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Stem cells and regenerative medicine: scientific, political and social aspects

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INTRODUCTION

Stem cell research represents a possibility of cell and tissue renewal, damaged by serious diseases or accidents. There are countless possibilities for applying regenerative medicine using stem cells; damaged cartilage and bone replacement, acceleration of wound healing and skin renewal, heart muscle replacement after myocardial infarction and treatment of neurodegenerative disorders including Parkinson's, are only some of them. Next to personalized medicine and nanomedicine, regenerative medicine is one of the most propulsive areas of future medicine *(1, 2, 3).* Research in that field has started to develop in the last decade of last century and has intensified over the past 20 years. EU Commission has accepted in 2008 the proposition for the *Directive of the European Parliament* as well as *Council on Standards of quality and safety of human organs,* mainly with the purpose of transplantation. Nowadays, transplantation is the only possible therapy for certain terminal organ insufficiencies such as liver, kidney and bone marrow.

Increase in the number of chronic patients related to population aging has caused an increased need for transplants which seem to be fewer in number. In fact, there is a decrease in the number of potential donors. For many patients transplantation is the only possible way of treatment and can be lifesaving. Therefore, the need for a bigger number of transplantations and alternative forms of treatment is clear. It seems that regenerative medicine, or stem cell application, is the real alternative.

In 2001 at the EU parliament in Brussels, a meeting was held on the topic of „Stem cells: for the freedom of research in Europe", where one of the authors of this article was invited as an expert. The meeting was organized by a Transnational Radical Party, instigated by patients suffering from serious illnesses *(4).* The meeting discussed cloning and so called therapy cloning as well as the application of nuclear transfer method with the purpose of treatment. It took 8 years for Food and Drug Administration to approve the first clinical trial using therapy founded on applying embryonic stem cells for the treatment of patients suffering from acute bone marrow injuries. In „Human stem cell research and regenerative medicine", published by the European Science Foundation in 2010, it is noted that regenerative medicine probably has the highest chances in the renewal of bone and cartilage *(5).* A plausible explanation is reduced complexity and good accessibility of those tissues compared with myocardia and neuronal tissue. One should mention that there is large competition in the field of research and new tissue renewal biomaterials.

The development and fate of stem cell research is heavily influenced by the ethical surrounding in which that research is being conducted.

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TABLE 1

A glossary for stem cell biology. Adopted from EMBO Stem cell research: status, prospects, prerequisites and ref. 20.

Term	Definition
Stem cell	Cell that can continuously produce unaltered daughters and has the ability to produce daughter cells with different, more restricted, properties
Mesenchymal stem cell	An adult multipotent cell derived from a well-characterized population that can form fat cells, cartilage, bone, tendon and ligaments, muscle cells, skin cells and even nerve cells
Self-renewal	Cycles of divisions which repeatedly generate at least one daughter equivalent to the mother cell with latent capacity for differentiation. The defining property of stem cells
Totipotent	Self-sufficient to form an entire organism: capacity of zygote and of plant meristem cells, not demonstrated for any vertebrate stem cell
Pluripotent	All cell lineages of the body including germ cells plus some or even all extraembryonic cell types; example: embryonic stem cells
Multipotent	Multiple lineages that constitute an entire tissue or tissues; example: hematopoietic stem cells
Clonal analysis	Investigation of properties of single cells. Essential for formal demonstration of self-renewal and potency
Embryonic stem cell	Derived *in vitro* from pluripotent cells in the pre-gastrulation embryo
Tissue stem cell	Derived from, or resident in, a fetal or adult tissue, with potency limited to cells of that tissue. Sustain turnover and repair throughout life in some tissue
Progenitor cells	Generic term for any dividing cell with differentiation capacity. Includes putative stem cells in which self-renewal has not yet been demonstrated
Stem cell homeostasis	Persistence of tissue stem cell pool throughout life. Requires balancing symmetric self-renewal and differentiated divisions at the population level, or sustained asymmetric self-renewal
Regenerative medicine	Reconstruction of diseased or injured tissue by activation of endogenous cells or by cell transplantation
Cell replacement therapy	Reconstruction of tissue by functional incorporation of transplanted stem cell progeny. Distinct from „bystander“ trophic, anti-inflammatory, or immunomodulatory effects of introduced cells
In vitro stem cells	Self-renewal *ex vivo* in cells that do not overtly behave as stem cells *in vivo*. Occurs due to liberation from inductive commitment signals or by creation of a synthetic stem cell state
Reprogramming	Increase in potency. Occurs naturally in a regenerative organism („differentiation“). Induced experimentally in mammalian cells by nuclear transfer, cell fusion, or genetic manipulation
Plasticity	Notion that tissue stem cells may broaden potency in response to physiological demands

