activities are unheard of. A few centuries ago, however, Galileo’s research created a gigantic wave of controversies in Europe lead

by the Roman Catholic Church against his observations. We had to wait until 1992 for an official declaration by the Pope that Galileo did nothing wrong [9]. Other scientific theories, such as the Darwinian Theory of Evolution were strongly opposed by influential theologians when first formulated. Recent years showed a renewed opposition to this theory – on religious grounds – despite the public apology in 2008 by the Church of England for misunderstanding Darwin’s work [10]. Controversies caused by scientific research and applications are not always the making of religious institutions: nuclear energy is strongly opposed by some for its long-term ecological imprint and at the same time advocated by others as the cleanest option at the moment for the sake of the planet. Those two opposing visions claim the moral ground without any theological justification.

The stem cell field, and in particular embryonic stem cell science, touches both the everlasting human fantasy of eternal life (either by regenerative potential or through cloning) and the central aspect of societies’ and individuals’ lives: reproduction and progenies. It contains all the ingredients for small differences in opinion to result in heated and passionate oppositions. There is a wide support in western societies for investigations with human embryonic stem cells [11, 12] strongly linked with the enormous potential this research might carry in treating currently incurable diseases (e.g., Alzheimer’s, diabetes, Parkinson’s, spinal cord injuries, etc.). But powerful lobbies often make this support seemingly marginal.

27.4 Secure a Scientist’s Support

A scientist’s primary mission (and they receive training in consequence) is to perform good and reliable science. The day-to-day conduct of scientific research being rather obscure for a professional from other fields, there is a widespread perception that scientists are a category of autistic human beings, and their work becomes worth mentioning when it reaches a stage of everyday life application. Anyone having been for even a short period of time in a laboratory knows how scientists are approachable and eager to share their passion with visitors. Yet in times of misunderstanding, voices from the political sphere often acknowledge the wide interest in science from the general public but blame scientists for failure to fulfill this interest [7, 8].

On the side of scientists now, there is a growing frustration of being allocated tasks that are not within their sphere of competence – research and teaching. Their administrative burden has been increasing over the last decades and if researchers need to be professional communicators in addition, the fulfillment of their primary mission can only suffer. Some top scientists in stem cell research went “back to school” to improve their communication skills or strategies. For them it is a matter of long-term survival to secure financial support from politicians (impossible to obtain or severely restrained if the “man in the street” objects). Thus it is also necessary for scientists to anticipate how non-scientists perceive the message. Linking stem cell research with the preservation of human lives remains a key to success in our

mission – that is, to promote interest and well-informed discussions based on scientific knowledge and leading to its consequences for the society – expected benefits and type of experimentation that is required.

Regardless of the priorities set by scientists for the use of their time, part of their duty is to correct false understanding of scientific issues and fuel interest in science. When it comes to confronting large and well-organized movements of opposition, knowledge itself is insufficient to correct misleading interpretation of science, and communication professionals should be involved in the process. Now, between rigorously correcting the mistakes in understanding scientific reality – which is within the call of duty of scientists – and expressing personal opinions over the moral relevance of a particular research – which is the right of any well-informed citizen – a distinction should be made. The latter choice requires a larger personal investment, both in terms of training and time allocation. It should not be compulsory for scientists, but for those who decide to choose this path, support should be given to allow them to do it efficiently, in a coordinated way and with minimal negative consequences in their work and career. As a most urgent priority, support should come from institutions to allow their laboratories to recruit not only their own administrative assistants but also skilled managers and other advanced professionals to let top scientists evolve efficiently in the global science-related societal context. If this is true for any field, it is especially crucial to keep alive activities under attack – such as the stem cell field – and let lawful science develop in relative serenity.

27.5 Outreach and Academia

27.5.1 Outreach Needs Academics

Modern research institutions seek visible outreach activities, as it positively reflects on their reputation, their ability to attract enough and/or more promising students, attract and/or keep more competent staff, and facilitate obtaining support from local politicians.

The participation of academics in outreach activities is necessary, but there is little to gain for them in doing so. Often, they are “recruited” on a voluntary basis, which changes into compulsory activity if volunteers are insufficient. This approach works in an acceptable manner, thanks to the widespread interest of scientists in sharing their enthusiasm and passion for their field. However, it also comes with consequences, especially frustration – of being forced into something and for lacking any meaningful acknowledgement for their contribution. As long as links are missing between public engagement activity and recognition by their peers, it has indeed the flavor of distraction from their research and from advancement in their career.

I should not fail to mention that there are already some benefits for academics to be active in outreach activities. An obvious one is the recent trend of large

cooperative research grants (such as the European Commission Framework Program) preferentially supporting research projects that include a reasonable amount of non-scientific activities (related to the impact of the project on the society, such as training, outreach, ethics, law, etc. [13]). Increasing the chances to receive money for research is obviously attractive and does not place the focus on outreach for what it is, but rather as a side activity to be done with pragmatism. For the very few who have a large-scale involvement with the media, there are other obvious benefits, but that corresponds to exceptionally rare cases in science. Smaller-scale benefits also exist, but cannot by themselves justify an implication in dissemination. An example: for activities taking part in high schools, the possibility of identifying early and trying to attract future PhD students with an interesting personality or approach, etc....

Coming back to my previous point, rewards should exist to flag down academics who are good at communicating their expertise to non-specialists, in addition to fulfilling their “standard” academic duties. When that is in place, the success and continuity of outreach activities are greatly enhanced, with positive consequences for both the actors of the activity and the institution itself.

Putting relevant rewards in place need not involve real monetary input. What primarily counts for academics is publications, and to some extent awards distributed by their peers. This is not traditionally linked with outreach activities, but it is simple when designing new dissemination programs to include a reporting component directed to academic journals (often on the methodology or a statistical analysis on its efficiency). Offering to academics helping the implementation of the activity to be co-authors of (or at least acknowledged in) the publication makes the approach far more appreciated in their eyes. In addition to a more eager and enthusiastic contribution to the event, their experience will support the reporting aspects of the program – reporting that will be far more convincing to the funder/orderer of the activity.

As a very recent trend, a growing number of scientific journals are now open to publishing from time to time articles on dissemination issues, but often have difficulties finding authors. *Science*, for example, proposes an award whose reward is the possibility of publishing an article on dissemination activity [14]. Very specialized journals are no different, judging by the *Stem Cell Research*’s having published a four-page letter on an art exhibition based on stem cell pictures [15]. However, it is not only journals that reflect the growing interest for dissemination to be recognized as academic contribution alongside the classical fields. For example, in 2008, during the 9th World Bioethics Congress, a full session called a “performance session” was allocated to exploring forms of art to extend the reach of bioethical debates. An exhibition complemented the performance arts with visual arts used for the same purpose. At the same congress, another session was dedicated specifically to bioethics and outreach. I could multiply the examples as most of the major scientific congresses (talking about the stem cell field, as with the case of the ISSCR annual meeting) now have an outreach component, though not always (yet) as part of the official program. A new academic field comes to be established in science/ethics/outreach, but while waiting for it to be widely recognized and

represented, other academics can at a personal level benefit from academic recognition from their outreach contributions.

Coming back to art as a science and ethics dissemination tool, it is important to mention that outreach activities aim at bringing knowledge to civil society. This requires communication support that can be easily adapted to different linguistic, cultural, and religious contexts and is true between countries, but also within different communities and minorities inside a single country. Art (in its various forms) seems to be the ideal means of dissemination that can be used across boundaries.

27.5.2 Interest in Science Versus Neutrality

One of the risks in communicating science is failing to clearly separate what we do as scientists and as citizens. Scientists think rationally, and must share reason and knowledge with others. This usually results in a tutor-novice type of relationship, which is useful and indeed most of the outreach activities are based on it. As citizens, we all have opinions and beliefs and we often wish to express them, share them or use them to convince others. Those personal views, however, do not entirely (if at all) derive from the application of scientific methods to our thoughts.

Being key stakeholders in science-related issues and typically enthusiastic about science, scientists have strong opinions they wish to push forward, and outreach activities are the ideal stage to do so. I do not condemn this practice; I even think it is by definition one aspect we are expected to cover. However, personal opinions need to be clearly introduced as such, in order not to confuse the audience with “neutral” scientific reality. Scientific truth, which should be used as a basis for creating personal views, needs to be separated (even with a single sentence) from the expression of individual opinion.

A directly related component regards the distance felt between scientists (experts who deliver the knowledge) and society (non-experts on this topic who receive it). A long-term success in engaging the public requires audience and scientists walking to meet each other halfway. In order to achieve this, it is useful to offer opportunities for the public to meet scientists in a more informal and dialogue-friendly (maybe even equal) context. As an example, an art exhibition has been produced from scientific pictures whose authors (from junior researchers to heads of institutes) looked at their own creations from a distance and complemented them with an artistic title. By itself, it is part of a learning process for scientists who are asked to see something other than their very familiar stem cell, neuron, feeder cell, etc. – and by doing so understand better what a non-scientist might feel towards images taken from scientific research. This exhibition has been shown in the hometowns of many of the artists who had a chance to discuss with visitors on an equal basis (art) while promoting, in an unforced way, interest in the science behind [15]. This is typically a complement in the standard “set” of outreach activities, and in my opinion is very beneficial to science. It also reaches citizens in a more democratic way as it equally attracts people regardless of their

general knowledge and understanding of science. At more standard outreach events (science festivals, scientific cafe, etc.), the vast majority of visitors, if not all, are from a science-literate minority.

27.6 Whom Should We Educate?

Outreach activity is easy to set up where there is a demand for it, a structure to receive it and a related professional or study background between those who deliver and those who receive it. This explains why the education system (from primary to high schools) is the focus of the majority of scientific outreach. Slightly marginal but following the same path are educational events organized outside the schools (i.e., personal tutoring in public hospitals, prisons, etc.). Some institutes have developed advanced programs to educate school teachers. For example, the EMBL institution (European Molecular Biology Laboratories) is actively involved in this type of dissemination [16]. Science teachers are the most obvious target, but multidisciplinary activities can be set up by including ethical, legal, and even linguistics aspects of the research. In school context this could involve teachers of philosophy, moral or religion; law or civic education; and language (often English, but not necessarily); which is a strong asset to receive attention and support from the highest levels of the education system representatives. These examples show activities that are typically implemented with the help of school teachers (having a similar work-“culture” with researchers) and reaching a limited sized audience. The same is true for outreach programs aiming at patient groups. If patient audiences are easy to communicate with (due to their personal interest and often a fairly good understanding of the basic science behind their condition), there is an emotional component in biomedical outreach that intimidates many scientists. The balance between maintaining hope and avoiding creating hype beyond what is realistic and predictable in the near future (a treatment development agenda that counts in decades makes little sense to a patient!) is the trickiest parameter and a key to long-term communication success.

There is another target that is often more complicated to work with but allows a far larger reach: professionals from the mass media. Educating the educators (from teachers to journalists) produces a larger and longer societal impact from the resources used, but gets quickly out of our control and therefore needs more experience to design it “safely.” One way, when there is a consequent budget for it, is to produce material in collaboration with experts of diffusion media (documentary movies, educational games on the Internet, etc.). The other approach is to “recruit” journalists to the cause we want to defend. The latter requires long-term collaboration (in order to build trust), to feed to the general public exciting announcements about discoveries and improvements (which might clash with academic reality) and sometimes results in being asked to comment on scientific topics unrelated to our specialty. It is naïve to think that we might properly educate journalists, though we can surely enhance their awareness of the issues that matter to us. For those who are in a position to initiate

a long-term communication strategy, the focus should be on students of journalism. At this stage of their careers, they are still accessible, open to new perspectives and willing to create their personal address book. I strongly advocate the establishment of workshops where scientists and students in journalism work on a scientific topic, identify and try to solve misunderstandings and communications pitfalls. This is an ideal arena to create a long-term relationship of trust and expose journalists to training they will most likely never take time to acquire later in life.

Regardless of the target audience, a key component of success is to focus part of our energy into educating the scientists themselves. The optimal design for creating a dialogue is for each party to reach the other half-way. For someone with a PhD in science, it is a difficult task to address a non-scientific audience on his/her field of expertise in such a way that the message makes sense, captivates and produces a desire to know more. The first component is for scientists to realize at which level of understanding their audience is. Scientists need to be guided to look at their academic work from a distance (e.g., "If I don't know that it is a neuron, what impressions would this picture promote?"), and think of day-to-day life analogies for each key scientific phenomenon. An increasing number of graduate schools now makes it compulsory for all PhD students to be involved in some sort of outreach event, and in practice it doesn't require large efforts to adapt existing outreach programs for their educative effects to benefit both the audience and the ones who deliver its content. This produces a second and durable wave of benefits to the field and its perception by lay citizens. What is an additional benefit when directed to a general audience becomes nearly a compulsory precondition when it comes to interaction with the mass media. Collaborating efficiently with journalists requires from the researcher some understanding of their working habits and priorities. There are often courses available for academics to obtain basic knowledge on media communication; the acquisition of more advanced experience comes from practice and exposure to the media.

27.7 Breaking Self-sustained Debates

We live an era of communication. Powerful new tools such as the Internet drive ideas and opinions throughout traditional boundaries and can reach virtually any part of the planet. It is therefore surprising to notice how strongly those old boundaries still influence the scope of debates. Talking about controversies surrounding the stem cell field, a specific set of aspects would be widely discussed in a given culturally, religiously, ethnically, historically and/or linguistically defined group while other aspects would be simply ignored. When we expose an audience to valid arguments that are unusual to them, we are suddenly in a strong position to bring reason to the center of the debate. It is not necessary to support those arguments but to allow the audience to listen to the full spectrum of opinions for them to build up their own opinion with some level of independence from their context. It is otherwise far more difficult to challenge our opinions on their universality, consistency, logic, etc.

Internet-based discussion forums that are fed from a multitude of places are a example of relevant support to bypass this problem, despite the linguistic limitation (ideally, people would express their views using their mother tongues). The Internet in general has many simple applications that greatly enhance the impact of outreach events. The European Commission's embryonic stem cell research program named ESTOOLS, for example, organized ethics debates on stem cell research between high schools across the European Research Area. Besides an internet connection, very little is required to make it possible. The appropriate network of international outreach agents is definitely an advantage – and regular efforts should be made to build and maintain such networks of contacts.

I could mention two such debates that ESTOOLS organized. One bridged online high school students in Milan (Italy) and in Stockholm (Sweden) and was a great opportunity for them to learn about their counterparts beyond clichés and to challenge their approach on the concept of life and relevance of stem cell research. The second event that illustrates the power of this approach was done between Jerusalem (gathering Jewish Israeli, Arab Israeli and Palestinian students) and Bonn in Germany. Despite the very high level of tension in the Middle East at the time of the event, the division of opinions most of the time was according to gender and not religion or politics. The whole event, in both locations, showed very respectful participants, and was driven by a real desire shared by students to understand the diversities of views they were facing.

