Report

of the

Central Ethics Committee for Stem Cell Research (ZES)

Sixth Report after enactment of the Stem Cell Act (StZG)

Reporting period: 1 December 2007 to 30 November 2008

1. The Central Ethics Committee for Stem Cell Research

The Central Ethics Committee for Stem Cell Research (ZES) is an independent, interdisciplinary expert body that reviews and assesses applications for the import and use of human embryonic stem cells (hESCs). The activities of the Committee are governed by the Act ensuring the protection of embryos in conjunction with the import and use of human embryonic stem cells (*Stammzellgesetz* – StZG) of 28 June 2002 (BGBl. I p. 2277) (http://217.160.60.235/BGBL/bgbl1f/BGBl102042s 2277.pdf) (amended by the Act amending the Stem Cell Act of 14 August 2008 (BGBl. I p. 1708) (http://www.bgblportal.de/BGBL/bgbl1f/bgbl108s1708.pdf)) and the Regulations concerning the Central Ethics Committee for Stem Cell Research and the competent authority pursuant to the Stem Cell Act (*ZES-Verordnung – ZESV*) of 18 July 2002 (BGBl. I p. 2663) (http://217.160.60.235/BGBL/bgbl1f/ bgbl102s2663.pdf). ZES makes recommendations on the applications to the competent body pursuant to StZG, the Robert Koch Institute (RKI).

The Committee's 18 members and deputy members were appointed for the first time with the entry into force of StZG in 2002 for three years by the Federal Government. The third term of office began during the reporting period. Following the departure of one member, all members and deputies were reappointed and one new member was appointed. The members and deputy members of ZES perform their duties on a voluntary basis. Pursuant to § 8 StZG, ZES currently has two members from the field of biology, three members from the field of medicine and four members from the fields of philosophical, medical and theological ethics. A deputy was appointed for each member (Table 1). Both the members and deputy members participate in the deliberations on the applications pursuant to ZESV.

The task of ZES is to review applications for the import and use of human embryonic stem cells for their ethical acceptability pursuant to § 5 StZG and is specified in § 9 StZG. According to these provisions, an application must be accompanied by scientific substantiation that the research project serves research purposes of premium importance for the acquisition of scientific knowledge (§ 5 No. 1 StZG), that the scientific questions have been pre-examined in other systems including animal models (§ 5 No. 2a StZG) and that there are probably no alternatives to human embryonic stem cells in order to obtain the desired scientific knowledge (§ 5 No. 2b StZG). Both natural scientific and ethical aspects play an important role when reviewing and assessing the submitted applications. Based on four votes prepared from the circle of members and deputy members from the various fields, ZES summarises the results in a written opinion.

The work of ZES requires the ongoing monitoring and consideration of the latest scientific developments in the field of stem cell research. Here, the focus is particularly on research on pluripotent cells of various origins. ZES followed with interest the debates in the run-up to the amendment to the Stem Cell Act. Committee members had an opportunity, especially at presentations and parliamentary public hearings, to input their expertise concerning scientific developments and ethical problems of stem cell research. The amendment to the Stem Cell Act that entered into force on 21 August 2008 placed, amongst other things, the cooperation of German research groups with European partners on a sound legal basis through the amendment to § 13.

The annual report of ZES, which is published by the Federal Ministry of Health (BMG) (§ 14 ZESV), and the previous ZES reports can be accessed on the BMG website (www.bmg.bund.de).