In 2002 European Science Foundation reported on stem cells, a harmonized approach of various national EU governments was suggested to find the ways of using adult stem cells for future therapy, which will be acceptable to all interested parties and to the general population *(6).*

Legislation across Europe is different. Twenty five countries have adopted legislation which explicitly prohibits human reproductive cloning (excluding Poland, Lithuania, Croatia, Ireland and Luxembourg). Seven countries allow human embryonic stem cell (hESC) research and the derivation of new hESC clones from supernumerary IVF embryos by low (Belgium, Sweden, UK, Spain, Finland, Czech Republic, Portugal). The same countries allow stem cell nuclear transfer by low except Finland, Czech Republic and Portugal who neither prohibit nor allow it. Three countries have adopted legislation to allow the creation of embryos for research purposes under strict conditions (Belgium, Sweden, UK). Seventeen countries allow the procurement of stem cells from supernumerary embryos. Six countries have not adopted legislation regarding human stem cell research (Bulgaria, Croatia, Cyprus, Luxembourg, Romania and Turkey). Differing legislation may lead to differences and imbalanced access in Europe to treatments deriving from hESC research. Patients may travel from one country to another where they may have access to a treatment that is forbidden in their own country leading to unequal distribution of benefits and burdens of stem cell research. Certainly, patients should not be denied treatment. The access to objective information regarding stem cell treatment should be made widely available for all European citizens.

TABLE 2

Comparison between embryonic and adult stem cells. Adopted from EMBO Stem cell research: status, prospects, prerequisites.

Embryonic stem cells	Adult stem cells
Are a ubiquitous component of the embryo with a well-understood function and life history	Are rare, often difficult to identify, of unknown origin, and partially understood function and life history
Are defined by position in the embryo (the inner cell mass of the blastocyst)	Are defined by a complex list of features such as cell-surface markers, behavior *in vitro*, and in some cases, position in the tissue
Can divide symmetrically indefinitely in culture without changing characteristics	Can divide few or many times (up to 200 or more) in culture, but not indefinitely
A single cell can give rise to a colony of genetically identical cells with the same properties as the original cell	In some tissues, absolutely consistent precursor cells can be identified and cultured; that remains to be shown for all tissues
Pluripotent: can give rise to all three tissue types of the embryo (endoderm, mesoderm, ectoderm)	Sometimes multipotent: most only give rise to their tissue of origin, but some may be able to give rise to different cell types within the larger groupings of endoderm, mesoderm and ectoderm (so-called plasticity)

Avoiding ethical (medical) tourism is extremely important *(5)*.

Naturally, investing into research brings substantial gains, as well as in the case of stem cell research. The National Institute of Health (NIH) has, in the period from 2005 to 2008, invested 3.5 billion $ for stem cell and regenerative medicine research, out of which 260 million $ was invested into human stem cell research. This resulted in a turnabout in stem cell research; reprogramming of adult stem cells into embryonic like stem cells, induced pluripotent stem cells (iPS). This discovery could not only push the boundaries of regenerative medicine, but could also solve all morally-ethical dilemmas over stem cell application *(7)*.

In December 2008 the International Society for Stem Cell Research (ISSCR) has published „Guidelines for the clinical translation of stem cells". The document is partially supported by the EUROCORES programme EuroSTELLS, financed within the FP6 framework. This document served as a basis for the before mentioned NIH publication *(5, 8)*.