27.8 A Matter of Duty

27.8.1 A Scientist's Duty

There are three sources of funding for science: public funds (national – from local to governmental levels – or international such as the United Nations or the European Union); charity-based funds (from individual patronage to charity institutions); and corporate funds (mainly research and development). The latter often requires a certain level of confidentiality and if some communication is arranged, it usually follows the company communication strategy. Public- and charity-funded research projects that are supported by public funds ought to provide some feedback, at least to the general public. I consider it to be a matter of duty and responsibility for scientists to report back to the population, regardless of the audience's age (people can support charity before voting or paying taxes). A particular focus should be given to stakeholders, and in the embryonic stem cell field, this might mean in particular the young generation (the most likely to benefit from future treatments) and patients having incurable diseases. In general, any group of the society deserves to be informed – without distinction by gender, ethnicity, age, religion, background, scientific knowledge or status in the society; unusual institutions (e.g., public prisons) might be as relevant as high schools or science museums.

27.8.2 *Freedom of Choice*

The two central questions, in my opinion, are to allow citizens to be responsible for their choices, which is a pre-condition of democracy, and, taking into account the importance of science in civilization's heritage, to expose citizens to the techniques and knowledge that contribute to the definition of our identities.

To be a citizen means having a collective decision-making responsibility, resulting – in “healthy” democracies – in societies that reflect the moral positions and notions of the priorities of its members. In principle, a citizen is supposed to make informed choices, otherwise he/she abstains or votes according to arguments relayed by external influences that he/she does not fully understand. Scientific understanding often requires more than information to understand its validity, impact and rationale. It is to the benefit of any society to make its scientific and technical improvements its own. Ideally, people would first gain enough knowledge and then be exposed to lobbies in order for the process to be democratic and respectful to individuals. That can happen in the field of stem cell research only with a strong implication of specialists who would widely disseminate the required knowledge.

Society should be the last judge of what is acceptable as scientific research and what is against its conception of humanity. Society should have power over what is allowed. We know the consequences of the opposite, where lobbies or self-appointed officials are able to determine the only acceptable way of thinking for everyone.

27.8.3 *Legal Dimension of the Debate*

In some countries, there is an opposition to stem cell research based on rejection of “human parts” patenting. As embryonic stem cells carry more than just our genome – they are one of the closest biological entities in vitro to a fertilized egg – there is a fear of monetizing human beings.

The main argument is that patents (and therefore personal incomes beyond a well-deserved salary) should not be obtained from work funded by public money. This situation is standard in other fields, i.e., where a young artist – for example, a writer – often relies for a while on national grants allowing him or her to produce books which any commercialization will not pay back to the grant-provider.

Using public funds for research should indeed result in benefits for the population who support it through its taxation. In our capitalist society, patentability is supposed to ensure just that. The result of restricting or forbidding patents in one country or groups of countries might be moral or ethical, but is only possible in isolation. The consequences in a worldwide economy are that publicly-funded discoveries are translated into patents overseas and the same population will pay to benefit from potential applications that it already supported financially to develop.

In a debate with lay citizens even without a legal background, some simple arguments – such as those described above – can be made, and counter-arguments listened to. The final goal might not always be to convince, but to understand the logics and

rationale behind a different opinion and to respect it. When this is achieved by both sides, the outcome can already be considered a success.

27.9 Dealing with Lobbies

Lobbies represent opinions that are typically at the edge of the spectrum. If their arguments make some sense to some people, the vast majority of the civilian society naturally stands in a more central position. Lobbies' voices attain their influence by claiming the moral ground and therefore silence other viewpoints. As an idea gains wider support in society, lobbies usually radicalize their arguments, either to stay as a force (in case their views are adopted) or otherwise to keep their supporters away from an opinion swing. On stem cell research, I have been at events where laboratories using human embryonic stem cells were compared to concentration camps, using the argument that one such cell equals a potential human person, so a laboratory with millions of them in culture (which might actually derive from a single embryo) is experimenting on/destroying millions of potential lives.

It is obvious that the best answer to these types of claims is reason, and academics are particularly good at that – when given enough opportunities to speak. But we always have to keep in mind not to play “the lobby game,” and in my opinion this means not systematically silencing other points of views and not using emotion-based analogies as our central arguments. When the context is right (meaning, when the audience can be exposed first to scientific reality and reason), inviting lobby representatives to share their own analysis with the audience is actually a very efficient way to reduce their impact. It is easy for a conference chairperson to discredit, in the name of “respect” and “tolerance,” those who look for a direct confrontation. What lobbies are gaining – if the event is visible enough – is to be not excluded. What they fundamentally lose is the universality of their moral stand (by being alongside other equally “universal” stands presented in parallel), and it dilutes their arguments. If the ultimate justification of a lobby is faith, it is useful to have three or four large religions represented with different approaches – the audience will be exposed to a variety of fully relevant connections between, let's say, embryonic stem cell research and faith or relation with God. A Catholic listener will not change his religion but will in the future remember that “interestingly, Muslims make this association,” etc.

We have the tendency to fear lobbies that oppose the type of research we see as useful and urgent, forgetting that groups who “over-support” research in our field can be as dangerous and damaging. In many countries, and maybe surprisingly, not only in the developing world, there are scientists and medical staff that exploit the hype related to stem cell research and the tragedies patients and their families are going through. From conducting medical trials before sufficient proof of concept is validated (hoping to be the first), to setting up “medical” units on a fully commercial basis, we academics have the duty to condemn those misconducts and criminal behavior. Those people do as much as possible to achieve legitimacy by fraternizing with other academics (by participating in congresses, publishing in specialized

journals, etc.) and it is a matter of responsibility to make a distinction as often as necessary. The consequences for patients of such practices need to be exposed as much as the need of therapies (or therapy improvements) for patients suffering!

27.10 Keep It Simple

A way to monitor the success of outreach material is to evaluate its impact beyond the public originally selected and directly engaged. Indeed (and for an ideal design), when some of the audience passes it on and creates a second wave of exposure at its own level, there is no doubt that we have fulfilled our mission. In practice, successful events – even to a lower extent – have in common that they are simple and often relatively cheap to implement. They need to be flexible in order to fit in various contexts; as an example, an ethical debate in high schools that typically includes various religious representatives might need to be modified to host theologians instead in secular states (where priests might not be allowed in the school). Logistics should also be minimal, as that allows the program to be passed on to colleagues (locally or in other regions/countries). Costs need to be appropriate, as continuity of the program is an important parameter; academics and voters would equally readily condemn a science dissemination program that costs more than the research itself. As simplicity often correlates with simple means of communication, and it helps its adaptation to other contexts as well (including other languages).

One of the direct benefits of a simple design for the coordinator of a program is that in the long run it can work as a network-creating tool. Indeed, it makes it easier for remote colleagues to adapt it and results in efficient and relatively painless collaboration. That is in essence the ideal context to build trust and get ready for future and more advanced/complicated collaborations.

Finally, there is already quite a lot of material available to communicate on the stem cell field, from playing cards to documents and movies. There is little sense in replicating them. However, with the rapid development of communication tools, there is a wide variety of support that is under-used and fully complementary to what exists in terms of the population it reaches – that includes educational computer games, social-network Internet site applications, mobile phones or MP3-compatible materials, etc.

27.11 Conclusion

This chapter aims to define the wide societal context in which the stem cell research was born and is developing, and to guide the establishment of dissemination methods that correlate with the reality of modern society. An important component of success is securing quality contributions from academics, by providing them with increased

experience, meaningful acknowledgement and proper support that we would expect from a member of our own working team. The conduct of outreach programs should fully adopt the democratic ideal: citizens' freedom of choice needs to be reinforced by scientific knowledge, with respect for diversity of opinions and beliefs. This construction places scientific reality – which is in essence fully neutral – above personal views in order to feed them. The scientist's duty to report to society complements the citizen's duty to make informed choices. Finally, a long-term plan in dissemination programs allows the building of networks of science communicators and relationships of trust that are crucial for the continuity of science developments, especially in research fields that are not unanimously supported.

At the end, we ought to ask ourselves the question: In a democracy, should we influence society in order to allow or forbid specific activities, or should society influence us and dictate acceptable moral boundaries? If the latter is meaningful, we must consider that giving proper decisional tools to citizens would be a republican duty, and support for science advocacy leaders should be institutionalized.

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Part VIII
Translational Stem Cell Research and Its
Psychological Implications

Chapter 28

Psychosocial and Cultural Factors Affecting Judgments and Decisions About Translational Stem-Cell Research

Melissa L. Finucane and Andrew E. Williams

Abstract Stem-cell research is touted by some as a medical revolution giving rise to unprecedented hopes. Others view it as a violation of fundamental human values. Decisions about the acceptability or non-acceptability of translational research agendas will ultimately depend on a reconciliation of many psychosocial and cultural factors affecting judgment and decision processes. In this chapter we discuss some of the key elements defining risk perceptions and influencing risk debates, including affect, qualitative characteristics of the technology, worldview, values, decision style, and social networks. Recommendations for policymakers are provided to help improve communications among stakeholders.

Keywords Cultural values • Decision making • Emotion • Intuition • Risk perception • Social networks • Worldview

28.1 Introduction

Stem-cell research is touted by some as a medical revolution giving rise to unprecedented hopes. Others view it as a violation of fundamental human values. Recognizing the ethical and scientific complexities of this controversy, the International Society for Stem Cell Research [1] has issued guidelines to facilitate the use of the technology in an “acceptable” way. They specify a regimen for clinical testing and outline some of the social justice issues related to translational stem-cell research. More is required, however, to move this research from its experimental phase to an acceptable form of routine medical care. The translation process needs to address the dynamic psychosocial and cultural systems that influence perceptions of and decisions about technological risk. Policymakers need to

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bear these systems in mind as they set a risk-management agenda. Understanding how science is interpreted and translated into action by diverse members of society is key. Scientists, patients, religious organizations, government agencies, the media, and the general public are constantly negotiating what is an acceptable or unacceptable risk. Lay people often raise questions that are not answered by technocrats, and technocrats provide answers that miss the point of what was asked because they do not understand the public's anxiety. The aim of this chapter is to articulate some of the gaps between scientific and social rationality regarding translational stem-cell research in an effort to facilitate the debate and improve decision processes.

28.2 Intuitive Understandings of Risk

When members of the public first hear about biomedical research involving human embryos, they often report a feeling of repugnance, even if they have difficulty articulating their reasoning. Called the “yuck factor” [2], this repugnance has been described as an “emotional feeling of deep wisdom,” that leads people to “intuit and feel, immediately without argument, the violation of things that we rightfully hold dear” [3]. This affective response reflects an evolutionarily old way of assessing risk, one that is fast, instinctive, and experiential. Relying on affect and emotion is a quick and easy way to determine whether something is dangerous or not. Intuition provides an efficient assessment of potential threats as we navigate through our complex and uncertain world.

Relying on an affective response contrasts with the traditional, “expert” analysis of risk that typically focuses on calculating the chance (probability) of injury, damage, or loss [4]. The different approaches of lay people and experts represent alternative ways of dealing with risk. The intuitive approach brings fast, instinctive, and affective reactions to the problem of determining danger. The probability-focused, analytic approach brings logic, reason, and scientific deliberation to bear on hazard management. Both intuitive and analytic processes are important components in what have come to be known as dual-process theories of information processing [5–9]. These theories provide an account of how evaluations and choices may arise in two different ways, or as the result of two different processes (intuition and analysis). These processes may act independently or may interact to influence each other during risk judgments and decision making.

In addition to affect, public responses to stem-cell research are likely to reflect perceptions of qualities of the technology such as unknown risk (the extent to which the technology is unknown, unobservable, unfamiliar, and has delayed consequences) and dread risk (the extent to which the technology is seen as dreaded, uncontrollable, fatal, not equitable, high risk to future generations, not easily reduced, involuntary, and potentially catastrophic). In general, stem cells and their derivatives represent an entirely novel product and their relative

newness means that they are perceived as being poorly understood by scientists. Since animal models of many diseases do not accurately reflect human disease processes, immune or other biologic responses in patients are not fully predictable. Stem cells also appear difficult to control and cellular transplants may persist for many years with potentially irreversible actions. These unknown and dread characteristics mean that the technology will likely evoke predictable social phenomena: high perceptions of risk, calls for strict regulation, and intense media scrutiny [10].

In sum, people may use both intuitive and analytic processes to navigate a complex and uncertain world. Both have advantages and disadvantages. Sound science is key to informed societal decision making, but to the extent that public opinions of stem-cell research are based on intuitive processes and qualitative aspects of the technology, attempts to communicate about the translation of the technology will need to address these dimensions.

28.3 Worldviews and Values

People's worldviews and values also shape their judgments of risk. Like affect, values are important in judgment processes because they "serve as stable, individual-level predispositions to accept or reject particular types of arguments" [11]. They provide an efficient and powerful means for screening information and determining risk acceptability [12, 13]. Differences in general attitudes towards social organization, trust in government, and other socio-political factors result in different attitudes toward technology and its expected impact on society. Different worldviews and values matter because they explain why stakeholder groups support different safety standards and practices. To facilitate a multifaceted and nuanced debate about translational stem-cell research, we need to articulate stakeholder differences in these guiding principles.

Central to the stem-cell debate are views about the value of scientific freedom of inquiry versus the status of the human embryo. A central assumption held by the scientific community (and their funders) is that when it comes to research, scientists should be free from direct regulation and political control [14]. This scientific "exceptionalism" is complemented by emphasis on liberalism in the economic marketplace in the USA and Europe, where stem-cell research is considered vital to the competitiveness of the biomedical industry [2].

In contrast, religious groups cast the debate as a moral issue. Whether the human embryo deserves the respect afforded a person or whether it is merely a fertilized ovum is key. In the West, Christian and Kantian philosophies of human dignity contribute to the debate. These philosophies reflect the notion that human life has a higher moral place than the rest of the natural world and that humans should be treated as ends and not as means [15]. Historically, beliefs about the status of the human embryo have varied with changing cultural values and new scientific knowledge [16]. Varying views are also found in our relatively secular, modern world.

A strict prohibition of embryo research in Germany, for instance, reflects a strong commitment to respect for natural processes and a rejection of the actions of the Hitler regime. In contrast, the UK has relatively liberal legislation that promotes embryo research.

Reconciling the constitutional principles of “freedom of inquiry” with “respect for human life” will be an ongoing challenge as long as there is a lack of consensus concerning the status of human embryos. Nonetheless, some authors [16] emphasize that an acceptable solution must satisfy the condition that human dignity is valued more than unrestrained freedom of research and that open public debate must come before research, at least in the most controversial areas.

Understanding the different values held by scientific and religious (or other) groups related to stem cell research is important because they reveal different beliefs about how best to debate the issue. A central assumption of many scientists is that increasing public understanding of the issue will lead to increased support for research. That is, scientists typically value a fully informed public who they believe will deliberate the issue in depth and sort through misinformation and emotional appeals. However, as described above, people rely on more than a deliberative approach to risk controversies, especially when the issue is scientifically and ethically complex. Religious groups tap into these intuitive responses by defining the debate as a moral issue. They mobilize public opposition to research by emphasizing key values such as human respect and dignity [17]. In short, diverse views and values shape the debate in a complex and dynamic way. Facilitating the debate will require a thorough understanding of how various predispositions impact what and how information is used to determine risk acceptability.