Field	Member	Deputy Member
Biology	Prof. Dr. rer. nat. Hans R. Schöler Max-Planck-Institut für Molekulare Biomedizin Münster	Prof. Dr. rer. nat. Martin Zenke Institut für Biomedizinische Technologien Abt. Zellbiologie RWTH Aachen
	Prof. Dr. rer. nat. Anna M. Wobus **(Deputy Chairman)** Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK) Abteilung Zytogenetik Gatersleben	Prof. Dr. rer. med. Ursula Just Biochemisches Institut Christian-Albrechts-Universität Kiel
Ethics	Prof. Dr. phil. Ludwig Siep **(Chairman)** Philosophisches Seminar Westfälische Wilhelms-Universität Münster	Prof. Dr. phil. Jan Beckmann Institut für Philosophie FernUniversität in Hagen
	Prof. Dr. med. Claudia Wiesemann Institut Ethik und Geschichte der Medizin Georg-August-Universität Göttingen	Prof. Dr. med. Giovanni Maio, Institut für Ethik und Geschichte der Medizin Albert-Ludwigs-Universität Freiburg
Medicine	Prof. Dr. med. Gustav Steinhoff Klinik und Poliklinik für Herzchirurgie Universität Rostock	Prof. Dr. med. Mathias Bähr Neurologische Klinik Georg-August-Universität Göttingen
	Prof. Dr. med. Marion B. Kiechle **(Deputy Chairman)** Frauenklinik und Poliklinik Klinikum rechts der Isar Technische Universität Munich	Prof. Dr. med. Ricardo E. Felberbaum Frauenklinik Klinikum Kempten Oberallgäu
	Prof. Dr. med. Anthony D. Ho Med. Universitätsklinik und Poliklinik Abt. Innere Medizin V Ruprecht-Karls-Universität Heidelberg	Prof. Dr. med. Ulf Rapp Institut für Medizinische Strahlenkunde und Zellforschung (MSZ) Bayerische Julius-Maximilians-Universität Würzburg
Theology	Prof. Dr. theol. Klaus Tanner Wissenschaftlich-Theologisches Seminar Lehrstuhl Systematische Theologie/Ethik Ruprecht-Karls-Universität Heidelberg	Prof. Dr. theol. Hartmut Kreß Evangelisch-Theologische Fakultät Abteilung für Sozialethik und Systematische Theologie Rheinische Friedrich-Wilhelms-Universität Bonn
	Prof. Dr. theol. Dr. phil. Antonio Autiero Seminar für Moraltheologie Katholisch-Theologische Fakultät Westfälische Wilhelms-Universität Münster	Prof. Dr. theol. Konrad Hilpert Lehrstuhl für Moraltheologie Katholisch-theologische Fakultät Ludwig-Maximilians-Universität Munich

Table 1: Members and Deputy Members of the Central Ethics Committee for Stem Cell Research (ZES), status November 2008

2. Deliberation and review of applications pursuant to § 5 StZG during the reporting period

During the reporting period ZES held eight meetings at which a total of 10 applications for the import and/or use of human embryonic stem cells and one extension application for an already approved project were extensively discussed. All the applications were viewed positively by ZES. Two applications, for which ZES had already handed down a positive opinion during the previous reporting period, were only approved by the Robert Koch Institute during the current reporting period. As, pursuant to § 11 StZG, data on applications may only be published after approval, this report contains information on these two applications. Table 2 gives an overview of the applications that were viewed positively by ZES and approved by RKI during the reporting period.

The subject of the first project (Approval 25) is the systematic examination of molecular processes on the levels of the transcriptome, the proteome and the epigenome that steer the development of pluripotent stem cells in early differentiation stages. The starting point for this is the cultivation of human embryonic stem cells under defined chemical conditions.

The second research project (Approval 26) addresses the development of improved methods for the cultivation and differentiation of pluripotent human embryonic stem cells. By means of magnetic activated cell sorting, MACS, that involves separation of human embryonic stem cells by using suitable surface markers, highly purified populations of human embryonic stem cells and differentiated cells can be obtained and characterised.

In the fourth project (Approval 28) a comparison of the differentiation potential of human embryonic stem cells and induced pluripotent stem cells (iPS cells) in cardiac tissue using various scaffold materials will be performed. The main question is whether human cardiac tissue suitable for transplantation can be produced from either pluripotent cell type. Hence, on various carrier materials and in three-dimensional scaffolds, differentiation into cardiac cell types is to be optimised using different methods and protocols. There are also plans to transplant tissue complexes differentiated *in vitro* into a rat myocardial infarct model.

Two projects look at the neuronal differentiation of human embryonic stem cells:
One project (Project 3, Approval 27) focuses on the examination of the early neuronal differentiation of man, in particular neural tube development on a three-dimensional matrix. It is expected that complex three-dimensional cellular structures will be formed. The research will seek to clarify whether differentiation during the development of the structures can be controlled and whether the spatial arrangement of the cells in the cell structures can be influenced during cultivation.

