Report

of the

Central Ethics Committee for Stem Cell Research (ZES)

Seventh Report after the enactment of the Stem Cell Act (StZG)
Reporting period: 1 December 2008 to 30 November 2009

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 (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 (*Stammzellgesetz* – StZG) of 28 June 2002 (BGBl. I p. 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 p. 1708, http://www.bgbl.de/Xaver/start.xav?startbk=Bundesanzeiger_BGBl&bk=Bundesanzeiger_BGBl&start=//*[@attr_id=%27bgbl108s1708.pdf%27]), 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://www.gesetze-im-internet.de/zesv/index.html).

The Commission has a total of nine members from the fields of biology, medicine as well as philosophical, medical and theological ethics and nine deputy members from the corresponding disciplines (see Table 1). Both the members and the deputy members regularly participate in the deliberations on the applications pursuant to ZESV. The members and deputy members of ZES perform their duties on a voluntary basis. They were appointed for the first time with the entry into force of the Stem Cell Act on 1 July 2002 for three years by the Federal Government. The Committee is now engaged in its third term in office.

The task of ZES to scientifically review applications for the import and use of hES cells for their ethical acceptability pursuant to § 5 StZG is specified in § 9 StZG. In each case it must be determined whether the use of hES cells applied for serves research purposes of superior interest for increased scientific knowledge (§ 5 No. 1 StZG), whether the required preliminary tests are available for the scientific questions (§ 5 No. 2a StZG) and whether the desired scientific knowledge can probably only be achieved with hES cells (§ 5 Nr. 2b StZG). Based on four votes by the members and deputy members on each application, ZES summarises the results in a written opinion. The opinion is given to the competent authority pursuant to StZG, the Robert Koch Institute (RKI).

As consideration must be given to the latest natural scientific aspects in addition to ethical aspects when reviewing and assessing the applications submitted, the work of ZES requires the ongoing monitoring and consideration of the latest scientific developments in the field of stem cell research. During the reporting period the Committee once again took a comprehensive look at international standards for research on hES cells and monitored, more particularly, the development of research on human induced pluripotent stem cells (hiPS cells). Given their properties and their differentiation potential they are frequently examined in comparison with hES cells.

ZES prepares an annual report which is published by the Federal Ministry of Health (BMG) (§ 14 ZESV). The previous ZES reports can be accessed on the BMG website (www.bmg.bund.de) and the RKI website http://www.rki.de/DE/Content/Kommissionen/ZES/Taetigkeitsberichte/taetigkeitsbericht_node.html).

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 November 2009

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 15 applications for the import and use of hES cells were extensively discussed. ZES has already handed down a positive opinion on 14 applications. These definitively approved projects meet the preconditions of § 5 StZG and are ethically acceptable within its intendment (§ 9 StZG). One application is still being deliberated. Another application, for which ZES had already handed down a positive opinion in the previous reporting period, was approved by RKI at the beginning of the new reporting period (in December 2008). As, pursuant to § 11 StZG, data on an application may only be published after its approval, this report only contains information on applications approved by RKI during the reporting period. Table 2 gives an overview of the applications that were viewed positively by ZES and approved by RKI.

Number.	Applicant	Research area	Date of positive ZES opinion
1 (35)	Prof. Dr. Thomas Skutella Anatomisches Institut, Eberhard-Karls-Universität, Tübingen	Comparative characterisation of human adult germline stem cells and human embryonic stem cells	17.11.2008
2 (36)	Prof. Dr. Thomas Eschenhagen Universitätsklinikum Hamburg-Eppendorf	Studies on the generation of artificial heart tissue from human embryonic stem cells	15.12.2008
3 (37)	Max-Planck-Gesellschaft Max-Planck-Institut für Molekulare Biomedizin, Münster	Reprogramming of human somatic cells by defined factors and comparative studies of human induced pluripotent stem cells (hips cells) and human embryonic stem cells	15.12.2008
4 (38)	Medizinische Hochschule Hannover	Cultivation, characterisation and differentiation of human induced pluripotent stem cells compared with human embryonic stem cells	21.01.2009
5 (39)	Zentrum für Integrative Psychiatrie gGmbH, Kiel	Studies on the pluripotency of human induced pluripotent stem cells and embryonic stem cells using whole genomic and proteomic approaches. Development of algorithms to assess the presence of cellular pluripotency	23.02.2009
6 (40)	Max-Planck-Gesellschaft Max-Planck-Institut für molekulare Genetik, Berlin	Comparative studies on the molecular basis of pluripotency and on the targeted differentiation of human induced pluripotent and embryonic stem cells	23.02.2009
7 (41)	Frau PD Dr. med. Sonja Schrepfer Universitäres Herzzentrum Hamburg	Study on the immunological properties of human embryonic stem cells	22.04.2009