28.4 How Value Predispositions and Knowledge Interact

Given the centrality of knowledge and values in the stem cell debate, it is important to ask whether and how they interact to affect risk judgments. Nisbet [2] hypothesized and found that the strength of value predispositions influence the extent to which there is a positive relationship between knowledge of and support for a technology such as translational stem cell research. He reported a significant interaction between the strength of religious beliefs and knowledge (the extent to which someone reports having heard, read, or seen information about the issue) on support for stem cell research. He showed that among moderately religious and non-religious individuals, greater knowledge is related to greater support. In contrast, highly religious individuals show low levels of support, regardless of their knowledge of the issue. For all levels of religiosity, uninformed individuals showed strong initial opposition (the “yuck factor”).

Similarly, Ho and colleagues [18] report that religiosity, ideology, and deference to scientific authority moderate the effect that scientific knowledge has on support for stem-cell research. They showed that people might interpret the same information

differently, depending on which predispositions are most salient when making sense of that information. Highly religious and conservative individuals may use their dispositional lens to override the potentially positive effect of scientific knowledge. In addition, they suggested that people who view scientists as making decisions in the best interests of the public are more likely to form opinions based on their scientific literacy.

Importantly, value predispositions may not be as stable as suggested by previous research [11]. For instance, people may be oriented (e.g., by reading a simple paragraph) toward interdependence evoking context-dependent information processing or independence evoking a cognitive mode focused on objects, independent of contexts [19]. Other sources of information (e.g., from the mass media) may thus play an important role in risk judgments. Consequently, public opinion may remain somewhat volatile in the short term, as it shifts in relation to changes in media attention and tone, at least in Western contexts. In short, value predispositions are influential, but not necessarily stable. How they might vary and what this means for judgments and decisions about translational stem-cell research needs to be better understood.

28.5 Cultural Differences in Decision Processes

The approaches of the religious and scientific groups described above reflect a typically Western perspective. In their efforts to gain support for their preferred policies, these groups have offered deliberation and analysis focused on their own particular logic or rules for or against stem cell research. This reflects a value in Western (European and American) cultures that tends to place power in the individual. Daily life is imbued with a sense of choice (and an absence of social constraint) and the personal freedom to debate, discover rules about the nature of surrounding objects and events, and create causal models of them. The construction of these models is typically done by categorizing objects and events and generating rules about them for the purpose of systematic description, prediction, and explanation [20].

In contrast, Eastern cultures typically share a sense of reciprocal social obligation or collective agency. The Chinese, for instance, tend to feel that individuals are part of a collective and that individual behavior should be guided by expectations of the group. The main moral system of China, Confucianism, elaborates the obligations between people; the emphasis on collective agency emphasizes the need for in-group harmony and discourages debate. According to Lee [21], a typical Confucian thinks that we all live in a moral community, which means we have strong moral commitment to each other. One implication is that if some members of society deeply care about the interests of embryos, then the rest of society should seriously consider their concerns. In this instance, society should develop policies that address their concerns (e.g., avoid deriving stem cells from embryos). However, another Confucian value is to relieve the pain of others. Lee states that this value outweighs the moral standing of

embryos, suggesting that stem cell research may be permissible if it helps to produce a cure for those suffering from illness.

Valuing individualism (emphasizing the goals of individuals over groups) versus collectivism (downplaying the goals of individuals in favor of group goals) may thus lead to people from different cultures directing their attention to different aspects of the stem cell debate and using different approaches to solving the problem. Recent work by Nisbett et al. [20] indeed argues that the considerable social differences that exist among different cultures affects not only their values but the nature of their cognitive processes. In general, people may possess essentially the same cognitive processes as their tools, but the tools of choice for the same problem may be habitually very different from one culture to another. Moreover, people from different cultural backgrounds may perceive the same stimuli as posing the different problems. Consequently, debates about translational stem cell research in the West and the East may focus on different concerns and be resolved by different processes. Of course, infrastructure and governance challenges in the East [22] also contribute significantly to the East-West differences in rate and nature of progress in translational stem-cell research.

28.6 Diffusion of Innovations Through Social Networks

Another important element of the translation of science into medical practice relates to the social amplification of risk [23] generally, and the role of social networks specifically. Individuals often defer to or imitate the attitudes and behaviors of those around them rather than thinking through the risks of a new technology. Thus, attitudes toward new technologies are partly determined by influential members of their social network. When new technologies are introduced within a health-care system, for instance, the opinions of influential peers is more important than purely rational risk-benefit calculations in determining the rate at which the technology is adopted [24]. The number of connections each member of a social network has with peers (the network's degree distribution) often follows a logarithmic power law distribution so that the individual with the second most connections has exponentially less than the person with the most, the person with the third most has correspondingly fewer than the person with the second most, and so on. Networks also exhibit community structure [25] in which tightly knit groups are joined together by a small number of between-group connections. Both of these properties affect whether and how quickly an innovation diffuses throughout a network.

In the case of translational stem cell research, discrepancies between the most influential members could perpetuate a non-productive dialog in which the most passionate or senior members of stakeholder groups provide an incomplete or misleading picture of the real interests and concerns of their groups. The most influential members of an advice network have a disproportionate influence on the

attitudes and behavior of the entire group regardless of their official status within the institutional hierarchy. Individuals who are sought out by many others for advice often have more influence on risk perception than their boss, clergyman, or family elder despite their lower status. These individuals function as informal gatekeepers for the group. Typically, their status as trusted advisors has been earned by a history of careful and conservative vetting of new innovations. In contrast, people who adopt new technologies and products soon after their development (called “early adopters”) tend to be on the periphery of these advice networks. They are usually far less connected to the rest of the group. These more pro-innovation members in the advice-seeking network tend to be more risk-seeking than others.

Group members’ influence and representativeness can contrast sharply to their passion and ability to participate in a public dialog about an innovation. Early adopters are often the most passionate and vocal participants in such dialogs. People in positions of authority are often the “natural” choice as spokespersons for a stakeholder group, but their own attitudes and behaviors might reflect the official rather than the actual attitudes of the group.

28.7 Recommendations for Policymakers

Balancing scientific and social rationalities is a complex task, but determining the extent to which public perceptions of risk should be central in risk debates is essential in any policy-making effort that purports to be democratic. Indulging demands for risk regulation that ultimately harm or restrict others might be unreasonable, even in a democratic society. Or it might be appropriate if moral repugnance is consistent with democratic values like equality and liberty. Thus, our first recommendation is that policymakers need to help stakeholders with diverse views to converge on empirically and morally sound principles for guiding the translation of basic stem cell research into acceptable standards of medical practice.

A second recommendation for policymakers is to evaluate the dynamics of intuitive processes and value predispositions. Finding risk-communication strategies that help people accept new information and potentially change their minds, *without* experiencing a threat to their cultural identities, requires an in-depth understanding of the psychosocial and cultural factors that affect decision processes [26]. Policymakers should take advantage of diverse social science methods to better understand what is important to individuals and why it is important so that misperceptions can be addressed and legitimate differences in stakeholder concerns and priorities accommodated.

Next, bridging the science-society gap requires partnerships that facilitate shared, deliberative decision making. The partnerships should provide contextualized information that efficiently answers real-world questions about what translational stem-cell research is needed and acceptable. These partnerships could also serve as forums for discussion and facilitate a bottom-up approach to building credible

institutions and policies. The outcomes of any portfolio of policies are unknown, but policies can be modified as multiple perspectives and processes are integrated and the space of possible action options is enriched.

Our final recommendation is to identify the most influential members of stakeholder groups and include them in the dialog. These individuals might not be the most senior or most passionate participants who are naturally drawn to participate in or seek to influence policy. Understanding the role of gatekeepers within social networks and learning to identify and include them will help to ensure that the existing structure used by the group for vetting new innovations is directly engaged in the policy formation process.

28.8 Conclusion

Translating stem cell research into medical practice is a particularly difficult process because the technology features several characteristics that evoke negative affect and heighten public concerns. Diverse worldviews and values also influence judgments about the technology. The attitudes and behaviors of influential members of social networks may be relied on by people as a guide about what to do in the face of scientific and ethical complexity. Decisions about the acceptability or non-acceptability of translational agendas will ultimately depend on a reconciliation of many psychosocial and cultural factors affecting judgment and decision processes. Clarifying the dynamic systems influencing the debate will help facilitate communications among stakeholders. In turn, this should help to improve decision processes and outcomes and ultimately help to build multifaceted agreements with cross-cultural legitimacy and a sense of ownership for all involved.

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Part IX
Ethical Evaluation of Translational Stem
Cell Research Projects in Research Ethics
Committees

Chapter 29

Ethics and Uncertainty: Considerations for the Design and Review of Translational Trials Involving Stem Cells

James A. Anderson and Jonathan Kimmelman

Abstract Once we set aside issues related to the ontological status of the human embryo, many of the ethical issues presented by translational stem cell trials resemble those presented by other areas of clinical research. Does the trial present a favorable balance of risks and benefits? Will the selection of subjects be fair? Will the consent of participants be informed and voluntary? The familiarity of these questions, however, belies difficulties faced by researchers and regulators involved in the design and review of translational stem cell trials. In this context, issues are problematized by high degrees of uncertainty concerning the nature and probability of study risks, the absence of accepted normative standards or frameworks for risk assessment, and the limited expertise concerning stem cell science on most institutional review committees.

Keywords Phase 1 • Research ethics • Risk • Stem cells • Translational research

29.1 Introduction

Once we set aside issues related to the ontological status of the human embryo, many of the ethical issues presented by translational stem cell trials resemble those presented by other areas of clinical research.¹ Does the trial present a favorable balance of risks and benefits? Will the selection of subjects be fair? Will the consent of

¹For the purposes of the following, “translational research” refers to first-in-human (FIH) phase 1 trials of genuinely novel interventions (i.e., interventions exploiting a novel pathway or platform). “Translational stem-cell research,” thus, will be used to refer to FIH trials of stem-cell based interventions.

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participants be informed and voluntary? The familiarity of these questions, however, belies difficulties faced by researchers and regulators involved in the design and review of translational stem cell trials. In this context, issues are problematized by high degrees of uncertainty concerning the nature and probability of study risks, the absence of accepted normative standards or frameworks for risk assessment, and the limited expertise concerning stem cell science on most institutional review committees.

In this chapter, we discuss central ethical challenges for the design and review of translational trials involving stem cells. Of course, translational stem cell trials raise a number of issues that fall outside the traditional remit of the research ethics committee (IRB) including scientific integrity [1–3], stewardship of the scientific enterprise [4], and public communications and expectation management [5]. Due to space considerations, we will center our review on issues that will need to be considered by investigators when presenting protocols involving early phase studies of cell-based interventions for IRB approval. In addition, we defer to others a fuller discussion of ethical conduct surrounding the procurement of cell materials for trials. The chapter concludes with a series of recommendations for the design and review of translational trials involving stem cell-based interventions.

29.2 The Challenges: An Overview

The ethical challenges posed by translational stem cell research can be grouped into at least three broad, intersecting categories: technical, normative, and social. From a technical perspective, the issues are numerous, including, but not limited to, problems related to the purity and characterization of cell materials, limitations of animal models for many of the conditions for which cell-based interventions are being developed, the invasiveness of delivery strategies, and the complex composition and properties of cell-based interventions.

From a normative perspective, there are at least two closely related problems. First, despite the recent proliferation of policy initiatives designed to spur translational clinical research [6–12], there are no widely accepted frameworks for deciding if and when the initiation of a first-in-human (FIH) studies are justified [2]. Second, notwithstanding general requirements that studies enable the production of scientifically/socially valuable knowledge, there are no widely accepted standards or frameworks for balancing risk and scientific/social value when testing highly novel interventions [13–15].

Finally, from a social perspective, investigators and reviewers face at least three more problems. First, IRBs may lack the relevant independent scientific expertise to review trials of these extremely innovative and complex interventions [16, 17]. Second, stem cell research is particularly susceptible to social pressures that influence the design, conduct, and reporting of trials. For example, investigators in translational stem cell studies are often also the designers and developers of the intervention; both professional and financial interest conflicts must be carefully

managed. Stem cell trials also have a high public visibility that is related to controversies surrounding the ontological status of human embryos and the high level of public expectation surrounding their clinical promise. Third, technical and social standards are often underdeveloped in new research arenas. For example, methods of quantifying the purity and potency of cell agents will vary across different trials. This complicates the assessment of study value, since the lack of well-developed standards for the design and execution of studies makes difficult the comparison of findings and pooling of outcomes from other relevant studies.

As we shall see, these challenges play out in different ways across the various stages of the design and review process. With the exception of the ontological status of the human embryo, ethical challenges in attempting clinical translation of cell transplantation are akin to those encountered for gene transfer [2]. Indeed, with growing interest in induced pluripotent stem cells (iPS) and the application of gene transfer vectors to somatic stem cells, the distinction between gene transfer and stem cells may be fast becoming a historical artifact.

29.3 Assessment of Risk

The primary ethical challenges posed by translational stem cell trials derive from the high degree of uncertainty concerning both the nature and probability of study risks. First, like gene transfer, cell-based interventions pose a distinctive set of risks [18]. Whereas standard small molecule interventions are passive and depend entirely on the bodily processes of the host for their distribution and metabolism, cell-based interventions are living and active, carrying out their own characteristic biological programs [18]. Since, at this time, control of these programs is imperfect, cell-based interventions present risk of integration into unintended tissues, differentiation or dedifferentiation into unintended cell types, formation of teratomas or malignancies, failure to function in the desired fashion in a host of other ways, and/or emergence of latent and/or irreversible toxicities [19–21]. If cells were prepared using tissues or sera derived from animal sources, another risk that should be considered is the possibility of xenosis (that is, transmission of animal pathogens to human beings).

Second, some toxicities associated with stem cells will be immunologic in character [19, 22]. Though this would typically result in rejection of transplanted tissues, inflammation – caused, for example, by surgical delivery – can potentially favor the proliferation of undifferentiated ES cells, thus promoting the onset of a malignancy [23]. These are not well modeled in animal systems, especially when the “work-horse” animal system for preclinical research is the immunodeficient rodent. As such, immune reactions may be difficult to anticipate in translational trials [21].

These risks interact with difficulties raised by the composition of cell-based interventions. Interventions are typically complex, involving a mixture of different cell types; purity is also an issue. Due to differences in the original donors, the methods used to create the cells, and the number of passages undertaken before

transplantation, different batches of cells may exhibit genetic or epigenetic variations. These differences can translate into risks of infection (e.g., with genetic disease), and risks of teratoma formation. Furthermore, though various procedures for the evaluation of purity have been proposed [24], just which tests provide meaningful information concerning risk to human subjects remains controversial [16].

There are also risks related to the method by which cell-based interventions are administered to patient-subjects. Many cell-based interventions require invasive delivery. To use an extreme example, consider cell-based interventions for Parkinson's disease. These require inoculations to structures deep inside the brain. Based on studies examining complication rates associated with similar surgical procedures [25–28], surgery-related permanent or serious neurological deficits are likely to occur with a frequency of 0.73% per inoculation [5]. Given that most trials involve multiple injections, the risk of causing permanent neurological deficit is considerable.

