Report

of the

Central Ethics Committee for Stem Cell Research (ZES)

Eighth Report after the enactment of the Stem Cell Act (StZG)
Reporting period 1 December 2009 to 31 December 2010

1. The Central Ethics Committee for Stem Cell Research

The Central Ethics Committee for Stem Cell Research (ZES) is an independent and interdisciplinary expert body that reviews and assesses applications for the import and use of human embryonic stem cells (hES cells). 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 (Stem Cell Act – StZG) of 28 June 2002 (BGBl. I page 2277, http://www.gesetze-im-internet.de/stzg/index.html), amended by the Act amending the Stem Cell Act of 14 August 2008 (BGBl. I page 1708, http://www.bgbl.de/Xaver/start.xav?startbk=Bundesanzeiger_BGBl&bk=Bundesanzeiger_BGBl&start=//*%5B@attr_id=%27bgbl108s1708.pdf%27%5D) and the Regulation concerning the Central Ethics Committee for Stem Cell Research and the competent authority pursuant to the Stem Cell Act (ZES Regulation – ZESV) of 18 July 2002 (BGBl. I page 2663) (http://bundesrecht.juris.de/zesv/index.html).

The Commission was appointed for the first time at the entry into force of the Stem Cell Act of 1 July 2002 for a period of three years by the Federal Government and is now working in its third appointment term. The altogether 18 members and deputy members of the Commission perform their duties on a voluntary basis. They represent the fields of biology, medicine as well as philosophical, medical and theological ethics (see Table 1). In accordance with the ZES Regulation the deputy members participate in the same way as the members regularly in the deliberations on the applications.

§ 9 StZG defines the task of ZES which consists in determining, on the basis of the documents submitted by the applicant, whether the project submitted for review meets the criteria of § 5 StZG and is ethically acceptable within this meaning. In this connection it has to be reviewed whether the use of human ES cells applied for serves high ranking research objectives for increased scientific knowledge (§ 5 No. 1 StZG), whether the required preliminary clearing for the reviewed scientific issues is available (§ 5 No. 2 a StZG) and whether the desired scientific knowledge is likely to be achieved only by using human embryonic stem cells (§ 5 No. 2b StZG). Based on four votes, which are prepared by the members and deputy members, ZES summarises the results of the review of the application in a written opinion. The opinion is then transmitted to the competent authority under StZG, i.e. the Robert Koch Institute (RKI).

For the assessment of applications, the activities of ZES require also the monitoring and consideration of the latest scientific developments in the field of stem cell research. At present there are indications of new developments in fundamental and application-oriented research. On the basis of hES cells, application options for certain therapeutic indicators can be identified and the development of active substance and toxicity test systems, in particular test systems for embryotoxicity on the basis of human pluripotent stem cells, increase on a worldwide level.

The Annual Reports of ZES, which are published by the Federal Ministry of Health (BMG) (§ 14 ZESV), can be accessed on the BMG (www.bmg.bund.de) and the RKI (http://www.rki.de/DE/Content/Gesund/Stammzellen/ZES/Taetigkeitsberichte/taetigkeitsbericht__node.html) websites.

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 Chairperson)** Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK) Abteilung Zytogenetik Gatersleben	Prof. Dr. 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 für 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 Chairperson)** Frauenklinik und Poliklinik Klinikum rechts der Isar Technische Universität München	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 Max-Planck-Institut für Biochemie Abt. Molekularbiologie München
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 München

Table 1: Members and deputy members of the Central Ethics Committee for Stem Cell Research (ZES), status: December 2010

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

During the reporting period ZES held seven meetings at which a total of nine applications for the import and use of human ES cells and an application for extension of an already approved project were discussed. ZES submitted a positive opinion on seven applications and the application for extension. These projects meet the prerequisites of § 5 StZG and are ethically acceptable within its intendment (§ 9 StZG). One application (51st approval under StZG) was already deliberated in 2008 and reviewed positively; at the request of the applicant, the procedure had, however, been suspended and only continued in January 2010; the positive review of the application by ZES was maintained. Another two applications have already been given a positive opinion by ZES but are still in the approval procedure. Table 2 provides a summary overview of the applications that were viewed positively by ZES and approved by RKI during the reporting period.