8 (42)	Prof. Dr. Agapios Sachinidis Institut für Neurophysiologie, Universität Köln	Optimisation of the cultivation and cardiac differentiation of human embryonic stem cells	22.04.2009
9 (43)	Paul-Ehrlich-Institut, Langen	Studies on the biology of the retrotransposons of human embryonic stem cells	18.05.2009
10 (44)	Fraunhofer-Gesellschaft e.V. Fraunhofer-Institut für Bio-medizinische Technik (IBMT), St. Ingbert	Optimisation of the cultivation and differentiation of human embryonic stem cells	18.05.2009
11 (45)	Universitätsklinikum Essen	Targeted differentiation of pluripotent human embryonic stem cells and induced pluripotent stem cells into haematopoietic stem cells	16.09.2009
12 (46)	Frau Dr. Insa Schroeder Martin-Luther-Universität, Halle-Wittenberg	Analysis of the pancreatic differentiation of human embryonic stem cells and induced pluripotent cells for the purpose of studying the pathogenesis mechanisms of diabetes	16.09.2009
13 (47)	Prof. Dr. Jürgen Hescheler, Institut für Neurophysiologie, Universität Köln	Characterisation of the human *T-cell leukemia* 1 (Tcl1a) oncogen in human embryonic stem cells	14.10.2009
14 (48)	Max-Delbrück-Centrum (MDC), Berlin	Targeted differentiation of human embryonic stem cells into somatosensory neurons for pain sensation	14.10.2009
15 (49)	Frau Prof. Dr. Elly Tanaka, Technische Universität Dresden	*In vitro* reconstruction of human retina formation using human embryonic stem cells and their use in the transplantation of photoreceptor precursor cells in animal models of retina degeneration	14.10.2009

Table 2: Overview of projects that were approved during the reporting period by RKI following a definitive, 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).

In several of the research projects reviewed by ZES during the reporting period (Approvals 35, 37, 38, 39, 40), hES cells are mainly used for comparative purposes for the characterisation and differentiation of pluripotent cells of diverse origin. In the first project (Approval 35) hES cells are to be compared with novel stem cells from human testicular tissue (human adult germline stem cells, haGSCs). The project seeks to provide answers to the questions about whether and, if so, to what extent human adult germline stem cells and hES cells have identical properties, whether their characteristics differ and the reasons for the possible differences.

The third project (Approval 37) looks at new and improved strategies for the induction of pluripotency in somatic cells. hES cells are used firstly for a direct comparison of the properties of hiPS cells. Secondly, various stages of further differentiated cells of different cell types generated from the hES cells are themselves to undergo reprogramming. Here the goal is to gain better understanding of the molecular processes during reprogramming.

The fourth project (Approval 38) is to undertake comparative studies of hES cells and hiPS cells, manufactured using different methods, in order to characterise the properties of both cell types on the levels of the transcriptome, epigenome and during early differentiation in conjunction with embryoid bodies and teratomes. The goal here is to test and establish the cultivation of these cells and their differentiation in larger volumes than on the laboratory scale. The studies likewise aim to help to elucidate functional commonalities or differences regarding the differentiation potential of both cell types into heart, lung and liver cells as well as cells of the haematopoietic line. The production of disease-specific human induced pluripotent stem cells of patients suffering from rare genetic lung diseases is planned in the longer term.