Finally, assessment of both the size and probability of (most of) these risks is complicated by a lack of reliable evidence. Although there is a great deal of clinical experience with some cell transplants (i.e., transplant of autologous hematopoietic stem cells for blood disorders), experience with their use for treating other disorders in human beings is limited. Assessment of risk-benefit is also complicated by the inadequacy of animal models for many of the conditions for which cell-based interventions are being developed. Even though researchers can use animal studies to derive estimates concerning the size and probability of risks, confidence in these estimates is very limited. Furthermore, because the full range of adverse events is generally not well understood at the point where novel cell transplantation trials are initiated, prudent risk minimization dictates that interventions should be considered riskier than indicated by preclinical studies [5, 29, 30].

29.4 Assessment of Direct-Benefit

All influential codes of human clinical research ethics demand that risks posed by medical research be proportionate to the foreseeable benefits. In the research ethics literature, a distinction is drawn between two kinds of benefits: benefits to participants and benefits to society. In ethical late-phase clinical research, risks of study interventions should be offset by the potential for direct therapeutic benefits for participants. In translational stem cell trials, however, it is far from clear whether the risks associated with participation can be justified in this way. For one, because it is always easier to disrupt a system than correct it, many of the problems that beset the assessment of risk apply to an even greater degree for the assessment of direct-benefit. The lack of clinically reliable evidence, for example, makes the assessment of both the size and probability of risks *and* direct benefits to participants very difficult. Again, since we currently have very limited experience with many of the cell platforms that are entering clinical testing, and lack reliable animal models for many conditions, our best estimates concerning both the nature and probability of direct benefits will typically have limited predictive value.

Second, even if these epistemic concerns are set aside, the aims and architecture of translational trials create difficulty for the claim that risks of study intervention are justified by the prospect of direct benefit for subjects. The primary goal of early phase studies is the shaping of uncertainty such that a clear, testable hypothesis can be formulated [31]; as such, translational trials are designed to narrow the range of uncertainty concerning the conditions under which an intervention can elicit a therapeutic response. Questions that such studies address include: Does the agent reach the intended target in humans? Does it have unintended (non-specific) effects? How is the agent metabolized? What is the optimal route and timing of administration? What is the optimal dose? Does the agent produce enough response to warrant further development? Trials designed to answer these questions typically constrain therapeutic action to such an extent that, even if the study agent can produce clinically meaningful effects, at least some of the participants will not receive the intervention under conditions required to produce those effects. For example, dose escalation studies generally involve administering subtherapeutic levels of an agent to initial subjects, and giving levels of an agent that trigger toxicities to the final subjects. In general, then, it is only later in the development process, when most of these variables have been nailed down and the range of uncertainty is sufficiently narrow, that the risks of receiving drugs can reasonably be justified by appeals to possible therapeutic benefits. For these reasons, the risks posed by well-designed cell-based interventions under study in a translational trial should be justified on grounds of social value [32].

29.5 Assessment of Social Value

If ethical trials must be justified on the basis of social value, adequate frameworks or metrics for prospectively assessing social value, and for balancing risk and social value are needed.

Currently, the scientific and social value of translational trials is determined with reference to what one of us has called the “regulatory model” [2]. According to this model, the value of a phase 1 study is assessed in terms of its ability to facilitate the progression of novel interventions from phase 1 to phase 2 testing and beyond (“progressive value”) [2].

There are good reasons to doubt the appropriateness of the regulatory model in translational settings. For one, phase 1 trials frequently lack social value according to this model. This can be demonstrated by pointing to the fact that in novel research areas, the majority of phase 1 trials do not lead to phase 2 trials [2]. According to the regulatory model, these trials are without social value because they do not attain progressive value. Given that participation in these trials involves nontrivial and sometimes significant risks to participants, and that these risks are not justified by therapeutic benefits, it follows that many phase 1 trials are ethically unjustified.

There are additional reasons to reject the regulatory model. One of the major problems is that this model does not reflect how first-in-human (FIH) phase 1 studies (if not all phase 1 studies) in fact accrue value. Citation patterns indicate that the information gathered from novel agent studies is much more likely to be incorpo-

rated into preclinical studies than new phase 1 or phase 2 studies [33]. Assuming that citation numbers reflect how peer researchers value a study, this finding suggests that progressive value may not be the best measure of the actual value of FIH studies. Rather, because these studies are cited more frequently in preclinical studies than in new phase 1 or 2 studies, their value may be better measured in terms of “reciprocal value” (see below).

We advocate the adoption of a “translational model of value,”² according to which phase 1 trials can produce useful knowledge by motivating further preclinical studies of an intervention (“reciprocal value”), by prompting modified phase 1 trials of the same novel agent (“iterative value”), or by informing other areas of loosely related research practice (“collateral value”) [2]. The translational model has a number of advantages over the regulatory model, two of which we will mention here. First, as the above already suggests, the translational model more accurately reflects the way that phase 1 studies of novel agents actually accrue value than the regulatory model does. Second, precisely because the translational model emphasizes reciprocal, iterative, and collateral value, this model will favor study designs that allow for the meaningful interpretation of negative results and, thus, the development of new lines of experimentation. In this way, the translational model maximizes the social value of translational studies even when progressive value fails to materialize.

Accordingly, we urge that all translational trials involving novel agents like stem cells incorporate five elements that enable capture of iterative and reciprocal value [2]. First, all FIH trials should be supported by a solid preclinical evidence base. As will be discussed shortly, this helps promote meaningful interpretation of negative results. Second, cell-based interventions should be flexible so that failures can be addressed by modification of the intervention. Third, agents should be thoroughly characterized and described in the protocol. This requirement is especially critical for interventions, like cellular agents, that involve mixed composition and multiple components. Source material should be archived so that, if needed, it can be characterized using assays developed subsequent to the trial. As well, investigators should use standardized protocols for characterizing composition and function so that trial results can be pooled. Fourth, in addition to measuring clinical outcomes, protocols should test molecular and cellular events along key points in the causal pathway. Last, researchers should have a well-developed contingency plan in the event that results in human beings are discordant with those in animals.

29.6 Assessment of Favorable Risk-Benefit Value

Even if we settle upon a model of scientific/social value, however, investigators and reviewers still face a very difficult question: how should risk/social value trade-offs be evaluated? At present, there are no widely accepted standards for balancing social

²Similar sentiments have been expressed by a many translational researchers [34, 35, 74].

value and risk [2, 13–15]. Existing proposals, furthermore, are typically too restrictive for FIH studies (e.g., the standard for demarcated research risks proposed by Alex London [36]). The lack of standards raises concerns that ethical evaluation of risk for FIH trials is susceptible to arbitrary and inconsistent judgments. To protect FIH trial participants and the integrity of drug development, then, we need a normative standard for assessing when an FIH study possesses sufficient research value.

Any proposed standard must satisfy at least three *desiderata*. First of all, it must say something about the location and nature of uncertainty warranting trial initiation. Second, an alternative framework must spell out what counts as an informative FIH trial result – one that can justify the considerable risks of drug administration in FIH trials. Should translational trials be designed exclusively with progressive value in mind – as the regulatory model suggests? Or should they be designed to maximize reciprocal, iterative, and collateral value – as suggested by the translational model? Finally, whichever model an alternative framework endorses, it must also provide guidance concerning how to design trials in order that information gain is maximized and risks to participants minimized.

Again, at present, there are no widely endorsed normative standards for any of these judgments in the context of translational research. However, one of us (JK) has recently proposed a normative standard – the principle of modest translational distance – that moves toward satisfying the three *desiderata* noted above [2]. When researchers initiate FIH trials, the prediction that outcomes observed in animals will also occur in human beings relies on a series of background assumptions. The number and strength of these assumptions can be likened to a kind of “translational distance.” The more extravagant the assumptions (e.g., the assumption that outcomes in immunodeficient mice predict outcomes in immunocompetent human beings is more extravagant than the assumption that immunocompetent non-human primates predict human outcomes), the greater the translational distance. The principle of modest translational distance holds that translational trials should involve no more than a modest translational distance between completed preclinical and/or related clinical studies and projected human trials. The principle is intended as a heuristic device, focusing the attention of researchers and reviewers on the credibility of assumptions linking preclinical and clinical studies. In general, the principle requires that the number and size of assumptions linking preclinical and clinical studies be minimized (by, e.g., maximizing the internal and external validity of preclinical studies and the correspondence between conditions used in preclinical studies and those used in clinical trials). According to the principle of modest translational distance: (1) the initiation of FIH testing is scientifically warranted only at the point where modest translational distance is achieved; (2) an informative FIH trial result is one that maximizes translational value (i.e., reciprocal, iterative, and collateral value) and contributes to closing translational distance; and (3) FIH trials that traverse no greater than modest translational distance and involve greater than minimal risk ensure that the welfare of participants is protected because an adequate threshold of scientific value is achieved.

29.7 Design and Evaluation of Preclinical and FIH Studies

Clearly, prospective judgments concerning the scientific and social value of a translational trial will turn, at least partly, on an assessment of the quality and appropriateness of a study's design. In any case, a study that lacks validity also lacks scientific and social value. It is important to emphasize, furthermore, that the appropriateness of design decisions in FIH trial will depend to a large extent on the quality of preclinical evidence (concerning optimal dosage, method of delivery, location of delivery, etc.) informing these decisions. If the preclinical studies informing the design of a translational trial are themselves poorly designed, this may significantly diminish the trial's value because results – particularly negative results – will be difficult to interpret (experimenter bias, for example, is a trivial explanation for a negative result that cannot be ruled out if poor preclinical methodology was used).

Unfortunately, preclinical studies often contain methodological flaws. Practices aimed at reducing bias that are routine in clinical research – random allocation, a priori statement of hypothesis, blinded treatment allocation and outcome assessment – are only sporadically applied in preclinical research [37–40]. There are additional concerns about publication bias [41–43] and about the reproducibility and robustness of preclinical findings [44].

Translational trials themselves frequently suffer from methodological flaws as well, particularly when they are assessed from the perspective of the translational model of value. From the latter perspective, FIH studies should enable meaningful interpretation of negative as well as positive findings. One way to achieve this goal, already alluded to above, is by building research components into a clinical trial that test an intervention's effect at key points in a causal pathway. Thus, if a study does not cause the intended therapeutic responses in subjects, these study components enable researchers to identify why effects seen in animal models are not recapitulated in human beings. With this knowledge, investigators can troubleshoot their intervention, and other research teams can build on insights derived from human studies. At present, many translational studies do not incorporate such research components. Absent quality preclinical data concerning optimal conditions for clinical testing, furthermore, investigators will often lack the information needed to implement these design features, or to interpret their results. The scientific and social value of translational trials, therefore, requires high quality standards in both preclinical and translational settings.

29.8 Subject Selection

Translational trials inevitably invite debate about subject selection. On the one hand, patients with advanced disease and no other options (the “treatment refractory”) have less to lose by participation because the opportunity costs of participation are lower [5]. Because they are treatment refractory, their decision to enroll does not deprive them of effective therapy or prevent them from pursuing alternative treatment

options. Furthermore, both the quantity and quality of their remaining life-time will typically be lower than that of patients whose symptoms are currently under medical control or who have yet to explore all of the established treatment options available.

On the other hand, treatment refractory patients are in a worse position *vis-à-vis* voluntary informed consent than healthy or medically stable volunteers. Because they lack treatment alternatives, desperation may cloud their judgment or that of proxy decision makers [16, 20]. Indeed, as is well known, many patients enrolling in phase 1 trials do not appreciate ways that study design can interfere with their therapeutic objectives (therapeutic misconception); patients also frequently overestimate benefits and underestimate risks (therapeutic overestimation) [45, 46]. Furthermore, trials enrolling healthier subjects may be more informative. Subjects with less advanced disease are often in a better position to respond to treatment [16, 47] and, when adverse events occur, the relative absence of co-morbidities simplifies causal attribution [5]).

Though consensus on this issue has yet to be achieved, we will offer our own view. Given the high degree of uncertainty surrounding translational stem cell trials, and the position that risk in FIH trials is ethically justified by social value rather than therapeutic benefit, we believe that non-maleficence should trump other considerations when it comes to subject selection. This means that where protocols are subject to major indeterminacy, enrollment should generally be restricted to treatment refractory populations in order to minimize risks, with stringent measures taken to ensure adequate informed consent (described below). Where trials are performed in medically stable or healthy volunteers, furthermore, the protocol should exclude volunteers at greater risk for adverse events [2]. This position is consistent with the recommendations of other commentators [20].

29.9 Justice

Subject selection in the context of translational stem cell trials also raises questions of justice. For example, are the benefits and burdens of research distributed equitably? This question is particularly pressing when disadvantaged populations are recruited. In the USA and other countries where many lack adequate health coverage, for example, justice issues may arise if the underinsured are targeted for recruitment in risky research. Justice concerns also arise when trials are conducted transnationally (i.e., studies are conducted in a disadvantaged country by researchers located in an advantaged one). In these scenarios, there is concern that the unfair disadvantages of host populations will be used to advance the health interests of advantaged populations.

Since debates surrounding a series of placebo-controlled trials of short-course AZT for the prevention of vertical transmission of HIV, research ethics statements have evolved two policies to promote justice in transnational studies involving disadvantaged host communities. The first is responsiveness: “[m]edical research is only justified if there is a reasonable likelihood that the populations in which the research is carried out stand to benefit from the results of the research” [48]. The second is post-trial access: “[a]t the conclusion of the study, every patient entered

into the study should be assured of access to the best proven prophylactic, diagnostic and therapeutic methods identified by the study” [48]. This is a standard of post-trial access. Though specific language varies, both conditions were taken up by numerous other authoritative policy statements and reports, including CIOMS [49], the Nuffield Council [50, 51], the National Bioethics Advisory Commission [17], European Group on Ethics and Science of New Technologies to the European Commission [52], and South Africa [53]. Some policies, like India’s [54] and Nepal’s [55], endorse the former but do not specifically address the latter in the context of studies involving economically disadvantaged populations.

Standards of post-trial access and responsiveness, however, were developed largely with late-phase trials in mind. As a result, it is often unclear how to apply these standards to translational trials. Consider the standard of responsiveness. Since translational trials almost never provide conclusive evidence of efficacy, what does “responsiveness” mean in this context? We suggest that in order to ensure the responsiveness of early phase trials of cell transplantation, these trials should address an urgent health need of the host community, and they should aim at producing knowledge that expands the capacity of health-related social structures in the host community to meet urgent health needs [56]. Where these conditions cannot be passively achieved, they can be addressed by a well-developed plan to achieve responsiveness through pharmaco-philanthropy, discriminatory pricing, equitable access-licensing, or advance-purchase agreement (reviewed in [2]).

Concerns with issues of justice in the context of translational trials are increasingly salient as stem cell research goes global. Several key translational stem cell trials have involved HIC sponsors and/or collaborators recruiting subjects from LMICs. For example, an Italian trial testing genetically modified CD34(+) cells for adenosine deaminase deficiency recruited patients from LMICs; this recruitment decision was driven by a desire to find patients who were ineligible for costly enzyme replacement therapy [57, 58]. Some early translational stem cell trials in cardiology involved HIC sponsorship and/or leadership plus recruitment of patients from LMICs [59, 60]. It is very likely that this trend will continue. To forestall the unjust exploitation of patients living in LMICs, it is essential that translational stem cell research be held to standards of responsiveness and post-trial access [56].