No.	Applicant	Research topic	Date of positive ZES opinion
1 (50)	Albert-Ludwigs-Universität Freiburg, Zentrum für Biologische Signalstudien (bioss)	Studies on the chromatin dynamics of human induced pluripotent stem cells and human embryonic stem cells	14.12.2009
2 (51)	Westfälische Wilhelms-Universität Münster	Studies on the endothelial differentiation of human embryonic stem cells and human induced pluripotent stem cells	19.05.2008
3 (52)	Max-Planck-Gesellschaft, Max-Planck-Institut für Molekulare Biomedizin, Münster	Differentiation of human embryonic stem cells in myocardial and nerve cells	22.02.2010
4 (53)	Dr. Hans-Jörg Bühring, Universitätsklinikum Tübingen	Comparative studies of human human adult germline stem cells and human embryonic stem cells	14.04.2010
5 (54)	Dr. Dr. Tomo Šarić, Institut für Neurophysiologie der Universität Köln	Comparative studies on cell aging processes of human mesenchymal stem cells and human embryonic stem cells.	19.05.2010
6 (55)	Medizinische Hochschule Hannover	Comparative study on the glycosylation patterns in human embryonic and induced pluripotent stem cells	13.10.2010
7 (56) (57)	Prof. Dr. Albrecht Müller, Institut für Med. Strahlenkunde und Zellforschung im Zentrum Experimentelle Molekulare Medizin, and Prof Dr. Anna-Leena Sirén Neurochirurgische Klinik und Poliklinik, Universität Würzburg	Comparative studies on the neurogenesis of human parthenogenetically produced pluripotent stem cells and human embryonic stem cells	15.11.2010
8 (58)	Prof. Dr. Dr. Bernd Fischer, Martin-Luther-Universität Halle-Wittenberg	Analysis of the influence of metabolic determinants and environmental contaminants on the adipogenic differentiation of human embryonic stem cells	15.11.2010
Extension of an already approved application			
9 Extension of the approval (16)	Prof. Dr. Sigurd Lenzen, Medizinische Hochschule Hannover, Institut für klinische Biochemie	Targeted destruction of non-differentiated embryonic stem cells in pancreatic differentiation cultures	22.02.2010

Table 2: Overview of research projects that were approved during the reporting period by RKI following a final positive assessment by ZES. The numbers in brackets in the left column correspond to the approval numbers in the RKI register (http://www.rki.de/DE/Content/Gesund/Stammzellen/Register/register_node.html).

The research projects submitted to ZES during the reporting period focus on fundamental issues concerning developmental biological processes in humans. Furthermore, hES cells serve in several cases comparative purposes for the characterisation and differentiation of human induced pluripotent stem cells (hiPS cells; projects 1, 2, 6) or pluripotent cells of a different origin (projects 4, 5, 7).

The first project (50th approval) concerns the study of dynamic processes of chromatin, which are responsible for maintaining the essential properties for pluripotency in human ES and iPS cells. Furthermore, it is to be examined whether and in how far there are measurable differences in dynamics and mobility of certain chromatin associated proteins between hES and hiPS cells and/or between hiPS cells produced in different ways. Finally, the reprogramming process of somatic cells on the level of chromatin is to be followed.

The isolation of endothelial precursor cells from hES cells, their comparison with precursor cells isolated from hiPS cells and an in-depth analysis of their *in vitro* potential for endothelial differentiation are covered by the second project (51st approval). Endothelial precursor cells isolated from the two stem cell types are then to be studied for their suitability in the therapy of ischaemic diseases. They are to be compared in different mice models, with special emphasis on neovascularisation as well as the integration of the transplanted cells into murine endothelia. Furthermore, the project deals with the question whether endothelial cells which are isolated from hiPS cells of patients with ischaemic diseases have different properties compared to endothelial cells which are isolated from hES cells and hiPS cells of healthy test subjects.