The classification of hiPS cells, hES cells and also of their differentiated derivatives regarding the specific phenotype that is characteristic of pluripotent cells using total genomic and proteomic methods is the subject of the fifth project (Approval 39). It aims to undertake comparisons of both types of stem cells (hES cells versus hiPS cells) within various lines of the same pluripotent cell type, within the same stem cell line (e.g. after longer *in vitro* cultivation) and between various hiPS cells from the same cell source (e.g. hiPS cells from the same patient, that have been produced separately from one another). In addition, after the differentiation of hES cells into fibroblasts, neural cells and blood cells, a comparative study is to be undertaken of differentiated cells from various cell sources, and also of differentiated and non-differentiated cells.

The comparative use of hES cells and hiPS cells for the analysis of molecular processes, which play a role in maintaining pluripotency, in triggering specific differentiation processes and in long-term cultivation, is the focus of the sixth project (Approval 40). It is a supplement to the ninth approval from 2005. The planned comparison of neurons that have been differentiated from various cell sources (from hiPS cells from Parkinson patients, healthy individuals and hES cells), seeks to improve the characterisation and understanding of neurodegenerative diseases.

Approval 36 (second project) involves the continuation and stepping up of work conducted since 2005 with the approval of RKI. The goal is to establish effective protocols for the *in vitro* production of human heart tissue (Engineered Heart Tissue, EHT) from hES cells which could then be used as an *in vitro* heart muscle model and as a substitute for heart tissue. It also aims to create the foundations for an *in vitro* test system for reliably predicting the effects of medicinal products on heart cells. To this end, the transcriptome and proteome of the resulting heart cells are to be compared with hES cells. Furthermore, the factors involved in cardiac differentiation are to be identified and analysed – whereby the emphasis is on the analysis of the expression of genes for G protein-coupled receptors. Strategies are likewise to be elaborated for the enrichment of differentiated heart cells.

Two further projects (Approvals 41 and 45) reviewed during the reporting period look at the immunological properties of hES cells and cells derived from them. As the use of cells or tissue differentiated from hES cells may be of importance in the long term for allogenic transplantations in patients with degenerative diseases, studies on the immune status of hES cells and on the question whether and, if so, to what extent their immunogenicity is changed by differentiation, are an important research goal.

The seventh project (Approval 41) looks at the expression of genes in hES cells whose products are of immunological relevance and which may undergo a possible change in expression during cardiac differentiation of hES cells. There are also plans to develop strategies to influence the immune response to hES cells and to cardiac cells derived from them. The immune response triggered following allogenic transplantation of hES cells and the cardiac cells derived from them are to be comprehensively analysed in the animal model.

Studies with a similar goal are envisaged in the eleventh project (Approval 45). The initial focus here is on the targeted differentiation of hES cells into human haematopoietic stem cells and the characterisation of their properties, viability and immunogenicity. The development of methods to reduce the immunogenicity of hES cells and haematopoietic stem cells manufactured from them as well as the analysis of their rejection behaviour after transplantation in suitable mouse models are further goals of the project. All studies in hES cells are to be conducted by way of comparison in hiPS cells, too.

The eighth project (Approval 42) aims to optimise the protocols for the cultivation and effective cardiac differentiation of hES cells. It aims, more particularly, to monitor and quantify the influence of growth factors and specific molecules on the cardiac differentiation of hES cells. Furthermore, there are plans to establish and optimise differentiation in conjunction with miniaturised bioreactors under the influence of small molecules. The work also entails comparative studies of the cardiac differentiation of hES cells and hiPS cells.