29.10 Informed Consent

Due to the complexity and novelty of the science involved, standard informed consent procedures may be inadequate in the context of translational stem cell research [20, 61]. Concerns about therapeutic misconception and overestimation are also heightened. A compelling pathophysiologic rationale may obscure uncertainties and technical challenges in clinical translation, engendering unsustainable expectations in both investigators and potential participants. Press reports may also amplify expectations. The enrollment of treatment refractory participants might also promote higher therapeutic expectations in subjects, as studies have

indicated that severely ill subjects are prone to viewing early phase studies in therapeutic terms and to mis-estimating the benefits of participation [62, 63]. Finally, individuals confronting high expected losses, like patients with terminal illnesses, tend to be less averse both to risks [64] and to high degrees of uncertainty about benefit [65].

Additional steps for informed consent in the context of stem cell research have been proposed by various commentators [20, 61]. Furthermore, informed consent guidelines devised for gene transfer [66] are applicable to stem cell research. These provisions range from the inclusion of clear language concerning the primary purpose of the trial and the likelihood and size of risks and benefits, to the administration of questionnaires designed to check the extent to which participants have understood key elements of the study. In order to make an informed decision to participate, participants should understand that the intervention has never been tried before in humans for the indication under study; that researchers do not know whether the intervention will work or even if it is safe; that the vast majority of participants in early phase trials do not receive major clinical benefit from participation despite the risks that participation entails; that the research team has financial and professional interests in the research (if they do); and that the study involves procedures and methods that are necessary for scientific validity, but might interfere with the subject's therapeutic objectives.

29.11 Privacy and the Procurement of Stem Cell Tissues

When translational stem cell research involves the transplantation of non-autologous stem cells, a major safety concern is the transmission of infectious agents or genetic conditions through transplanted materials (as we already mentioned above). In order to forestall or at least minimize these concerns, researchers need up-to-date information about the health status of cell donors [67]. For this reason, regulators like the FDA require screening and testing of donors at the time of donation, as well as provisions for tracking donated materials to their original donors [68].

The problem is there is often a considerable lag between the donation of biological materials used to derive stem cells and the trials involving their transplantation [61]. This presents three challenges. First, even if donors were properly screened and tested at the time of donation, new risks may become apparent in the ensuing period. For example, genetic conditions yet to manifest at the time of donation may emerge. Second, unless consent for recontact is obtained at the time of donation, recontacting donors violates their privacy. Third, absent consent for recontact from donors, review committees are placed in a difficult dilemma: either they violate the privacy of donors in order to ensure that trial participants are not put at undue risk, or they protect the privacy of donors and put participants at risk.

In order to strike an appropriate balance between the need to protect participants and the need to respect the privacy of donors, some commentators urge that permission to recontact donors be obtained at the time of donation [61]. According to this

view, review committees should assure themselves that provisions for recontact and confidentiality are in place, along with upfront testing of and linkage to donors [61].

29.12 Independent Review

A final challenge for committees reviewing translational stem cell research is the problem of finding adequate, disinterested expertise for independent review. Due to the complex and innovative nature of cell-based interventions, local review committees typically lack the relevant independent scientific expertise to review these trials [16, 69]. Though many institutions carry out separate scientific and ethics reviews, this practice has come under fire in recent years [70]. Central to the mandate of ethics committees is the assessment of risk/benefit (direct and social) proportionality, and it is difficult to see how review committees can provide meaningful assessments of risk or benefit absent an understanding and appraisal of the science involved in the study [71, 72].

In light of these concerns, some commentators (including one of the present authors) have advocated centralized/coordinated review of translational stem cell protocols by specialized stem cell research oversight committees [73]. National oversight committees like the Recombinant DNA Advisory Committee in USA (set up to review the science and ethics of gene transfer research), furthermore, provide useful precedents for the development of specialized stem cell research oversight committees [71]. Finally, effective independent oversight helps to protect fragile research domains. Here the case of gene transfer research is illustrative: ethical missteps in a trial involving a hereditary liver disorder, OTC deficiency, had cascading negative effects on the broader field [2, 4]. If such problems are to be avoided, it is essential that stem cell researchers avoid ethical pitfalls by maintaining high scientific and ethical standards.

29.13 Conclusion

Translational stem cell studies raise issues similar to those encountered in other novel platform translational studies. The particular challenges relate to the high degree of uncertainty concerning risk, the absence of articulated normative standards or frameworks for risk assessment, and limited independent expertise at the level of institutional review. These factors together present great difficulty for the goal of non-arbitrary, systematic ethical decision-making.

Though this chapter was primarily aimed at presenting a comprehensive overview of the issues facing researchers and reviewers, we also offered a number of recommendations for addressing these challenges (see Fig. 13.1). We suggest that following these recommendations will place translational stem cell studies on a more secure ethical footing, while helping to avoid some of the missteps and setbacks that have in the past taxed the development of other novel platforms.

Figure 1: Recommendations

1. When uncertainty is great, assume the protocol is higher risk.
 - *Use “upper bound” rather than “best guess” estimate of risk.*
2. Risks in translational stem cell trials should be justified by knowledge benefits.
 - *Exclude direct benefits (i.e., therapeutic benefits for subjects) from the risk-benefit calculus.*
3. Adopt the “translational model of value” for translational stem cell trials.
 - *The translational model maximizes the social value of translational studies even when progressive value fails to materialize.*
4. Maintain modest translational distance between preclinical and clinical experiment.
 - *Minimize the number and size of assumptions linking preclinical and clinical studies by e.g., maximizing the internal and external validity of preclinical studies and the correspondence of preclinical and clinical study conditions.*
5. Restrict enrollment to treatment refractory populations.
 - *Where trials are performed in medically stable or healthy volunteers, furthermore, the protocol should exclude volunteers at greater risk for adverse events.*
6. When pursuing trials in disadvantaged populations, ensure “responsiveness.”
 - *Sponsors/research team should show evidence that study addresses urgent health needs of host community and that results can be deployed in that setting – either directly or indirectly through philanthropic intervention or intellectual property agreements.*
7. Due to the complexity and novelty of the science involved, additional steps for informed consent are required.
 - *The inclusion of clear language concerning the primary purpose of the trial and the likelihood and size of risks and benefits, and the administration of questionnaires designed to check the extent to which participants have understood key elements of the study.*
8. Protect the privacy of donors by seeking permission for recontact up front.
 - *In order to strike an appropriate balance between the need to protect participants and the need to respect the privacy of donors, permission to recontact donors should be obtained at the time of donation.*
9. Create specialized stem cell research oversight committees for the centralized/coordinated review of translational stem cell protocols.
 - *Due to the complex and innovative nature of cell-based interventions, local review committees typically lack the relevant scientific expertise to review these trials.*

Note

Readers may be interested in two recent publications by the authors related to the content of this chapter. Concerning section 29.4: Anderson JA, Kimmelman J. Extending Clinical Equipoise to Phase 1 Trials Involving Patients: Unresolved Problems. *Kennedy Institute of Ethics Journal* 2010; Mar; 20(1): 75-98. Concerning section 29.12: London AJ, Kimmelman J, Emborg ME. Research Ethics. Beyond access vs. protection in trials of innovative therapies. *Science* 2010; May 14; 328(5980): 829-30.

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Part X

**Looking at the Future of Translational
Stem Cell Research and Stem Cell-Based
Therapeutic Applications: Risks, Long-
Term Effects and Priority Setting**

Chapter 30

Unruhe und Ungewissheit: Stem Cells and Risks¹

Nils-Eric Sahlin, Johannes Persson, and Niklas Vareman

Abstract This paper focuses on the risk of unknown and uncertain long-term effects of stem cell research and its applications. Research on human embryonic stem cells and induced pluripotent stem cells are used as examples. We discuss some problems that such uncertain knowledge creates for decision makers, and describe how difficult decision making in this context really is. A method for handling this type of hard choice situations is presented and discussed.

Keywords Decision-making • Epistemic risk • Knowledge uncertainty • Risk • Value uncertainty

30.1 Introduction

“Die Unruhe und Ungewissheit sind unser Theil”, — writes Goethe in a letter to the German novelist Sophie von la Roche in 1774. But is it the incalculable and indeterminate that cause disquiet, or is it our bustling pursuit of knowledge that makes us uncertain?

Contemporary psychologists have taught us a great deal about the way we perceive risks and about the way affects and emotions influence our behaviour. Their research has shown that, as decision makers, as risk-assessors, and as risk-controllers, we are short-sighted, one-eyed and prone to serious errors of refraction. Who would have guessed that? We generate too few, and too narrow, hypotheses. We gather information, or evidence, in favour of our guesses that is too narrow, readily available,

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and skewed in favour of preferred beliefs. Once we have a pet hypothesis, we look for confirmatory evidence, neglecting countervailing evidence. We are simply not rational — not in the way our theories of rationality (logic, probability and decision-making) assume, at any rate. This is an alarming fact, considering the serious risk assessment and risk management tasks that lie ahead of us.

This fact of irrationality (the phrase “fact of irrationality” seems fair, since the claim is supported by a vast amount of empirical evidence) should not just bring about unrest; it should make us think — think at least twice about our state of knowledge, in particular when the task is to make a serious risk-assessment in a convoluted situation [1]. We must not go gentle into that uncertainty.

In this chapter we will focus on a particular type of risk: the risk of unknown and uncertain long-term effects. The problem here is one of not knowing what will happen, and when we know it will, when; and of not being acquainted with the consequences, and therefore being unable to value the unfamiliar. Doing this our center of attention will be human embryonic stem cells and induced pluripotent stem cells. Adult stem cells are not as interesting – they do not bear the same type of risks and moral difficulties.

The paper urges risk analysts to take a Socratic approach to their discipline.

30.2 Stem Cells: Some Risks and Benefits

If money is a measure of hope, the amount of cash spent on stem cell research speaks for itself. Stem cell treatment is often presented as the key to progress in the battle against disease and the suffering ill-health causes. Its only serious competitors today are nanotechnology and GWAS (genome-wide association study).

What does stem cell research promise? Stem cells have the ability to self-renew and are pluripotent (can differentiate into a diverse range of specialised cell types). It is these properties that make the treatments they may provide potentially powerful. The aim is to develop therapies that replace, or restore, damaged tissues, and rejuvenate our malfunctioning organs.

Turning to the reverse of the medal, in stem cell therapies pluripotent stem cells have a tendency to cause cancer (teratomas) after transplantation. This is a recognized difficulty, and as yet we do not know how to circumvent it [10] — an uncertainty of some import. A problem to be solved, perhaps, in a year or ten? The bottom line, however, is that future stem cell therapies are permeated by potential cancer risks: epistemic uncertainties combined merged with undesired consequences, and risks hard to measure and put a value on. In cases like this we should not forget what the psychologists taught us.

But cancer is but one of many examples. It is also predicted that stem cell treatments will help patients with spinal cord injuries to regain their ability to move and walk. Early last year (2009) the FDA (Food and Drug Administration) in the US gave approval for a phase-1 trial of GRNOPC1 in patients with acute spinal cord injury. GRNOPC1 is a therapy derived from human embryonic stem cells (hESC). In the

course of this treatment hESC-derived oligodendrocyte (brain) progenitor cells are injected directly into damaged parts of the injured spinal cord. The hope is that the treatment will result in the patient's spinal cord function being restored.

There are many other examples of potential stem cell therapy. In every case, however, the translation of preclinical research into an effective treatment — the step between “in theory” and “in practice” — is long, slippery and winding; lined with known and unknown risks.

Today haematopoietic stem cell transplantation (bone marrow transplantation) is the only stem cell therapy used successfully on a regular basis. With some exceptions, it is used to treat life-threatening conditions such as leukaemia, and certain immunodeficiency syndromes and inborn errors of metabolism. This is because it is not entirely risk free; approximately 1:10 patients do not survive treatment. Haematopoietic stem cell transplantation may well be the only type of stem cell therapy whose risks are not (completely) unknown or indeterminate. It has been used for decades, and we have various prediction models for estimating the risks that a course of treatment carries (for the individual patient).

But stem cell treatment requires stem cells. In an effort to reach higher aims, researchers are now looking beyond adult stem cells, towards the much more potent embryonic stem cells (ESCs). It is well-known that research on the latter provokes moral concerns and involves the greatest unknown risks.

The alternative to using hES cells is to use induced pluripotent stem cells (iPSC), i.e. reprogrammed somatic cells. The hypothesis is that iPS cells and hES cells are “identical” and inter-changeable; but we do not *know* this. This lack of (robust) knowledge is a deep source of uncertainty and unrest. The good thing is that with iPS cells, the most burning moral questions (relating to the hESC) — for example, those having to do with the status of the embryo — are circumvented. The use of iPS cells raises other ethical questions, but they are not the topic of the present paper.

So what are the uncertainties? And why do we feel that there are serious outcome risks *and* epistemic risks to be accounted for? That is, what requires us to explain both the risks associated with the consequences of our actions, and the choices and risks involved in the way we use the knowledge we have?

First, there is the identity problem [3, 4]. What do we need to know in order to say that iPS cells and hES cells are “identical?” We need to know that they have the same biological properties — for example, growth properties. If the activity of the iPS cell is disturbed, if two cells with corrupt DNA sequences are produced, a disorder can propagate and harm the organism. The differentiation also has to take place in the proper order and stop when it is supposed to stop.

It is, of course, not necessarily true that they are “identical”. And why should they be? They are generated under rather different circumstances, from the inner-cell mass of an embryo via a fully determined cell that is forced into a pluripotent state. What we really need to know is whether they differ (in ways relevant to treatment), and if so, how. What is important is that iPS cells do the work they are supposed to do and carry acceptable risks. But a few well researched areas aside, our epistemic map of their differences is a blank sheet of paper.

We also need to know that iPS cells differentiate, not in a — “fashion similar to”, but the very same way as hES cells, into fully differentiated tissues — that is, that they are suited to “replace” hES cells. Different kinds of experiment have shown great similarities between iPS cells and hES cells. But is close good enough? Is “similar, but there is so much we do not know” an acceptable state of knowledge, an acceptable state of epistemic uncertainty? Of course not, and for obvious reasons.

Another thing we want to know is whether the reprogramming of a somatic cell also means normal epigenetic reprogramming. The methylation of a gene can prevent transcription, for example, and influence the presence and profusion of proteins in cells or tissues. The effects of an irregular methylation process can be substantial. Therefore, it is crucial not only for a perfect reprogramming to be guaranteed, but also that we know the reprogramming to be free of any unasked-for effects when the cell moves to a new somatic stage, i.e., when, so to speak, the tape rolls forward again (a version of the Dolly problem, premature ageing).

Recent research shows that differentiated (IMR90) cells turned into iPS cells have methylation patterns similar to those of hES cells. If iPS cells are to replace hES cells this is good news [5]. However, we only know that this happens at certain specific locations of the genome, not in general.

On the other hand, an even more recent article by A. Doi *et al.* gives a somewhat different picture of the present situation. It concludes: “In some cases, the methylation in iPS cells was indeterminate between differentiated fibroblasts and ES cells; this was true, for example, of *TEX5*, which encodes a transcription factor that is involved in cardiac and limb development. In other cases, methylation in iPS differed from both fibroblasts² and ES cells, suggesting that the iPS cells occupy a distinct and possibly aberrant epigenetic state” [6]. Doi’s research group also found that certain loci in iPS cells remain incompletely reprogrammed, and that other regions are aberrantly reprogrammed. We thus have evidence indicating that iPS and hES cells have different methylation patterns, giving rise to the obvious question: What does this mean? Today the answer is: We do not know! An important piece of uncertainty [7].