ALTERNATIVE APPROACHES TO USING STEM CELLS

The term *therapeutic cloning* implies transfer of the somatic cell nucleus to an enucleated egg cell, followed by propagation of the hybrid cell to a cluster which is further directed towards development into specific tissue. This kind of therapy involves individually tailored embryonic stem cell therapy for cell or tissue replacement. In this manner, the problem of immunological compatibility is resolved, together with cell or tissue rejection, which is often the case with transplants (9).

Due to ethical problems connected with embryonic stem cells, adult stem cells were suggested as an alternative. They are multipotent, exist in many adult organs and can theoretically serve as a potential weapon for future therapy. Adult stem cells can be separated and, in some cases, *ex vivo* expanded. Furthermore, they can be transplanted back into the adult individual where they can differentiate and function normally. There are many known cases of successful conversion of adult stem cells, e.g. turning bone marrow cells into neurons or hepatocytes, liver stem cells into neurons or neurons into cardiomyocytes. However, there are some problems related to adult stem cells. Their expansion is challenging since it is difficult to culture them in large numbers. Most likely they are not pluripotent and they are present in organs in low levels. Moreover, it is still not known whether they are affected by the process of aging. Finally, if the donor carries a certain genetic disease, adult stem cells may have the same genetic effect *(6, 10, 11)*.

Another source for isolating progenitor cells without asking ethical questions connected with hESC research are amniotic stem cells. Amniotic fluid can serve as a new source for the isolation of cells with totipotent properties which can differentiate into various cell types. Amniotic stem cells could be used for the treatment of congenital defects, wherein only 2 ml of sample is needed to separate these cells in large enough numbers to construct material which contains fetal cells and could be used as an implantation graft directly after birth.

Fetal cord blood stem cells can be used as another source for therapy. There are two types of such cells that are interested for therapeutic purposes: cord blood stem cells and fetal brain tissue. If treatments based on cord blood stem cells became available, the issue of social justice and social equality with regards to personal cord

TABLE 3

Recommendations for the development of stem cell research.

In order for stem cell research and its development in Europe to have a chance of fulfilling its large potential for contribution to health, biological science and economy, several recommendations follow *(5).*
• Prospective value of stem cell research for science, economy and medicine needs to be publicly recognized. Human stem cell research must enter the core of biomedical research, including disease modelling, research on cellular degenerative processes, the development of pharmaceutics, toxicological screening and regenerative therapy.
• Adult and embryonic stem cell research, which is pronouncedly complementary, needs to fully be aided, which implies understanding the processes of reprogramming, nuclear transfer, cell fusion and other techniques.
• Communication and professional education on stem cell research and its application must be promoted with active participation of scientists and clinicians together with ethicists and patient groups' representatives. Intellectual ownership related to the usage of embryonic stem cells and cell lines after their derivation from embryos need to be patentable, in order to encourage industry participation in translational stem cell research in clinical application.
• Stem cell research and application regulations should be clarified and harmonized, while legislative impediments for free scientific collaboration should be removed.
• Regulative claims should not be too restrictive and they should not represent an unreasonable financial and bureaucratic obstacle for clinical application, as it is the case nowadays.
• Efficient criterions and standard procedures for clinical use of stem cells must be developed. In an established clinical practice efficacy measures need to be at least at the level of the same measures for other safety controls.
• Stem cell banks with high level of quality assurance, as well as international approach of scientists and industry, should be encouraged.
• Greater harmonization and mutual interactions of clinical research and their results throughout Europe should be encouraged with transboundary studies and cell therapy evaluation. Such initiative should be aided by public financing and be of use to all citizens.
• Technology development should be invested in to secure large scale processing of stem cells for applications in pharmaceutical screening, toxicological testing and cell transplantation.

blood banking would arise. Given that such banking is expensive, only a small proportion of the population may be able to buy access to a personal cord blood bank *(5, 11, 12).* Fetal brain tissue from aborted fetuses has been used in the treatment of Parkinson's disease.