The development of new methodological approaches for the provision of highly enriched populations of functioning human neurons and cardiomyocytes is the focus of the third project (52nd approval). Through the transfer of corresponding reporter and marker gene expression cassettes in hES cells the processes of *in vitro* differentiation from the non-differentiated stem cell to the mature myocardial cell and/or to the terminally differentiated dopaminergic neurons and motoneurons are to be analysed and optimised. Moreover it is planned to make available cell models for neurodegenerative diseases on the basis of hES cells by the expression and/or inhibition of the expression of genes whose products play a role at the development of these diseases,. In a further step it is planned to compare these neuronal cells isolated from hES cells with corresponding cells which were differentiated from hiPS cells of patients with the respective neurodegenerative disease. In addition, studies on the cardiac differentiation potential of hiPS cells and the production of hiPS cells from patients with specific coronary diseases are planned. Within proof of principle studies the development of an *in vitro* test system for pharmacologically active substances is planned which is to be based on cardiomyocytes derived from hES cells.

Project 4 (53rd approval) deals with comparative studies of hES cells with stem cells from human testicular tissue (human adult germline stem cells, haGSCs). The project refers to a partial aspect of a project which had already been approved during the past reporting period (35th approval). Comparative studies in hES cells are to clarify whether surface molecules identified on haGSCs are also on hES cells and whether haGSCs and hES cells differ in terms of the presence and properties of these surface molecules.

In another project, hES cells are needed for comparative purposes to understand cell aging processes (54th approval). The focus is on studies on cell aging processes in multipotent mesenchymal stem cells (MSCs), which discontinue growth during their cultivation (replicative senescence). For the identification of factors and signal pathways which are relevant at the aging of MSCs, MSCs with lower and higher passage numbers are to be compared in terms of their gene expression profiles and the DNA methylation patterns as well as with hES cells which represent a non-senescent cell type. Processes of cell aging are also to be analysed in hiPS cells isolated from MSCs with different lengths of cultivation. hES

cells are also envisaged for comparative purposes to clarify whether the hiPS cells isolated from MSCs have typical properties of pluripotent stem cells.

In the sixth project (55th approval) the glycosylation pattern of hips and hES cells cells is to be studied in comparison. The goal of the project is to understand the influence of different culture conditions on the glycosylation of pluripotent stem cells and obtain information on the changes of the glycome, the proteoglycome and the proteome of the two types of pluripotent cells during the differentiation to cardiomyocytes. In the long-term the findings of this research project are to contribute towards the development of appropriate large-scale cultivation processes and the optimisation of purification protocols of cardiomyocytes differentiated from pluripotent stem cells for cell therapeutic applications.

Since for the seventh project a joint application with identical research activities was submitted, but the two applicants work for two different research institutions, two approvals (56th and 57th approval) were issued by RKI. The project deals with the question whether human parthenogenetically generated pluripotent stem cells (hpPS cells) can be differentiated to neural cell types in the same way as hES cells. This is to clarify whether the lack of a paternal genome in hpPS cells impairs the neuronal differentiation of these cells and/or influences the properties of the neural precursor cells derived from them. Apart from a comprehensive *in vitro* characterisation of the neural cells derived from the two cell types, the survival capacity of the cells and their integration into the neural network after transplantation into the experimentally damaged brain of immunosuppressed mice are to be verified. In this connection the significance of erythropoietin is to be reviewed at the neural differentiation of human stem cells. A focus of the project is the analysis of genomic imprinting which plays a role in neural development.