Another project (Project 5, Approval 29) plans to establish an *in vitro* model system with the properties of human neuronal networks. It is to be used in studies on the developmental biology of human neurons and to examine neuroactive substances. The *in vitro* system is also to serve as the basis for models of neurodegenerative diseases that could be used to compare transplantation of neuronal cells derived from human embryonic stem cells or from human umbilical cord blood cells.

Number	Applicant	Research area	Date of positive ZES opinion
1 (25)	Max-Planck-Gesellschaft Max-Planck-Institut für Molekulare Biomedizin, Münster	Studies on transitions of human embryonic stem cells from the pluripotent state to defined differentiation stages	14.01.2008
2 (26)	Miltenyi Biotec GmbH, Bergisch Gladbach	Development of magnetic activated methods for the enrichment of pluripotent human embryonic stem cells and their derivatives	16.07.2007
3 (27)	Frau Prof. Dr. Elly M. Tanaka Technische Universität Dresden, DFG-Zentrum für Regenerative Therapien	Establishment of a three-dimensional culture system for early human neural precursor cells to examine aspects of the development of the human neural tube	21.11.2007
4 (28)	Frau Prof. Dr. Maria Wartenberg Universitätsklinikum Jena	Comparison of bioengineering of vascularised cardiac tissue for cell transplantation using human embryonic and induced pluripotent stem cells	13.02.2008
5 (29)	Dr. Marcel Dihné Universitätsklinikum Düsseldorf	Production and characterisation of functional neuronal networks from human embryonic stem cells	13.02.2008
6 (30)	Frau Dr. Kaomei Guan Universität Göttingen	Comparative study of adult spermatogonial and human embryonic stem cells. Differentiation of human embryonic stem cells into multipotent cardiac precursor cells	17.03.08
7 (31)	Prof. Dr. Jürgen Hescheler Institut für Neurophysiologie der Universität zu Köln	Examination of the effects of harmful influences on differentiating human embryonic stem cells. Differentiation of hepatocytes from human embryonic stem cells and their characterisation	16.04.2008
8 (32)	Prof. Dr. Jan Hengstler Institut für Arbeitsphysiologie, Universität Dortmund	Differentiation of human embryonic stem cells into hepatocyte-like cells and the study of their suitability for the development of improved *in vitro* toxicity tests	16.04.2008
9 (33)	Prof. Dr. Harald von Melchner Klinikum der Johann Wolfgang Goethe-Universität Frankfurt am Main	Production of human embryonic stem cell libraries through conditional gene trap mutagenesis in human embryonic stem cells	19.05.2008
10 (34)	Frau Dr. Anja Moldenhauer Institut für Transfusions-medizin, Charité Berlin	Differentiation of human embryonic stem cells into erythrocytes and thrombocytes	14.07.2008

Extension to an approved application			
11 Extension to approval (1)	Prof. Dr. Oliver Brüstle Institut für Rekonstruktive Neurobiologie, Universitätsklinikum Bonn	Differentiation and transplantation of neural precursor cells from human embryonic stem cells	19.05.2008

Table 2: Overview of projects that were approved by RKI during the reporting period following a definitive, positive assessment by ZES. The numbers in brackets in the left column correspond to the approval numbers in the RKI stem cell register.

Project 6 (Approval 30) consists of two separate parts. The first part pursues a new perspective for the production of pluripotent cells. The intention is to isolate human spermatogonial stem cells (SSCs) from adult testicular tissue and to compare them with human embryonic stem cells. Given that multipotent adult germline stem cells (maGSCs) with specific properties of pluripotent cells can be isolated and cultivated from murine testis tissue, cell lines are to be established in a similar manner from human testicular tissue in order to examine them for the presence of the characteristics of pluripotent cells. Comparative studies of human embryonic stem cells and human SSCs are expected to produce knowledge about the ability of human SSCs of being reprogrammed into pluripotent cells.
In the second part the project conditions for the differentiation of human embryonic stem cells into precursors of cardiac cells are to be established. In this context cardiac precursor cells are to be identified using a receptor for the vascular endothelial growth factor (VEGFR-2), then isolated and differentiated into cardiac cells.