From the perspectives of risk analysis and risk management it is important to remember that similar does not mean “identical,” and that identical does not mean inter-changeable: hESC and iPSC may well be “identical” without being inter-changeable (and vice versa). There is no such thing as two identical cells. Similarity is not important; what is important is how the induced cells function, that they do the job they are supposed to do and nothing else. If we make a plain distinction between rigid comparisons *in vitro* and the cell’s dynamic life *in vivo*, it is the latter that may lead to unknown and/or unexperienced future consequences; and therefore a complicated risk analysis.

DNA methylation gives the cell a memory. An iPS cell should not be a cell without a memory. On the other hand, it should not have the wrong memories or be

²Of course, uncertainties emerge from the fact that the fibroblasts are grown *in vitro*; ordinary cells live under completely different environmental pressures.

disposed to remember things the hES cell is unable to recall. Why this stricture? It has to do with risk management. Epigenetic factors play a role in some congenital genetic diseases; and there is a risk that the wrong methylation pattern will cause cancer or heart failure [8].

By the way, to be noted is the combinatorial paroxysm of possibilities that emerges when we move from genetics to epigenetics. Mathematics is not an empirical science — we know that. But pure and simple combinatorics tells us that there are quite a few combinations (epigenetic patterns) to be accounted for. Of those, some will have causal (perhaps serious, perhaps negligible) effects, some will not. One day we will probably have tools that help us predict what will happen, but today we do not.³

The crux of the matter is that there are things we do know, but also many that we do not know; it is the known unknowns (and unknown unknowns) that cause unrest and make the risk-assessment so immensely difficult.

30.3 Why Risk Analysis?

There are many reasons for doing risk analysis and risk management. Two motives stand out. First, we do not want to hurt patients by taking unnecessary risks or because our knowledge is deficient. Second, we need the public's trust to be able to increase our knowledge through scientific enquiry. To create and maintain trust it is important to inform the public about what we *do* know, but also about what we *do not* know. What risks are known? How serious are they? What are the potential risks, not yet analysed and assessed? We need to take what might be called a Socratic approach to risk analysis and risk management.

30.4 Types of Decision

There are four paradigmatic types of decision. The complete picture is, of course, vastly more complicated than a simple four-by-four matrix. For one thing, the categories are continuous rather than discrete.

In *Type 1* situations the decision maker has extensive knowledge and information, expressed in terms of precise probability estimates. He has also clear and distinct preferences and values.

In *Type 2* situations the quality and quantity of information is poor, and it is difficult to represent the underlying uncertainty in terms of probability. On the other hand, the decision problem is one in connection with which the decision

³Those of us looking at this research area from without wonder why tailor-made differentiated cells are not the best alternative if we wish to avoid uncertainties, i.e., by way, not of inducing uncertainty, but blocking combinatorial possibilities. A multipotent, non-pluripotent, cell does not go off the rails as easily.

maker still has clear and distinct preferences and values: he knows what he wants and desires.

In *Type 3* situations the quality and quantity of information is good — good enough to assess precise probabilities. However, the decision maker lacks harmonious, clear and distinct preferences and values. Perhaps his appetites are out of keeping with his valuations.

In *Type 4* situations both information and preferences are unfixed or unreliable.

Type 1 situations are readily detected in the paradigmatic cases with which classical theories of rational choice and decision-making deal. But the traditional theories are ill-equipped to handle the three other types of situation. In situations of Types 2, 3 and 4 the traditional theories are no guides to action. Here we need theories that help us to represent indeterminate beliefs and imprecise values. We must introduce more complex, but also more complete, decision procedures. “Maximise expected utility”, the mantra of the classical theories, is no longer an available option. This is not the place to discuss generalised and competing theories of rational decision-making. It is, however, important to know that there are no ready-made theories, agreeable to all; the theoreticians are still bickering.

Which of the four types of situation best describes stem cell research and the risks of stem cell therapy? The answer is: all four. It is obvious, for example, if we are talking about iPSC, that even those sold on stem cells must admit that there are one or two things we do not know. Thus we lack a robust understanding of the way epigenetic factors influence the working of the iPSC.

In truth, the answer to the question depends on which experimental stem cell therapy we are looking at. High-risk neuroblastoma and haematopoietic stem cell transplantation can be a Type 1 situation. However, not all children respond to stem cell treatment, and in a case of recurring neuroblastoma we might be drawn into a Type 2 or Type 4 situation. What are the odds that a haematopoietic stem cell transplantation that failed to succeed the first time will work on the third or a fourth attempt? With the number of refractory instances, the relevant probabilities become harder and harder, if not impossible, to estimate; and as our epistemic state deteriorates, our desires and values become more and more woolly.

Examples involving nuclear power plants, BSE, GMOs, electro-magnetic fields, and nanotechnology have taught us that various kinds of factor create epistemic uncertainty. Good risk analysis requires careful inspection of the present epistemic state. It is simply not enough to identify and evaluate outcome risks, i.e., the negative consequences of our actions [9]. An estimation and evaluation of the current state of ignorance is crucial. We must know what type of knowledge and value situation we are in (Type 1, 2, 3 or 4).

Let us briefly mention some factors producing epistemic risk and value uncertainty. We can then stop and discuss one especially important factor in detail.

Research is a mechanism which, off-and-on, gives us incorrect or indeterminate results. Sometimes the machinery works flawlessly, sometimes chance has an

unfavourable effect on the results, and sometimes the investigation does not work at all. To accept the results without asking whether they are the result of a working mechanism or not is to wink at all forms of epistemic uncertainty.

As is well known, we might end up in Type 2 and Type 4 situations for moral or practical reasons. Sometimes it is difficult to carry out controlled experimental studies. A good example is toxicity testing, when, say, we are interested in the effects of extremely low doses over a long period of time. Sometimes the desired knowledge is not, for moral reasons, within reach. At this point we might have to rely on softer, indirect empirical evidence rather than evidence that is solid and direct. *In vitro* experiments and animal (rat) models have given us much of the bulk of the knowledge we have today relating to stem cells and epigenetics. Our cells do not live their lives in an artificial environment, and we are not rats — obvious, but important factors inducing uncertainty.

The fact of irrationality (see above) can also be a serious cause of epistemic uncertainty. Not only the man in the street, but also the esteemed scientist is disposed to generate too few, and too narrow, hypotheses, and to support them with evidence that is too narrow, too readily available, and skewed in favour of confirmation, neglecting falsification. Sometimes we perceive these biases. The fact of irrationality then pushes us in the direction of epistemic uncertainty. It propels us into Type 2 and Type 4 situations.

The factors so far mentioned all produce epistemic uncertainty, but there is another factor that pulls us in the other direction — that is, from epistemic uncertainty to illusory certainty. The fact of irrationality, when unnoticed, can make us far more certain than we should be. In particular, however, and for no good reason whatsoever, statistics and traditional (“mathematical”) methods of decision analysis always demand numerical precision. These contemporary tools and theories encourage us to act as if we are in Type 1 territory. They sweep under the carpet the simple possibility that we are in a situation bearing the stamp of uncertainty. The theories ask us to maximize or minimize. To do that we need unique probability distributions and well-defined utility functions. But we have neither. Risk analyses based on such theories force the risk managers to make decisions involving great epistemic risks.

A Socratic approach to risk analysis means that we honestly portray our present state of epistemic uncertainty; that we do not pretend that our knowledge and information is more precise or better than it is.

30.5 Time: Pushing and Tugging

Lack of experience results in Type 3 or Type 4 decisions, at least if — as many psychologists argue — experienced utilities differ from decision utilities [10]. An illustration is provided by the phenomenon of hedonic adaptation, where the affective intensity of favourable and unfavourable circumstances is reduced [11]. At least some of these adaptations involve changes in how we value our lives. For instance,

Tyc [12] found no difference in quality of life between those who had lost limbs to cancer compared with those who had not.

Experienced utilities can be reported instantly or in retrospect (as remembered utilities), but of course not prospectively. This creates a possible ontological contrast with values, and this contrast may explain differences between the four types of situation. If — as Kahneman’s title “Back to Bentham” suggests — we conceive of experienced utilities as especially relevant, there is a sense in which the future tugs us. We need to uncover these eventually experienced utilities in order to get hold of that kind of value.

In point of fact, if we look at the issue from the epistemic perspective, it becomes less certain that we will always have a convergence or increased stability in our perceptions of values of the kind that should make us expect — increasingly, with increasing knowledge and experience — a reduction to Type 1. Hedonic adaptation should illustrate the possibility of this situation as well. Certain kinds of experience bring with them an adaptation of our values and interests which makes them less stable than they were before we had those experiences.

One could argue that, in time, new discoveries and knowledge of a more robust, context-independent type are added to our body of beliefs, making them more useful and certain. This is certainly one reason why we invest so much in science. We are pushed into the future by the promise to make our sciences more informative and evidence-based. In fact, going for “more data” was long the favoured road to certainty in risk analysis. The acquisition of more data does not always facilitate a risk analysis, though, as the risk picture may be complicated by new knowledge. One tosses a coin, say. Knowledge that the coin is not biased and that the probability of tails is one half is welcome, since such data reduce uncertainty in this situation. In more complex areas, like the possible identity of hESC and iPSC, setting up experiments in order to reduce uncertainty about differentiating features may give rise to new questions regarding some other aspect of their identity. This can upset the quality of knowledge (and the quality of values) involved in the question whether or not

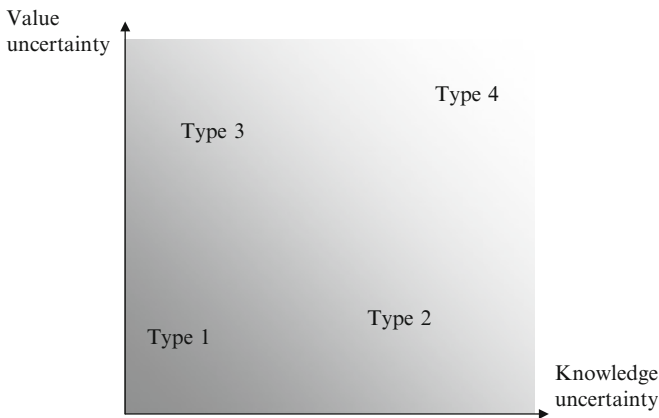


Fig. 30.1 The four types of decision situation

hESC and iPSC are identical. So we may start in a Type 2 situation and end up, as the result of an experiment to reduce uncertainty, in a Type 3 or Type 4 situation.

It seems that, in risk analysis, rather than being confronted with Type 1 decisions, the risk assessor is lost in the outer regions of the diagram in Fig. 30.1. If this is so, profound consequences for risk analysis as a decision procedure follow. In the risk assessment our ignorance needs to be mapped together with the knowledge we have. The dynamic nature of risk assessment and risk management must be emphasised even more [13]. The risk analyst needs to be like Socrates on his toes.

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Chapter 31

Looking at the Future of Translational Stem Cell Research and Stem Cell-based Therapeutic Applications: Priority Setting and Social Justice

Göran Hermerén

Abstract Two kinds of priority setting problems are discussed in this chapter: setting research priorities and setting priorities in health care. They are not unrelated, but the criteria are not the same. Focus is then, as the title suggests, on setting priorities in health care. The discussion is based in the assumption that in the future there will be stem cell based treatments of many current disorders, and we may have to choose within them or between them and other treatments, since resources are scarce. I argue that new standards are not needed for such problems; current criteria and standards can be used. Several problems in applying them are related to lack of clarity of distinctions, such as the one between vertical and horizontal priority setting, differences between different health care systems and the value premises used as starting point.

Taking a human rights-based approach as a point of departure, some relevant dimensions are outlined and incorporated into a model – to be used in the practical handling of priority setting problems. Problems of uncertainties and knowledge gaps are highlighted and their relevance to priority setting is explored. A take home message is that some of the problems in applying this model are not related to the value premises or dimensions of the model, but to the lack of knowledge of efficacy, costs, prevalence, patient needs etc. Cases of increasing difficulty are distinguished and illustrated. Fair access is not only a problem from a global point of view, it is also a problem in many countries, since wealth is unevenly distributed. Social justice aspects – discussed in the recent ISSCR guidelines – conclude this chapter.

Keywords Access • Priority-setting • Relevant dimensions • Social justice • Stem cell research and therapy

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31.1 Introduction

In the year 2030 at 11:00 p.m., a person suffering from severe life-threatening cardiac problems is rushed to the university hospital in Middletown, USA. Five years ago, he donated some of his somatic cells and \$2,000,000 to the hospital. The cells would be reprogrammed to a pluripotent state, cultivated and preserved, in case he would need them for acute treatment in the future.

At the very same moment, in a small province in Africa without university hospital and health care insurance, 60 children and women die from dehydration, starvation, poverty, and lack of clean water. Forty people in villages nearby die from malaria and undiagnosed problems. Neither treatment nor qualified medical staff are available here in this part of what once was Zimbabwe.

Is this futuristic scenario completely unrealistic? Obviously, this is hard to tell. But if at all plausible, it does raise some questions about social justice and priority setting, relevant to consider for the future of translational stem cell research and stem-cell based therapeutic applications.

31.2 Setting Research Priorities

Stem cell (SC) based therapies raise priority setting problems both in health care and in allocating resources for research. These two priority setting problems are not unrelated but they need to be distinguished. Since SC research is a hot area, attracting research money from many different sources, discussion and analysis of principles and criteria for setting priorities in research is highly relevant but not much discussed or studied.

Many research councils funding basic research provide instructions to their reviewers about what criteria to use. The information from the Swedish Research Council [1] illustrates the sort of questions the referees and priority setters are to consider, in addition to feasibility and project management:

Is the project original or groundbreaking? Does it challenge prevalent opinions or practice? Does the project include an innovative hypothesis or seek to remove key barriers to further progress in the research field concerned? Does the project entail new ideas, approaches or interpretations? If the aims of the project are attained, will it substantially enrich knowledge in the field concerned or our understanding of health and ill-health?

These guidelines and criteria merit further analysis. There is a certain liberty in interpreting and implementing them. From a historical perspective this becomes obvious. Studies in the history of research demonstrate that there are also fashions and fads in the world of science when it comes to setting priorities.

In order to rank applied research projects other questions also need to be answered, such as What are the chances for success? Are there any risks of adverse effects? Many important applications of the research results? How far ahead are

they? When are clinical or industrial applications to be expected? How much will they cost? How will they be financed?

One of the contemporary problems in European research funding is that, for understandable reasons, there is focus on funding research that is likely to promote European competitiveness, health and well-being of European citizens, providing job opportunities and promoting industrial development. For stem cell researchers, this means that in order to get funded, they have to promise something, some diagnostic or therapeutic outcome, even if the importance of their research may be to contribute to basic biological knowledge, to better understanding of processes of cell division, the differentiation processes, the role of epigenetics, etc. This invites hype in the applications that also raises ethical issues.