In the eighth research project (58th approval) the question is analysed whether and in how far glucose and environmental contaminants such as the plasticisers di-ethylhexylphthalate (DEHP) and bisphenol A (BPA), which are suspected of triggering and/or promoting obesity, have an impact on the early human embryonic development and in particular the differentiation in fat cells. In a model for adipogenic differentiation based on hES cells the obesogenic effect of substances with known and suspected obesogenic effect at different points in time of the differentiation is to be studied. Furthermore, the development of methods is planned to gain populations of adipocytes and/or their precursor cells as pure as possible. The research project will probably deepen knowledge about mechanisms which influence the fat cell differentiation in humans. Further development of the project could result in new *in vitro* test systems for the analysis of chemical substances for their possible harmful, in particular obesogenic effects before their placing on the market.

Within the framework of a planned extension of the 16th approval under the StZG issued in 2006 research activities were applied for which required a further discussion in the Commission about the existence of the prerequisites under § 5 StZG. For the studies on the differentiation of human ES cells to insulin-producing pancreatic beta cells the development of an experimental approach is planned with which non-differentiated hES cells can be eliminated from a population of differentiated cells. A so-called suicide gene (here the gene for thymidine kinase of the herpes simplex virus) is to be integrated into hES cells under the control of the gene for the transcription factor Oct4, expressed only in pluripotent stem cells, coupled with a reporter gene. After the selection, enrichment and differentiation to pancreatic beta cells, non-differentiated cells are to be selectively removed by treatment with the guanine analog ganciclovir (GCV). In this way the risk of formation of undesired teratomas after cell transplantation is to be reduced. The experimental approach is also to be transferred to the pancreatic differentiation of hiPS cells.

Information on the content of the approved projects, which have received a positive assessment by ZES, can be taken from the register of RKI (http://www.rki.de/DE/Content/Gesund/Stammzellen/Register/register__node.html).

The central arguments of ZES concerning the high ranking status of the research projects, their sufficient pre-clearing and the necessity to use human ES cells have been taken into account at the assessment of the research projects by RKI.

In 2010 seven applications of research groups which had so far not worked with hES cells and two applications by groups which had already received a corresponding approval in the past were reviewed by the Commission and then assessed. At present 44 groups at 32 research institutions conduct work with hES cells. Furthermore, results from approved research projects have by now been integrated into 37 original scientific publications of 11 research groups.

During its seven years of activities ZES has deliberated on a total of 61 applications for import and/or use of hES cells. In addition, four applications for extensions of already approved projects were assessed. As a result, 65 opinions were submitted to RKI of which 63 included positive votes. All applications supported by ZES during the reporting period have been approved by RKI.

The scientific state of knowledge which has to be taken into account at the review of the necessary pre-clearance in accordance with § 5 StZG and is submitted within the framework of the application procedure, has considerably changed during the last years. Within the framework of the submissions required under StZG for pre-clearing, the applicants increasingly refer to knowledge about human ES cells established in international research projects. In addition, human cells exhibit significant species-related differences compared to animal cells within the context of different scientific issues. For that reason it must be reviewed in each individual case whether and in how far studies in animal cells can lead to a gain in knowledge for the development of hypotheses and/or experimental approaches planned within the framework of the project and in how far specific pre-clearings of project issues in *in vivo* or in *vitro* animal models can be requested in a useful manner.

Developments and tendencies in stem cell research

Part of the applications assessed during the reporting period dealt with comparisons between hES cells and hiPS cells concerning the differentiation in certain cell types. Some projects based on hiPS cells strive for the establishment of cell models for human diseases, in order to be able, for instance, to analyse pathomechanisms of human diseases *in vitro*. The assessment of applications by ZES often involved aspects of ethical issues which can also occur at the isolation and use of hiPS cells. In opinions on projects in which the simultaneous use of hES cells and hiPS cells is planned ZES points out, by way of precaution, that before the removal of donor material to establish hiPS cells a vote of the Ethics Committee in charge of the responsible research institution has to be obtained.