Project 9 (Approval 43) addresses the possible influence of specific transposable elements (long interspersed nuclear element-1, Line-1, L1) on the genetic stability of various hES cell lines. It aims to elucidate whether and on what scale hES cells produce the gene products needed for retrotransposition and whether the retrotransposition rate of L1 elements in hES cells changes in the course of differentiation into liver, lung and blood cells. Furthermore, it aims to examine whether the frequency of retrotransposition increases during the long-term cultivation of hES cells and whether the integration of mobilised retrotransposons tends to occur at preferred sites in the genome of hES cells. Studies are to be conducted in comparison to hiPS cells.

The tenth project (Approval 44) focuses on the use and optimisation of novel techniques, so-called microfluid systems, for the cultivation and differentiation of hES cells. It seeks to examine the influence of various carrier materials, media and so-called small molecules on the growth and properties of hES cells and on their differentiation into cardiac and hepatic cells. Based on the evaluation of miniaturised cultivation systems, automated cultivation methods and encapsulation conditions for the cultivation and differentiation of hES cells, reproducible, standardisable and, where appropriate, xenogenous-free cultivation methods are to be developed that can also be applied to hiPS cells. The work is part of the EU project “High Yield and Performance Stem Cell Lab” (HYPERLAB). It is conducted with other partners from abroad and with Cologne University (see Approval 42).

Cultivation methods in bioreactors also play a role in Project 12 (Approval 46). Here the goal is to develop a pancreatic differentiation model for the *in vitro* reconstruction of islets of Langerhans with their insulin-producing cells. Via cells of the definitive entoderm and in co-cultivation with various human cell types, hES cells are to be differentiated into mature pancreatic beta cells. To this end, three-dimensional co-cultivation systems and miniaturised bioreactors are to be used. The functionality of the differentiated cells both *in vitro* and after transplantation in diabetic mouse models are to be examined. Furthermore, there are plans for studies on the effects of various active ingredients, used to treat diabetes, on the pancreatic cells derived from hES cells. One major focus of the work is a direct comparison of hiPS cells with hES cells in the differentiation into functional pancreatic cells. Thereby hiPS cells from patients with various forms of diabetes are also to be generated with a view to using them to analyse the mechanisms of disease onset.

The thirteenth project (Approval 47) looks at the role of the human Tcl1a oncogene (T-cell leukaemia/lymphoma 1a), in maintaining the pluripotency of hES cells. Following the inhibition of expression and after overexpression of the *Tcl1a gene* in hES cells, the phenotype of the resulting cells is to be comprehensively examined. There are likewise plans to identify and characterise the interaction partners of the Tcl1a protein in undifferentiated hES cells.

The fourteenth project (Approval 48) aims to help to improve understanding of the neuronal differentiation of hES cells into cell types of the peripheral nervous system which are responsible for the perception and transmission of pain stimuli. The focus is on the establishment of conditions under which sub-populations of somatosensory neurons that have differentiated from hES cells, can be harnessed, cultivated and propagated if possible in pure form. The work also aims to help create the foundations for new *in vitro* cell cultivation models in order to examine cellular processes of pain reception and transduction, to analyse the effect of analgesic substances on neuronal cells and, where appropriate, to identify new analgesic substances. The methods developed in the project are to be transferred to hiPS cells, too.

Project 15 (Approval 49) concentrates on the generation of cells from the human retina. Using three-dimensional cultivation systems protocols are to be developed and optimised that make possible the differentiation of hES cells into cells of the retinal pigment epithelium (RPE) and the neural retina. By combining both retinal cell types, the interaction is to be studied between cells in retina differentiation *in vitro*. Transplantation in the sub-retinal space of mice and in mouse models for retina degeneration are to be undertaken in order to examine their survival, their integration in recipient tissue and their functionality. Studies on the question whether hiPS cells and hES cells have a comparable potential for differentiation into retinal cells are planned, too.

Further information on the content of the projects supported by ZES and approved by RKI can be accessed 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 hES cells have been taken over into the assessment of the research projects by RKI.