Two of the research projects reviewed by ZES aim to differentiate human embryonic stem cells into cells with the properties of human hepatic cells. They are part of the ESNATS project supported by the European Community which promotes the development of new test protocols for the testing of toxic substances.

With the help of various differentiation protocols Project 8 (Approval 32) aims to develop human embryonic stem cells into hepatocyte-like cells, characterise them morphologically, biochemically and functionally, and then examine them for their ability to metabolise various substances. Furthermore, the properties of hepatocyte-like cells are to be compared with those of primary human hepatocytes and of hepatocyte-like cells derived from somatic stem and precursor cells. After optimisation of the differentiation protocols, the cells are to be cultivated in specific co-culture systems together with other cells (e.g. neurons) manufactured from human embryonic stem cells by other ESNATS project partners. The latter are to be used as target cells to test substances which are only toxified after metabolic degradation by hepatocytes.

The second of the two EU research projects (Project 7, Approval 31) consists of two parts. In the first part human embryonic stem cells are to be developed into hepatocyte-like cells using a differentiation protocol based on the enrichment of differentiated cells involving the expression of a reporter gene expressed under the control of a tissue-specific promotor. In the second part, cells from human embryonic stem cells spontaneously differentiating inside *embryoid bodies* are to be exposed to various noxae with potentially teratogenic effects. Differences in the gene expression pattern of the cells, which are due to the differentiation-dependent, time-dependent and dose-dependent impact of the specific noxa, are to be identified on the RNA and protein levels. The intention is to create foundations for new *in*

vitro test systems for teratogenic substances and medical active substances with unknown effects on development processes.

Systems of this kind for *in vitro* drug testing and for examining the metabolic degradation of potential pharmaceuticals could help to estimate the risk potential of drugs for use in humans and reduce this potential in future.

Another research project (Project 9, Approval 33) aims at quantitatively analysing gene functions of human embryonic stem cell lines. To this end a library of mutated human embryonic stem cell lines is to be established in which one gene has mutated in each of the cell lines. The gene trap strategy used, based on the use of retroviral vectors, should potentially allow to mutate all genes in the human genome. In this way genes that play a role in human disease could be identified and characterised.

The subject of Project 10 (Approval 34) is the harnessing of haematopoietic precursor cells from human embryonic stem cells in order to differentiate them into specific blood cells, i.e. erythrocytes and thrombocytes. The goal of the project is to obtain insights into factors and mechanisms involved in the embryonic development of haematopoietic cells and to establish a basis for reliable, reproducible protocols for the *in vitro* differentiation of human embryonic stem cells into cells of the erythroid and thrombocytic lines. The studies are to involve a comparison with haematopoietic cells generated from umbilical cord blood

An application for further work has been submitted for the first research project with human embryonic stem cells from 2002. This required an extension to the RKI approval and also renewed discussion by the Committee. The work spanning several years on the neural differentiation of human embryonic stem cells is to be used in the extended application to apply the knowledge acquired to studies on the treatment of epilepsy. Human embryonic stem cells and neural precursor cells derived from them are to be genetically modified in such a manner that they produce factors with anti-epileptic properties. Preclinical investigations are to be conducted on the basis of the transplantation of neural precursor cells derived from human embryonic stem cells in various rodent epilepsy models. They are supposed to produce information on later human therapeutic effects. Furthermore, there are plans to monitor the functional integration of the transplanted cells in the nerve tissue of the recipient animal using a molecular reporter system. In this way specific regions in the brain could be identified which are particularly suitable for the transplantation of neural cells.

Further information on the content of the projects supported by ZES and approved by RKI can be found in the RKI register (http://www.rki.de/DE/Content/Gesund/Stammzellen/Register/register__node.html). The main ZES arguments concerning the superior interest of the research projects, their sufficient preliminary clarification and the need to use human embryonic stem cells have been taken over into the assessment of the research projects by RKI.

In its work now spanning six years, ZES has deliberated on a total of *38 applications* for the import and/or use of human embryonic stem cells and *three applications for extensions* to already approved projects. In total *41 opinions* were handed down, *39 of them were positive.* All the projects supported by ZES were approved by RKI.