The assumption often made in public that hES cells are merely required for a transitory period and as simple comparison material for other pluripotent cell types, is confirmed neither by the development of research activities in Germany which transpire from the applications nor from the international scientific development. hES cells continue to be a separate object of research under which essential scientific issues are studied independently from research on hiPS cells. As opposed to hiPS cells, hES cells are native, epigenetically original cells. Furthermore, the two cell types are also used in combination, for instance in order to better understand general properties of human pluripotent cells. In addition, it becomes increasingly clear that the two types of pluripotent cells can have specific fields of application in future: in international research there are on the one hand numerous activities which target the identification of new active substances by means of disease-specific hiPS cell lines. On the other hand, more recent publications suggest efforts towards using hES cell lines as cell model for the early human embryogenesis within the framework of reproduction toxicology studies. In how far hiPS cells can represent a model for human embryonic development, is currently open.

Overall, the application reference of research involving hES and hiPS cells becomes increasingly clear in the deliberations on applications. According to StZG research activities using hES cells are also permitted with reference to diagnosis, prevention and therapy, i.e. with a view to future applications. Many applications submitted to ZES combine fundamental research with the development of new methods, for instance for the *in vitro* testing of active substances. It can be taken from the applications that such test systems could be available in a rather near future. In this way, too, hES cell research could make a contribution towards the protection of human health – earlier than for clinical applications. The development of such hES cell-based test systems, for instance in pharmaceutical research, is ethically acceptable under StZG according to ZES, if high ranking objectives are pursued and the other requirements of StZG are met. Whether the future use of hES cell-based test systems for pharmaceutical development will also involve an economic benefit for the applicant, is not relevant for the assessment of applications under StZG.

In applications submitted to ZES, the applicants also stated in some cases that as a result of the projects applied for, animal experiments could possibly be reduced. The objective of a potential reduction of laboratory animals cannot be relevant for a decision concerning the assessment of the applications under StZG. The development of hES cell-based alternative test systems with the goal of reducing animal tests, as required by EU Directives, is, however, basically seen as an ethically significant goal.

In connection with research on pluripotent stem cells the consequences of a possible differentiation of these cells in precursor cells of germ cells is discussed as well. The differentiation of pluripotent human stem cells in gamete precursor cells *in vitro* is presumably the only possibility to analyse the early human germ cell differentiation. The preparation of hiPS cells from patients with fertility disorders and their differentiation in germ line cells would, moreover, provide an opportunity to study defective developments at the germ cell differentiation. ZES has also taken note of the fact that new reprogramming techniques have resulted in new aspects concerning the understanding of the totipotency criterion (see opinion of Leopoldina of October 2009).

ZES is aware that hES cells have already been clinically tested within the framework of international developments. During the reporting period the US company Geron started the world's first phase I study with neural precursor cells derived from hES cells. In this study, designed for a period of two years, neural precursor cells derived from hES cells are transplanted into patients with subacute dorsal injuries. The primary goal is to focus on the tolerance and safety of the therapy; the efficacy of treatment will only be covered by later studies. A second US company (Advanced Cell Technology, ACT) has been granted permission in November 2010 by the US FDA to clinically test cells differentiated from hES cells. This concerns retinal pigment epithelia cells which are to be used for the treatment of patients affected by Stargardt's disease within the framework of a phase I study. The company has in the meantime applied for a further phase I study on the dry age-related macular degeneration. If German hospitals were to become involved in these studies on the transplantation of cells differentiated outside Germany, this would not require an approval under StZG, because StZG regulates the import and use of hES cells but not the handling of cells differentiated from them. Other laws would, however, have to be observed, such as the German Medicines Act for the approval of clinical trials by the Paul Ehrlich Institute or provisions of the Tissue Act. However, if the differentiation of hES cells to precursor cells and a subsequent transfer to patients were planned in Germany, the reservation of approval of StZG would have to be taken into account, too.

The eighth report was unanimously approved at the 56th ordinary meeting of ZES on 17 January 2011.