In order to overcome possible ethical problems connected with stem cells, in 2003 it was suggested that a stem cell bank should be formed. British Medical Research Council funded this effort to store every kind of stem cell created in the UK, together with hundreds of others from different countries, with 9 billion pounds. The purpose is to supply researchers from all over the world with fetal, stem and adult stem cells *(11).* There is an increasing interest among private companies to create personal cell banks where parents can pay for their child's umbilical cord to be stored for potential future therapeutic use. It is far from clear whether treatments will be available based on stem cells derived from cord blood.

Shortage of egg cells is a setback for therapeutic cloning. As a possible solution to this problem, fusing animal enucleated stem cell with human cell nucleus, thus creating chimeric embryos, was suggested. The newly formed cells have around 99,9% human genes and 0,1% genes of a cow or rabbit (oocyte mitochondrial genes). This procedure was considered for the treatment of motor neuron diseases (turning stem cells into neurons) with an uncertain final outcome *(5, 13).*

UK Parliament gave permission for the Human Fertilization and Embryology Authority (HFEA) to create animal/human hybrid embryos using stem cell nuclear transfer. However, recent evidence suggests that animal/human hybrids do not express the genes required for pluripotency *(14).*

THE MORAL FRAMEWORK AND THE INITIATIVE FOR CHARACTERIZATION OF STEM CELLS

There are many issues that must be addressed concerning the use of human stem cells for research and for clinical application. Our moral framework is based on an imperative – to preserve and sustain life. The key question is whether it is permissible to prevent death and agony of children and adults by using cells separated from a fertilized egg cell. How else can the legal approach to abortion or our readiness to remove ectopic pregnancy be explained? Human embryos have a limited potential to become human beings. Most of them get lost before the menstrual period. Contraception methods that destroy embryos are used widely and there is a general public

consent for *in vitro* fertilization (IVF); only 10% of transferred IVF embryos produce a baby, while 40 000 are generated through treatments in the UK and cannot be transferred.

Human embryonic stem cells are separated from the embryo in the early phase of its development. They can be isolated from inner cell mass as well as from morula, blastomere. These cells have the potential to develop into any cell or tissue and thus make for a potential source for transplantation and tissue engineering. First hESC lines were established in 1988 *(15).* Since then, and up to year 2010, almost 700 cell lines were established in different countries. Legalization of usage of surplus egg cells made this possible. Many of these cell lines are registered in the European Human Embryonic Stem Cell Registry which is planned by the European commission. One of long term goals of the Human stem cell website is to establish a platform for comparing results of clinical research in Europe *(12, 13).*

One of laudable initiatives during the last years was the characterization of hESC lines. This project was led by International Stem Cell Forum (ICSF), an organization made of academies and research institute foundations from 21 member countries. The purpose of the project was to stimulate international collaboration and receive support for stem cell research. The initiative is known as Stem cell initiative 1 (ISCI 1). 58 hESC in 19 laboratories throughout the world were characterized. Results confirmed that those cell lines have similar expression profiles for several markers *(15),* although there are many differences between specific lines. The follow up ISCI 2 initiative is examining conditions for growth and culturing of those cells, while ISCI 3 is examining hESC genetic stability, considering that those cells are going through genetic alterations during long culturing *(8).*

CLINICAL APPLICATION: PROBLEMS AND THERAPEUTIC STRATEGY

There are several possibilities of applying stem cells in regenerative medicine: researching fundamental aspects of stem cell biology, identifying new potential drugs, with the most important application being cell based therapy for injuries, diseases or disorders. European Tissue and Cell Directive (EUTCD) regulates the quality of cells used in human therapy and it demands good manufacturing practice (GMP) for the production of those cells, which includes their quality. In relation to human stem cells, purity quality and product procedure is very strict. Difficulties arise when products contain animal components such as mouse *feeder* cells or fetal bovine serum. Cells can absorb animal proteins which are immunogenic and can promote cell reactivity after transplantation. Such substances can contain infective agents which are difficult to remove after they had been exogenously imported. Hitherto, several hESC lines of special purity have been derived.