In Germany 26 working groups are currently engaged in research involving human embryonic stem cells. Experimental findings from the approved projects of six groups have been included in 18 scientific publications.

In 2008 the Committee definitively reviewed eight applications from research groups who had not previously worked with human embryonic stem cells as well as three applications by groups who already have permission to work with human embryonic stem cells. These

applications were already received during the first six months of the reporting period and required extensive deliberations by ZES.

On the occasion of its fifth anniversary, ZES invited members of parliament and other interested parties to a colloquium in September 2007 which looked at the situation of stem cell research and provided insight into the research projects approved in Germany. The papers that documented the national and international research situation were published in the Bundesgesundheitsblatt, Volume 51, Issue 9, Sep. 2008 (Table of contents available on http://springerlink.com/content/rt5280141x5t/?p=520af9244f334ae5898725092f4b858f&pi=0)

Outlook and final comments

Based on their experience drawn from the discussion of these applications, ZES sees opportunities for cell biological basic and applied medical research with human embryonic stem cells in several areas. In the field of basic research this involves more particularly analysis of the development processes of human cells. In this context basic research can also achieve indirect success by producing results that can be used for other, related research areas. This applies in particular to research on other stem cells, pharmacotoxicology or active substance research. Basic research of premium importance can open up entirely new research and application paths.
International research on human embryonic stem cells has become an established research area. The cultivation and differentiation of human embryonic stem cells has been developed on using standardised methods. By contrast, clinical applications or regenerative therapies based on human embryonic stem cells are not likely in the near future. The problem remains that cells differentiated from human embryonic stem cells may be contaminated with human embryonic stem cells which can lead to the development of tumours (teratoma, teratocarcinoma). Nonetheless, neural cells derived from human embryonic stem cells can be harnessed already today without any contamination with undifferentiated stem cells. The general expectation is, therefore, that the first clinical trials can be developed with transplantable cells derived from human embryonic stem cells for the regeneration of neural tissue.
Human embryonic stem cells play an increasing role as the standard in comparative studies with other pluripotent cell types, e.g. parthenogenetic embryonic stem cells (pESC), germline stem cells (GSC) and induced pluripotent stem cells (iPS). These “new” pluripotent cell types are increasingly under discussion as a potential source of cells for harnessing patient-specific cells. All the same, in the case of all donor cells derived from pluripotent cells, impurities with undifferentiated cells may still lead to the formation of tumours. In particular induced pluripotent stem cells, iPS cells, take on a special role in the field of research concerning pathogenesis mechanisms of human diseases. This is because, after reprogramming with the help of pluripotency genes, pluripotent stem cells from ordinary body cells (e.g. fibroblasts, harnessed from patients with a genetic disease) can be isolated and undergo molecular and cell biological *in vitro* analysis. It is expected that these research activities will produce important findings for the diagnosis and treatment of numerous diseases.
Furthermore, the use of human embryonic stem cells for the development of medical active substances, for the testing of medicines and for toxicity studies seems to be imminent. This is directly obvious from applications submitted to ZES, e.g. within the framework of the EU programmes, but also from international developments. Toxicological and pharmacological tests in human embryonic stem cells will also supply more reliable results for man than studies in animals or animal cells. This moves in the same direction as EU support for research, various streams in the ethical debate and research projects aiming to reduce the number of animal experiments and the high consumption of laboratory animals by establishing test systems with human embryonic stem cells. In particular the EU Regulation 1907/2006 REACH throws up major challenges for EU Member States, including Germany.

In this context, human embryonic stem cell lines of this kind that are established, cultured and stored, for instance in European or international stem cell banks, will become increasingly important for recognised test methods.

The StZG permits the import and use of human embryonic stem cells pursuant to § 4 para 2 StZG for research purposes. Against the backdrop of domestic and foreign research projects, there will have to be, in future, more exact clarification of whether and, if so, to what extent the broad use of human embryonic stem cells, e.g. within the framework of safety tests for medicinal products, is to be seen as a research goal within the intendment of the Stem Cell Act. For test purposes differentiation trials with embryonic stem cells would have to be staged regularly, for instance for studies on embryotoxicology.

The Sixth Report was unanimously approved at the 43rd ordinary meeting of ZES on 21 January 2009.