To sum up so far: The focus in basic research is on new methods, new knowledge, and on advancing understanding. But the balance between basic research and applied research, criteria of scientific excellence, likelihood of success and patient needs as well as ethical aspects of hype in project applications are some relevant topics. However, a sharp distinction between basic and applied medical research is not always easy to maintain. In order to be able to develop successful therapies, it is necessary to understand the causes of the diseases in question. This may require basic research at the molecular level.

An interesting and telling case story is the controversy over the funding of research at the NIH, described in some detail in [2]. In basic research there is always uncertainty about the result of the research – if this was not the case, it would simply not be basic research. That adds to the difficulties of those who rank project applications. Sometimes they rely on the earlier demonstrated merits of the researchers, sometimes on the merits of the application, often on a combination. The problems they face are different from those facing priority setting problems in health care.

A full discussion of the interpretation, weighing and ranking of the criteria hinted at above would require much more than a whole separate chapter. But since the focus of this book is on translational stem cell research and thereby also on future clinical applications, the problem of setting priorities in health care will now be addressed.

31.3 Setting Priorities in Health Care

The second problem is much discussed and studied, but so far not much within the context of stem cell based therapeutic applications. Major causes of this problem include increase of costs, aging population and increasing population as well as the lack of alternatives. If there is only one available treatment for a certain disease, the question arises of whether it will be offered or not, and to whom. If two treatments are available, which one, if any, shall be offered, and to whom? The general question here then boils down to: if and when competitive cost-effective SC-based treatments are available, to whom should they be offered? And on what grounds? If the answer

is “equitably and fairly,” this raises the further question: What are the criteria of equitable and fair distribution? Suppose the answer to this second question is “distributed according to need.” Then this raises further questions such as “Whose needs?” and “What kind of needs and what about costs?”

Distinctions and definitions are not always ethically neutral. For example, the notion of health care needs is not quite clear. There are borderline areas between needs of different kinds. Suppose a watchmaker and a dust collector both have eye problems and need cataract treatment. Let us suppose that objectively speaking, their vision is equally bad or has deteriorated equally. But the watchmaker is more dependent on his eyesight than the dust collector in order to earn his living. Are the health care needs of the watchmaker more acute than those of the other person, so he should be given preferential treatment, or do we take into account social needs as well as health needs, if we give preferential treatment to the watch maker?

The problem can be given a more general and perhaps even global twist by considering attempts to draw a clear line between catering for more basic health needs and for other kinds of needs. Where does, for instance, surgery on teenagers with obesity problems belong? In cultures where it is essential to be thin?

Priority-setting problems in the stem cell area are by and large hypothetical in the sense that we do not yet have stem cell-based treatments for many diseases, even if there have been successes in the use of stem cell-based treatment of hematological diseases, severe skin burns and some other conditions. But the frontlines are rapidly changing, important work is being carried out on different disease models, as is evident from other chapters in this book, and progress is also being made with other diseases, such as Parkinson’s, diabetes and others.

Bridging the gap from bench to bedside raises many problems. It is difficult to clarify the roadmap to the clinic in advance, and to agree on who defines the steps. Studies in the history of medicine – for example, the discoveries of insulin- or penicillin [2] before clinical trials are possible – suggest that current regulations would have been violated by many innovative breakthroughs in the past. These innovations have been tried by daring experimenters on themselves or as innovative treatments on patients in the sense explained by the ISSCR guidelines [3], when there was no other possibility.

31.4 Two Perspectives

Two perspectives need to be distinguished here. The first is the perspective of those who allocate resources to different health care areas (clinics, specialties, etc.), relying on statistics and other information about the current disease panorama, waiting times, availability of effective treatments, information about costs, etc. Here the approach is impersonal; transparency and fairness are the guiding principles.

The other is the perspective of the doctor who is sitting face to face with a patient, and has to take into account statistical evidence, if available, about cost-effective treatments, and apply that to the individual case. The patient in front

of him or her may be the exception. The patient may also have several diagnoses at the same time, which, combined with a lack of rationality documented by empirical research, complicates the application of the statistics. The approach has to be personal and empathic [4, 5].

The situations are not independent of each other. The decisions made at the impersonal level – viewing the problems from a distance – define and limit the area of possible interventions at the personal level, when the physician is being closely confronted with the problem.

31.5 New and Different Standards?

Do we need new and different standards for setting priorities in stem cell research and the distribution and use of stem cell-based therapeutic applications? In my view, the answer is *no*. There is no reason to introduce different standards and new criteria when stem cell research and stem cell-based therapeutic applications are discussed. The usual standards and procedures could be used, but the problem is that these standards are elusive and difficult to get a firm grip on.

Vertical priority-setting problems arise within a specialty, when different treatment alternatives for a particular condition are ranked according to patient benefits, cost-effectiveness and other criteria to be discussed below. Horizontal priority setting problems – which are more difficult – arise when decision makers have to compare and set priorities between health care interventions in different medical fields (oncology, pediatrics, geriatrics, etc.).

The distinction between “vertical” and “horizontal” priority setting is often used and rarely questioned in the debate on priority setting. But it is not as clear as it appears at first sight. First, it is relative to the notion of a “specialty,” and the emergence of a specialty has to be understood in a historical context, where forceful personalities, scientific breakthroughs, skilful marketing and administrative structures play a role and give the notion somewhat fuzzy edges. Besides, if specialties are related to organs, and organs, such as the heart, lungs and brain are interdependent, the notion of “vertical” becomes less clear; we cannot exclude “diagonal” connections in addition to the vertical and horizontal ones.

In spite of the difficulties, there is an analogy to such distinctions in priority setting in research. There is a difference between comparing and ranking research proposals within a certain area – say history, physics or stem cell research – and ranking projects between these areas. It is difficult enough to compare the merits of different research proposals in one area, but even more difficult to compare and rank research proposals from several different disciplines.

Priority-setting decisions are not ethically neutral. Some patients will get what they want and need, others not, or at least not immediately. The normative starting points, as well as trust in the system and legitimacy of the decision makers, are essential aspects of the problems, especially in health care systems largely financed by taxpayers.

31.6 Priority-Setting and the Health Care Systems

Priority-setting does not take place in a vacuum – it is always done in a certain historical situation and in a given health care system. However, praxis varies and there is no universal agreement about criteria and procedures. There are important differences between health care systems, cultural and social traditions in various parts of the world, as well as differences concerning what is – and what is not – covered by health care insurance, and so forth. This creates a variety of problems in different countries for both financing health care and for allocation of health care resources, which can be illustrated by comparing the situation in, for instance, Germany, the UK and the USA.

In Germany there is a complex system of health care insurance. There is the so-called “Gesetzliche Krankenversicherung” (statutory health care insurance) with macro- and micro-allocation on several levels, described in some detail by Fleischhauer in [6]. There is also private health care insurance, “Private Krankenversicherung,” as well as a mandatory social care insurance, “Soziale Pflegeversicherung.” Each of these ways of financing health care services gives rise to partly separate problems, and also to various problems of allocating the available resources. Several reforms of the system have been proposed, discussed by Fleischhauer.¹

The National Health Service (NHS) in the UK was created with the idea that everyone should get everything they need in health care for free. But it soon turned out that this idea was unrealistic. The costs have increased. The NHS was designed for a population much smaller than it is today, and the average life span of the population was shorter in those days. As a result, the disease panorama has changed, with increases in chronic diseases and demented elderly as some of the conspicuous consequences. This has created serious problems for the NHS. Several reforms have been proposed over the years, including the Private Public Partnership initiative. But in spite of numerous reforms, the politicians have not succeeded in containing the costs and eliminating the deficits.² The problems facing the system in the UK are not identical with those in Germany.

In the USA, there is a basic distinction between the private and public health care sector. In the private sector, there private group insurance as well as private individual insurance. Those who are not covered by any insurance will have to pay out of their own pockets. In the public sector, there is Medicare, Medicaid, the State Children’s Health Insurance program, as well as several other state programs (for details, see [6]). Millions of people are not covered by any health care insurance, and many more are insufficiently insured. This is obviously a big problem ethically, politically and economically.

¹See [6], pp. 52 ff.

²[6], p. 116.

31.7 General Normative Points of Departure

Since there is no general agreement about principles, procedures or practice in Europe, let alone globally, I will venture to propose a model, using explicit value-premises, based on key international human rights documents. The model can be applied to the priority setting problems, which will arise, if and when stem cell based therapies competing with others are available.

In many of these documents, for example the European Convention for the Protection of Human Rights [7] and the Oviedo Convention [8], “human dignity” is taken as a basic principle which is the basis for a number of rights, liberties and obligations – enabling and protecting the citizens. There is considerable literature on the precise interpretation of these various basic notions (for instance, Ashcroft [9], Brownsword and Beyleveld [10]), and also some criticism of their vagueness and ambiguity, for instance, in Macklin [11].

Sometimes it is easier to specify what these principles and rights forbid, or what would constitute a violation of them, than to define their positive meaning, and what they positively allow. But the advantage of using these starting points is that many of them have been discussed, amended and approved in political assemblies, so from transparency and democratic legitimacy point of view, they have some advantage over declarations, which express the views of a particular profession.

Thus, I will propose a human rights-based approach, without offering a philosophical defense or justification of the basis of these human rights here. This would require more space than is available – see, for instance, Gewirth in [12]. But I chose this approach for the pragmatic reasons suggested by Norman Daniels in [13].

In some priority-setting systems, like the one in Sweden, human dignity is interpreted in terms of human rights, as suggesting that everybody has the same human rights and the same right as everyone else to have their rights respected. This is then applied in the following way: it essentially forbids certain forms of stigmatization and discrimination, due to age, gender, ethnicity, race, occupation, or financial situation, as well as eugenics, trafficking and degrading treatments.

If various treatment options do not violate this basic principle, the next step is to go on and distribute available health care resources fairly according to health care needs – rather than according to demand, or social or economic status. Also the goals of medicine are relevant as a guide at this point.

Finally, the costs have to be related to the effects of the treatment. The easy situation is when the treatment of an individual has no effect. Then that treatment is not needed and should not be given. The difficult cases are when the treatment has effect but is very costly, especially if treatment with this costly medicine is required for a long time. That might very well apply also to some stem cell-based treatments in the future.

If the cases are rare, the cost will jeopardize the budget of the particular clinic or hospital. Then a solution might be to argue that money has to be provided by the region or the whole country. Rare cases occur, sometimes here, sometimes there,

and in the name of solidarity money is set aside to help these people from the larger area. But the situation is even more difficult, if these cases become more and more common, as is the case nowadays with some expensive treatments for certain forms of cancer.

31.8 Some Relevant Variables or Dimensions

Medicine is not just a set of techniques and methods, but techniques and methods used to achieve certain goals. The goal of medicine is thus one obvious starting point for the discussion, supplemented by the human rights documents and other declarations referred to earlier. The challenge is to break down these rights, underlying values and principles to a number of variables or dimensions, which can be operationalized and applied to the more or less hypothetical future situation, when SC based therapies have to be compared to others.

I will use the five-dimensional model I suggested elsewhere [14] for priority setting problems in health care generally, in the hope that in the future it will be more widely used also in the discussion involving choices of stem cell-based therapies.

Let us assume that the goals of medicine include combating disease, providing relief from suffering, and restoring, maintaining and improving health and quality of life. Some of the basic goals of medicine remain constant, whereas others – related to, and depending on, the economical and technical level of society – change.³

When this happens, the following dimensions or variables are relevant:

Seriousness. The seriousness or severity of the condition. Conditions that are life-threatening without intervention should be given priority.

Effects 1. The positive effect (“likely benefits”) of the intervention on the health and/or quality of life of the patient or patient group in question.

Effects 2. The negative effects to the patient (“possible harm”) including the secondary risks to which others may be exposed.

Costs. The costs of the intervention in relation to Effects 1 and 2 – which, of course, need to be distinguished from the costs in relation to volume.

Volume. The prevalence – that is, how common the disease or the condition is.

Each of these variables can be graded and quantified, at least roughly. The seriousness of the condition can be very severe, severe, somewhat severe and mild or slight. Effects 1 and 2 can in an analogous way be very significant, significant, small or insignificant. In a similar way, the risks can be divided into four sub-groups, obviously without sharp boundaries.

The two kinds of effects can be combined into a larger variable, which may be labeled “patient benefit.” But starting with this combined variable is likely to hide some difficult problems of weighing (comparing, subtracting) positive and negative effects.

³See [2] for details, especially the conceptual framework in Chap. 7.

The danger is that the benefit/risk ratio is presented in a misleadingly objective fashion, especially in cases where underlying relative frequencies are unknown or uncertain.

A complication is suggested by combining the distinction between “positive effects” and “negative effects” with the distinction between “certainty” and “uncertainty.” This suggests four different combinations: certainty about positive effects as well as about negative effects, certainty about positive effects but uncertainty about negative effects, and so on. The first combination is the easiest one to handle in a satisfactory way; the last is the most difficult. Thus there are many unknowns here.

What is known, however, is that public perceptions of risk vary; culture, ethnicity, gender, age and other factors play a role, as many studies by psychologists indicate (Paul Slovic, Melissa Finucane and others [15, 16]). Some people are risk-avoiders, while others are risk-seekers, or at least more willing to accept risks of unpredictable negative side effects in return for possible benefits. This willingness may also change with age, gender and with disease and its development. Is the disease progressing? Are there no alternative treatments? The question is what role we will give to evidence of this kind. Should it influence the way we set priorities in health care? If so, in what way?

Desperate patients may be willing to try anything if they suffer from an incurable disease and they are getting weaker. But the doctor has to believe that there is at least a slight chance to succeed, as the earlier mentioned ISSCR guidelines make clear. There is a moral problem here in that there can be clashes between what benefits the patient and patient autonomy. On the one hand, in the name of freedom, people may be inclined to allow patients in dialogue with their doctors to make their decisions on what risks they are prepared to take in return for some possible future benefits. On the other hand, this presupposes reasonably high moral standards of the doctors, so they don’t take advantage of and exploit the person who is desperate to try experiments that are not justified in the sense that there is no reasonable hope of success.

Besides, other distinctions need to be made. Suppose we combine affirmative and negative answers to the two questions: (1) Are the relative frequencies known? (2) Are the values stable? We can obtain four cases, where the first is the easiest one (the values are stable, and the relative frequencies are known) and the last is the most difficult.

A final and very important question is, of course positive – or negative – effects for whom? The patient? His or her family? The health care staff? The hospital? The taxpayers? Society at large? In other words, a lot of clarification is needed about benefits, harms, risks and chances before communication about these issues can be improved substantially.

31.9 Medical Tourism, Risks and Ethics

In a situation where there is increasing focus on patient’s rights and patient autonomy, this also raises the ethical issue of who should decide if and when certain risks are to be taken in the hope of possible treatment benefits – the patient, his or

her doctor, ethics committees, or the regulatory authorities. This is a question of power, a question of which values and whose values are most important, and thus an ethical question.

The existence of medical tourism for uncertain stem cell therapies – such possibilities are available also in Europe – shows that these issues are relevant to consider also in the present context. For example, the XCell-Center is a private clinic group and institute for regenerative medicine located in Düsseldorf and Cologne, Germany. On their web site they claim that since January 2007, more than 2,400 patients have safely undergone their various stem cell treatments.