In its work now spanning seven years, ZES has deliberated on a total of *52 applications* for the import and/or use of hES cells. In addition, *three applications for extensions* to already approved projects were reviewed. In total, *55 opinions* were handed down to RKI, *53 of the votes were positive*. All the projects supported by ZES, aside from one project, were approved by RKI. In the case of the one project that has not been approved up to now, the applicant requested the temporary suspension of the procedure. In Germany 36 research groups in 32 institutions are currently engaged in research involving hES cells. Experimental findings from the approved research projects of eight groups have been presented in 25 scientific publications.

During the reporting period the Committee definitively reviewed ten applications from research groups, that had not previously worked with hES cells as well as five applications from groups that had already been granted corresponding approval in the past. In the run-up to the decision on all these applications, for which in some cases ZES went back to the applicants with further questions, extensive deliberations by ZES were necessary.

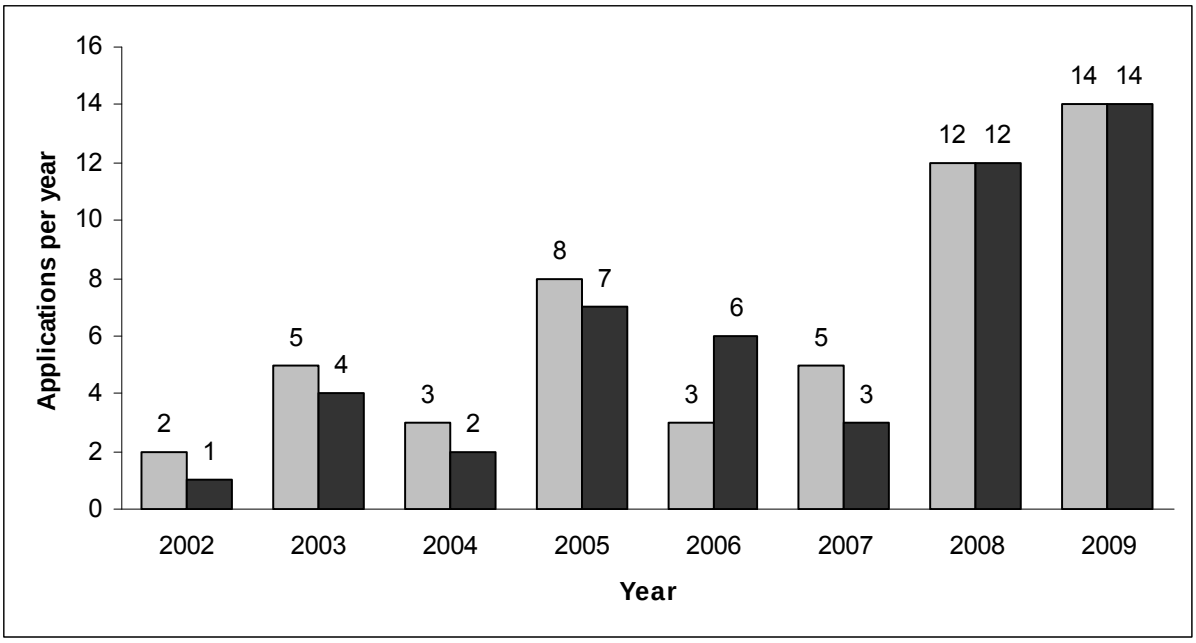

Fig. 1: Number of applications reviewed by ZES (grey columns) and approved by RKI (black columns) pursuant to StZG in the respective calendar year. Extension applications, on which ZES also handed down an opinion, are not included.

Outlook and final comments

The number of applications for the import and/or use of hES cells has risen markedly since 2009. In 2008 and 2009 just as many applications were examined and reviewed as in the previous five years since the entry into force of StZG (see Fig. 1). This can be attributed, amongst other things, to the research in hiPS cells that only began with a description of these cells at the end of 2007 and for which hES cells are required in many cases for comparative purposes.