According to the current clinical status, hematopoietic stem cells are often used for bone marrow transplantation, while adult stem cells are still in the phase of early clinical evaluation. Web sites European Community EudraCT (https://eudract.emea.europa.eu/) and US NIH (www.clinical trials.gov) give information over more than a hundred ongoing clinical tests based on usage of mesenchymal stem cells, a kind of adult stem cells which are soon expected to reach clinical use. Some of those trials are in phase III, therefore quite close to clinical exploitation. Considerably more of such research is in phase I and II, and is conducted in Europe. Main foci of the ongoing research are steroid-refractory graft-versus-host disease, periodontitis, heavy chronic myocardial ischemia, distal tibia fracture, osteoarthritis, multiple sclerosis, vascular diseases, diabetes, decompensated liver cirrhosis, fistulising Chron disease, etc. *(5).*

Before the treatment has started, it is necessary to choose the cell type and preparative culture. Various stem cells have their own specificities and advantages, as well as faults related to regulation and mechanism of action. The most plausible and easiest option for implementation is the use of autologous stem cells, as they can be derived from adipose tissue or bone marrow. However, those cells can have a different tissue renewal capacity which can be caused by patient's age, condition, nature of the disease, or a parallel medical treatment.

Another method of administrating autologous stem cells is generating iPS cells. There is, however, a potential risk of their inadequate „behavior", such as tumor formation, which should be seriously investigated before this therapy truly enters clinic *(7).*

On the other hand, use of heterologous stem cells allows for a logistically simplest method. Cells could be cultured and stored in banks, they could be significantly better characterized and even (genetically) modified before application. Yet, there is a risk of rejection *(4, 5).*

The choice of preparative cultivation of stem cells depends on the suggested application and on the type of cells which are being used. If adult stem cells were used, long term cultivation would not be necessary, and cells could be prepared and selected in the operation hall. Such method would be simpler, since the risk of transformation during culturing would be reduced/eliminated. Cells could be registered as a medical device, and not as Advanced Therapy Medicinal Products (ATPM). Conflictingly, if cultivation procedure would be utilized, a significantly more extensive cell characterization could be carried out before the application itself. Considering the chosen options, therapeutic index clinical outcome (efficacy/toxicity) would determine which approach is preferred in the specific clinical application.

One of the current main questions which bothers scientists working with human stem cells is therapy safety, including the question of epigenetic status and stability of cells. Thereat, the formation of teratoma after undifferentiated hESC/iSP transplantation is a critical question. Antiapoptotic gene surviving *(15)* plays a major role in the formation of tumorous tissue. This only illustrates the need for the development of efficient differentiation protocols based on generating fully differentiated and directed precursor cells. Moreover, there are several strategies available for avoiding undifferentiated hESC transplantation, such as sorting pluripotent cells using GMP-grade flow cytometry or using suicidal genes.

What is the current situation in stem cell research in Europe? In the last decade, stem cell research in Europe has been very intense and has become globally competitive. While media, on the one side, gave promises to the public, European scientists have seriously and responsibly approached the problem, making tremendous progress in fundamental research. With the advancement of research it became clear that tissue derived stem cells, hESC and iPS cells need to be studied in parallel. Well controlled clinical experiments were performed whenever it was convenient. With the growth of safety knowledge and different cell type functions, that area will finally come closer to its expectations. Legislation on safe use of stem cells is successfully implemented, situation with research funding is improved, and some questions connected to patients, previously unanswered, are finally addressed. There is hope that treatments for many serious illnesses could be soon implemented. Stem cells offer the possibility for revolutionary therapy in medicine. That challenge needs to go hand in hand with the ethical principles based on a mutual EU concept, although they might differ from country to country *(ESF Human Stem Cell Research and Regenerative Medicine: A European Perspective on Scientific, Ethical and Legal Issue) (5, 16, 17, 18, 19).*

In conclusion, any stem cell can be turned into any tissue given the appropriate conditions. More research has to be done before we understand whether there are restrictions on this process, whether it involves reprogramming that can lead to other unpredictable cellular behaviors and, finally, whether it even occurs at sufficiently high frequency to be clinically useful. Until then, there are no ethical and moral reasons to forbid embryonic stem cell therapeutic cloning.

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