At this point it becomes essential to distinguish between two kinds of medical tourism, one where patients travel to other countries to get what they also can get at home, but they get it better or more cheaply abroad. This sort of tourism is less problematic and clearly within the ideas and ideals of the EU, even though some disagreement may remain as to who should pay. The other form of medical tourism is when patients travel to other countries to get they what they cannot get at home because the resources do not exist, because it is forbidden by law, or because it is illegal or considered immoral. Fertility treatment, for example, will not be offered in Germany at present to patients who are over 65.

31.10 Problems of Uncertainties and Knowledge Gaps

An important complication that can hardly be overstressed is that the available evidence required for rational priority-setting, both in health care and in medical research, is usually very fragmentary and uncertain.

The evidence for the seriousness of a condition is different from the evidence for the effects of treatments or preventions. In the first case, statistics on mortality and morbidity, life expectancy, health and quality of life will play a central role. Panels or focus groups could be used to elucidate these problems. The evidence for the effects of treatments and preventive measures will involve causal hypotheses, tested in various types of clinical studies.

The golden standard, according to the traditional view, is randomized, double-blind clinical trials. But incidentally, this golden standard may for ethical reasons be difficult to apply to tests of new stem cell-based treatments for diseases like Parkinson's, when there already is a treatment available with some documented effect. There has to be genuine uncertainty about whether the new stem cell based treatment is better than the best available alternative. But the precise interpretation of this "equipoise"-requirement (whose uncertainty?) has been much debated [17], and so has "the best available" and "best proven" requirements (available where? proven how?) [18].

At any rate, the five dimensions or variables – as mentioned above – can be measured in different ways. This fact does not necessarily indicate that the methods used do measure the same thing or have a reasonable degree of validity, precision, stability and reliability. In most cases, it is an open question whether or not this is the case. Here there is room for further theoretical analysis and methodological development.

The knowledge gaps and the shortcomings in the available scientific information – obvious to anyone who has worked through problems of this kind – suggest that any hope one might have to be able to quantify these variables in an exact way will turn out to be an illusion. It is already quite an achievement if it will be possible to place the interventions compared in one of the four subgroups mentioned earlier under each relevant variable.

How does one compare treatments offered for Parkinson's disease? There is no once-and-for-all valid answer. Not long ago this disease was widely regarded as incurable, a condition about which not much could be done. Great advances have recently been made. The answer to the question above will depend on the alternatives available, whether the treatments are established or innovative, and on the other variables mentioned above, including cost in relation to effect, benefits and risks, etc. The picture is thus dynamic, changing over time, and not static.

31.11 Cases of Increasing Difficulty

If we assume that we can use “same” and “different” to classify treatments, diseases and health care systems, there are theoretically eight different combinations. Already this is a simplification in the sense that the options admit of degrees: treatments, diseases, and health care systems can be more or less different, and may differ in various respects. Treatments can also be more or less effective and the same disease more or less severe. But all of these possible combinations are not equally interesting. I will comment on four of them, which vary in complexity and difficulty.

The first case is represented by the challenge to compare different treatment options for the same disease in the same health care system. This is a classical example of what has been called horizontal priority setting. Let us consider stem cell-based therapies compared to pharmacological or surgical treatments of Parkinson's disease, using the variables suggested earlier. Then the variations in two of the four variables (severity, volume) are limited.

But there will be some variations in these variables, since stem cell-based treatments will not be used in all cases, only in chronic and/or severe ones. Besides, if stem cell-based treatments include transplantation of stem cell lines into the brain, they are not without risk. The differences are more considerable in the other cases. The variations in all four variables are then likely to be more conspicuous. Nevertheless, it is difficult enough, given the uncertainties, to compare the treatment options according to the two remaining dimensions or variables, but relatively easier than in the next steps.

A second case is comparing different treatment options for the same disease in different health care systems – for example, those of Sweden, Germany and Portugal. In an EU perspective – which is why I have chosen this example – medical tourism is becoming a growing problem, since one of the basic ideas of the EU is the free movement of goods and services. Against that background it becomes interesting and relevant for patients seeking treatment for a particular disease to compare different treatment options for the same disease in different health care systems.

If the systems are different enough, like those of Germany and Sweden, such comparisons become very difficult.

In the third case, different treatment options for different diseases are compared in the same health care system, such as NHS or the German system, for instance, pharmacological and surgical treatment of Parkinson's disease with (future) stem cell-based and pharmacological treatment of Huntington's disease. This is a straightforward example of one type of vertical priority setting problem within regenerative medicine. Then all four variables have to be checked.

The remaining uncertainties and knowledge gaps – at least at present – makes a non-arbitrary comparison very difficult. The reason is that intersubjective comparisons are presupposed, and all methods used so far (based on QALYs, DALYs, etc.) have been criticized for bias. So one has to be reasonable and realize that only very rough indications are possible. Thus one must be prepared for changes, if and when new evidence becomes available.

What does it mean to be reasonable in this context? One aspect is not to expect precision and exactness when it is not possible. Another aspect has been stressed by Norman Daniels who has argued convincingly that accountability for reasonableness,⁴ as well as the account he offers of fair, deliberate process,⁵ strengthens a human rights approach to health:

“It specifies the conditions under which negotiations among rights proponents, various stakeholders, and governments about progressive realization should be carried out. It provides a coherent rationale for those conditions. Accordingly, it adds legitimacy to the priority setting that must go on as the details of progressive realization are worked out.”

The fourth and most difficult case is that of comparing different treatments for different conditions/diseases in different health care systems. As has been shown, for example, by Kurt Fleischhauer's already-mentioned detailed comparisons of priority-setting in the USA, British and German health care systems, generalizations are extremely difficult. These health care systems differ in so many important ways – in financing, what is offered, how the gate-keeping systems work, what is covered by health care insurance, the extent of private care, etc.

Concluding his analysis of the financing and allocation of resources in the health care systems of Germany, the UK and the USA, Fleischhauer points out and exemplifies that there are big differences between them.⁶ Nevertheless there are also some important similarities. Due to new diagnostic methods, the relations between patients and doctors have changed in all systems. The costs of health care are rising in all countries, and all countries face difficult problems in financing their health care systems. But if the details of the gate-keeping system, the possibility of choosing your doctor, the role of commercialization, and so forth, are studied, many differences are related to the structures of the three systems.

⁴[13], p. 315.

⁵Op. Cit., Chap. 4.

⁶[6], p. 115.

An important complication is that the values of the dimensions can vary independently of each other. For one disease or intervention, the seriousness can be considerable, the risks small, the costs in relation to effects significant, and the volume small. For another disease or intervention, the picture might look rather different.

31.12 A Diagram

To illustrate the model, let us look at some concrete examples, although there are variations within each group, depending on the development of the conditions. I have chosen to compare cataract, arthritis of the hip, leukemia in children and early indications of changes in the walls of the aorta that, if untreated, would lead to aneurysm and abdominal aorta rupture (Fig. 31.1).

There are both difficult and easy cases. Unfortunately the easy cases are rare. An easy case would be if decision makers have to compare two alternatives, A and B, where A is a very serious disease, for which an effective treatment exists, and this treatment costs little in relation to effect, and the volume of the disease is small, and where B is a disease that is not very serious but for which a treatment exists with little effect, and where the cost is high in relation to the effect, and the volume of the disease is high.

The diagram is based on the simplified assumptions that cataracts are not very serious, the effects of interventions are very significant, the risk is low and the volume very significant, whereas, for example leukemia in children is very serious if untreated, the effects of interventions are significant, the risk is moderate, and the volume is small. I am aware of the fact that there are several different forms of

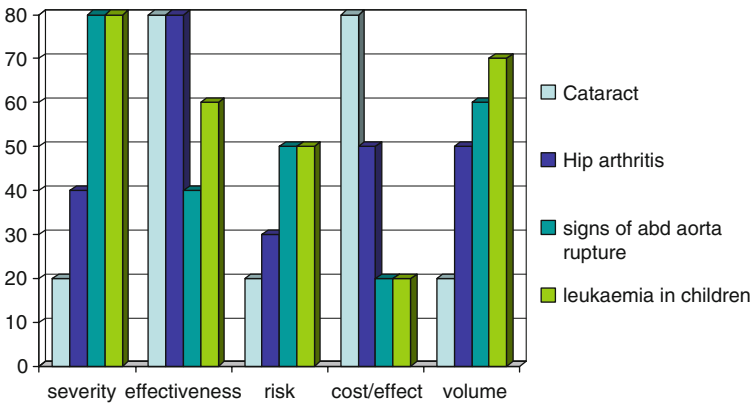


Fig. 31.1 The diagram compares four conditions with respect to severity of the condition, the effectiveness (possible benefits) of current treatments, the risks of these treatment for patients (potential harm), the costs in relation to effect as well as the volume of the various conditions

leukemia, and different treatments with varying price tags, so what I am saying is a deliberate simplification in order to drive home the point that values play a role, whatever the form of leukemia we compare and regardless of the actual price tags on the various treatments.

If there are fixed prices on cataract and hip replacement operations, this would obviously simplify the discussion. But if no reliable information about costs can be found, I will argue purely hypothetically: If the costs of a certain form of leukemia are much higher than, say, screening for aneurysms in the abdominal aorta, then.... In a sense, I do not want to say anything here about health economy, I want to call attention to the problem when the variables pull in different directions. If treatment for arthritis of the hip is more costly than for cataract, but cataract is more widespread and the treatment is more effective, who should be given preferential treatment – the person who needs a cataract operation or the person who needs a hip replacement?

It is thus a question about underlying values, not a question about health economy. These values may need to be ranked in importance, if all of them are accepted in our culture [19]. Different hypothetical questions can be asked given other assumptions, and we rely on the health economists to provide us with data – although they sometimes tend to exclude costs that are relevant but difficult to measure – for instance, the costs of care provided by family members of ALS patients.

The information condensed in the above diagram is based on interviews with doctors. But if possible I would like to avoid getting stuck in a debate concerning the details. I am aware of the differences between diseases in different phases and also of the existence between different individuals, due to age, and other diagnoses or diseases patients may have at the same time. The point of the diagram is something else; it illustrates the many difficult cases where the values of the variables point in different directions, and that a more holistic judgment then has to be made, both at the impersonal level – when types of interventions are compared – and at the personal level when the doctor is face to face with the patient.

31.13 More on the Role of Values and Norms

Thus for each intervention we have to try to achieve a more holistic assessment, balancing the values of the various variables against each other. The picture is also relative to time and can change quickly, depending on the existence of adequate infrastructure, skilled staff and the extent to which successful attempts to scale up the treatments have succeeded.

In a situation loaded with such difficulties, underlying values and norms will play a considerable role. Which view do we have of the mission and goals of health care? Should need – restricted to health and quality of life-related needs – or demands of patients or patient organizations be the basis for distribution of health care resources? Is health care a commodity, which can be bought or sold in the same way as sugar or salt, or rather a human right that citizens in a welfare society are entitled to – if and when they need it?

In such a situation, basic ethical principles can be of some help, even if it would be premature to propose or assume that every priority setting problem that will arise can be solved in an easy and elegant way.

If, then, the dimensions are ranked according to the principles of the Swedish governmental priority setting commission [20], the result would most likely be that the top candidates would be interventions to treat abdominal aorta rupture and leukemia in children. This is then based on the assumption that the severity of the condition and the effects of the treatment are more important to consider than costs in relation to effect and volume. This kind of reasoning should be applicable also to priority-setting involving stem cell-based treatments in the future.

31.14 Social Justice Aspects

The discussion of priority setting problems should be viewed in the wider context of theoretical, practical, empirical and normative controversies over social justice, recently stimulated in particular by the works of John Rawls [21] and Alan Gewirth and their collaborators and followers, such as Norman Daniels. This aspect has also been stressed, for example, in several recent EGE reports [22]. Problems of social justice have recently been discussed in the context of stem cell research in the earlier mentioned International Society for Stem Cell Research (ISSCR) guidelines. I will use that discussion as one of my starting points for the second half of this chapter.

As pointed out in these guidelines, there are additional reasons to consider justice in the context of stem cell translational research. The anticipated benefits have to be genuinely and justly available. There is a potential to develop therapies that could be widely shared internationally. Choosing which application to address (or select) for clinical development, and how, will then necessarily require attention to issues of social justice.

Public engagement and participation in debates over access, fairness, patient selection, research priorities and so on are essential for transparency and public trust. The community of researchers have an obligation to take concerns raised by patient organizations and other groups in civil society seriously and get involved in these debates.

In order to maximize public good, the ISSCR guidelines recommends – Recommendation 38 – that stem cell collections with diverse sources of cell lines should be established, that collaboration among researchers should be structured to maximize the fairness of the parties' roles, and increasing joint capacity and social benefit. Similar recommendations have been proposed by the EGE concerning, for example, stem cell and cord blood banks.

Fair access is important, but difficult – in view of the differences between the health care systems in the world, and what is often referred to as the 10/90 problem [23], roughly 90% of the world's health care research resources are focused on meeting the health care needs of roughly 10% of the world's population. It is now not only a question of how much money is spent, but also of what you get for the

money – and then the even greater disadvantage than the 90/10 proportions would indicate. In a global perspective, the access problems are pressing. It is not likely that the problems will diminish if and when stem cell-based therapeutic applications are considered; on the contrary, they may increase.

Will stem cell-based therapies be available more or less only for the already rich in the rich parts of the world, or will they be accessible on the basis of need globally? It is too early to try to answer this question now. It is common that new therapies start on a small scale, are quite expensive and thus at the outset limited to a few. But if scaling up is possible, and the benefits considerable, this therapy is likely to become available to more and more people.

Fair access is not only a problem from a global point of view, it is also a problem in many countries, since wealth is unevenly distributed, and in some countries millions are not covered by health care insurance. Moreover, access to specialists and competent general practitioners vary, as well as the difficulties of diagnosis; Parkinson's disease, for instance, is more difficult to diagnose early and correctly than Huntington's disease.

In the ISSCR guidelines it is stressed (recommendation 38c) that access will depend on financial terms and business models that are perceived as fair by all stakeholders. These stakeholders include patients, providers, payers, companies and governments. The problem is that all these stakeholders rarely perceive the same models as fair; there are always minorities – and they are considerable in a country like the USA – that want a change and do not consider the present arrangement as fair. The recent controversies over proposed reforms of the health care system in the USA illustrate some of these difficulties.

A particular problem, dealt with in previous chapters of this book, which is also relevant for issues of social justice, include models of intellectual property, licensing, product development, and public funding. They can drive up costs and block fair access. But they can also be developed to promote fair and broad access to stem-cell based diagnostics and therapies.

Against that background, broad public debate should be promoted, also to the extent to which alternative models need to be developed and assessed for patenting and other IPR issues discussed in earlier sections of this book.

31.15 Concluding Remark

If there is to be a bright future, to return to the title of this paper, for stem cell-based treatments, they have to be developed ideally for a disease from which many suffer, which is serious, and for which (1) there is currently no alternative treatment, or (2) the SC-based treatment is more cost-effective and less risky (having fewer adverse side effects) than other available alternative treatments.⁷

⁷I would like to thank Kurt Fleischhauer and Nils-Eric Sahlin for helpful comments on an earlier version of this chapter.

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