During the reporting period the number of human embryonic stem cell lines, whose import and use was approved by RKI, has also risen. Whereas between 2002 and the end of 2008 approvals were given for the import and use of 20 of the 21 lines in the NIH register available at that time, approvals have since been given to 23 “new” hES cell lines which were produced between 1 January 2002 and 1 May 2007. They are the following lines: HUES1, HUES2, HUES4, HUES6, HUES7, HUES8 and HUES10 of Harvard University (USA), the lines Shef-1, Shef-2 and Shef-3 of Sheffield University (England), the lines HS181, HS401 and HS415 of the Karolinska Institute (Sweden), the lines NCL3 and NCL4 of the Newcastle Fertility Centre (England), the lines SA121, SA167, SA181, SA348 and SA461 of the company Cellartis AB (Sweden) and the lines KhES-1, KhES-2 and KhES-3 of Kyoto University (Japan). The fact that applications were made for several hES cell lines for the conduct of various projects can be attributed, amongst other things, to the fact that the properties of various hES cell lines are not exactly the same. In many cases it can only be determined during the project which lines are suitable for specific purposes like, for instance, differentiation into a specific cell type.

It should be noted that various applications point out that studies in animal cells in the run up to work with hES cells only have limited meaning for the study question. For instance, murine embryonic stem cells in particular differ in some cases markedly from hES cells, e.g. concerning the molecular mechanisms of their pluripotency or their immunological properties.

Hence concrete project contents, that are to be elucidated using hES cells, cannot be clarified in a preliminary manner in many cases using animal embryonic stem cells. Furthermore, research on hES cells is growing faster than ever on the international level (in 2009 more than 400 original papers were published). Hence, planned questions, including specific methodological procedures, have sometimes already been examined abroad in hES cells. This means that further preliminary clarification in animal cells does not always make sense.

What is noticeable recently is that more projects are being conducted involving parallel studies with hiPS cells and hES cells. hES cells are deemed to have a high potential in basic research, in research into the pathogenicity of disease (as a cell model), in active ingredient and toxicity research and, in the longer term, in regenerative medicine. One goal of the majority of scientific projects reviewed during the reporting period is to examine the question whether and, if so, on what scale hiPS cells and hES cells are in fact the same. So far the mechanisms of reprogramming are still largely unelucidated. It is unclear whether the reprogramming of various human somatic cells (e.g. fibroblasts, cells of the umbilical cord blood, neuronal precursor cells, patients' cells) using various methods (for instance transduction of genes for various transcription factors with the help of various vectors, use of specific small molecules, treatment with a protein cocktail of various transcription factors) leads to pluripotent cells that have comparable properties to hES cells. This means that in research activities involving hiPS cells there is still a need for comparison with hES cells. Hence, the growing body of research with hiPS cells leads to the increased use of hES cells as, without comparative studies, the original properties of hES cells, like pluripotency and differentiatability, cannot be compared with the corresponding experimentally induced properties of somatic cells. However still a significant number of projects focuses on the study of a scientific question in hES cells which – only after successful examination in these hES cells – can then be transferred in part to hiPS cells, too. Based on the experience of ZES in the previous reporting period, hES cells are not just used for comparative purposes – as frequently stressed in the public debate – but also continue to be a separate subject of research.

A number of the applications reviewed by ZES also continue to pursue the goal of possible later cell and tissue therapies based on hES cells. However, the use of hES cells for purposes other than research is still not permitted in Germany. ZES has already drawn attention to this problem in earlier reports.

It has already been mentioned that many of the projects reviewed by ZES in the reporting period envisage comparative research of hES cells and hiPS cells. In its opinions on these projects ZES points out that a vote has to be sought from the Ethics Committee in charge of the responsible research institution if human material is to be harvested to produce hiPS cells from patients or volunteers within the framework of the project.

The remarkable increase in research projects with hiPS cells during the reporting period is also due to the fact that these cells are exposed to fewer public controversies than hES cells. When frequent use is made in public of the term "ethically safe" hiPS cells, this does, however, suggest that the handling of hES cells was in itself a matter of concern from the ethical angle. ZES, however, assumes in the review of the research projects within the intendment of the law that research with hES cells is ethically acceptable.

The Seventh Report was unanimously approved at the 49th ordinary meeting of ZES on 14 December 2009.