Report

of the

Central Ethics Committee for Stem Cell Research (ZES)

14th Report after the enactment of the
Stem Cell Act (StZG)
for the reporting period
1 January to 31 December 2016

1. The Central Ethics Committee for Stem Cell Research

The Central Ethics Committee for Stem Cell Research (ZES) was appointed for the first time when the Stem Cell Act (StZG) came into force in 2002. This independent and interdisciplinary expert body reviews and assesses applications for the import and use of human embryonic stem cells (hES cells) according to the regulations of the Stem Cell Act (StZG), issues an opinion on every application and sends it to the Robert Koch Institute (RKI), the competent authority under the StZG. The Committee's activities 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=//*[@attr_id=%27bgbl108s1708.pdf%27]), 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 interdisciplinary Committee conducts its work on an honorary basis. It is made up of nine members and nine deputies; in accordance with section 8 of the Stem Cell Act (StZG), five members represent the disciplines of biology and medicine, and four members the fields of ethics and theology (see Table 1). In accordance with the ZESV, both the members and the deputy members take part regularly in the meetings and deliberations on the applications.

According to section 9 of the StZG, it is the Committee's task to examine the ethical acceptability of the applications submitted to the RKI for the import and use of hES cells. On the basis of the documents submitted by the applicants, the Committee determines whether a research project intending to use hES cells, for which an application has been submitted, meets the criteria of section 5 of the StZG. Section 5 of the StZG requires that an application must prove in a scientifically substantiated manner (a) that the project pursues research objectives of high-level interest for an increase in scientific knowledge (section 5, no. 1 of the StZG), (b) that the scientific issues have already been subject to a preliminary clarification in other systems, for example in animal-cell models (section 5 no. 2a of the StZG), and (c) that the targeted increase in scientific knowledge requires the use of hES cells (section 5 no. 2b of the StZG). The ZES summarizes the results of its review in a written opinion and sends it to the RKI.

The ZES prepares its reports annually (section 14 of the ZESV). They are published by the Federal Ministry of Health (BMG) and can be accessed via the websites of the BMG (www.bmg.bund.de) and the RKI (http://www.rki.de/DE/Content/Kommissionen/ZES/Taetigkeitsberichte/taetigkeitsbericht_node.html).

Field	Member	Deputy member
Biology	Prof. Dr rer. nat. Hans R. Schröder Max-Planck-Institut für Molekulare Biomedizin Münster	Prof. Dr rer. nat. Martin Zenke Institut für Biomedizinische Technologien Abt. Zellbiologie RWTH Aachen
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Medicine	Prof. Dr med. Mathias Bähr Neurologische Klinik Georg-August-Universität Göttingen	Prof. Dr med. Wolfram H. Zimmermann Institut für Pharmakologie 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. Ursula Just Max-Planck-Institut für Herz- und Lungenforschung Bad Nauheim
Ethics	Prof. Dr phil. Dr med. h.c. Jan P. Beckmann Institut für Philosophie FernUniversität Hagen	Prof. Dr phil. Ralf Stoecker Professur für Praktische Philosophie Universität Bielefeld
	Prof. Dr mult. Nikolaus Knoepffler Lehrstuhl für Angewandte Ethik Universität Jena	Prof. Dr phil. Christine Hauskeller Department of Sociology, Philosophy and Anthropology University of Exeter England
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	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), last revised December 2016

2. Deliberations on and reviews of applications pursuant to section 5 of the Stem Cell Act during the reporting period

In 2016, the ZES held six meetings and discussed a total of 10 applications for the import and use of human ES cells. The ZES handed down positive opinions on all the applications. In addition, 7 applications for extensions of already approved research projects using hES cells were assessed and voted on using the written procedure.

Table 2 provides a summary overview of the applications that were assessed positively by the ZES and approved by the RKI during the reporting period. All the projects listed therein that were discussed by the ZES meet the preconditions of section 5 of the StZG and are ethically acceptable within its intendment (section 9 of the StZG).

No.	Applicant	Research topic	Date of the positive ZES opinion
1 (106)	Prof. Dr. Christian Rosenmund, Charité – Universitätsmedizin Berlin	Ultrastructural studies of the synapses of synapsin-1-deficient neurons derived from hES cells	17/02/2016
2 (107)	Prof. Dr. Katja Schenke-Layland, Universitätsklinikum Tübingen	Studies on the influence of biophysical stimuli on the differentiation and maturation of cardiomyocytes derived from human embryonic stem cells	29/02/2016
3 (108)	Medizinische Hochschule Hannover	Establishment of cell models for cardiac hypertrophy. Study of the effects of small open reading frame-encoded polypeptides	14/03/2016
4 (109.110)	TWINCORE – Zentrum für Experimentelle und Klinische Infektionsforschung GmbH (Centre for Experimental and Clinical Research into Infectious Diseases)	Establishment of cell models on infection with the hepatitis C virus (HCV) and human respiratory syncytial virus (hRSV)	14/03/2016
5 (111)	Dr. Micha Drukker, Helmholtz Zentrum München	Study on the influence of certain *Polycomb-group*-associated and *Trithorax-group*-associated proteins on pluripotency and differentiation of human embryonic stem cells	18/05/2016
6 (112)	Medizinische Hochschule Hannover	Stem-cell-based myocardial reconstruction in animal models	18/05/2016
7 (113)	Medizinische Hochschule Hannover	Extraction of human pulmonary cells from pluripotent stem cells and their functional *in-vitro* and *in-vivo* analysis	13/06/2016
8 (114)	Prof. Dr. Michael Schäfer, Universitätsmedizin der Johannes Gutenberg-Universität Mainz	Study of L1-deficient human neurons derived from hES cells to clarify pathophysiological mechanisms	17/10/2016
9 (115, 116)	Prof. Dr. Wieland Huttner, Max Planck Institut für molekulare Zellbiologie und Genetik, Dresden Prof. Dr. Svante Pääbo, Max Planck Institut für evolutionäre Anthropologie, Leipzig	Studies on the evolution of the human brain using cerebral organoids derived from hES cells	17/10/2106

10 (117)	Rheinische Friedrich-Wilhelms-Universität Bonn	Genome editing and neural differentiation of human embryonic stem cells for research into psychiatric disorders	16/11/2016
Extensions of already approved applications			
11 Extension of approval no. (62)	Evotec AG, Hamburg	Establishment of a cell model for Huntington's disease using human embryonic stem cells: characterization of neuronal dysfunctions and screening of potential active substances using high-throughput methods	03/02/2016
12 Extension of approval no. (90)	Dr. Anthony Gavalas, Technische Universität Dresden	Differentiation of human embryonic stem cells into pancreatic beta cells and motor neurons, and their functional characterization	27/04/2016
13 Extension of approval no. (55)	Medizinische Hochschule Hannover	Comparative study on glycosylation patterns in human embryonic and induced pluripotent stem cells	01/08/2016
14 Extension of approval no. (105)	Medizinische Hochschule Hannover	Pancreatic beta cells derived from hES cells: development of improved methods of their production, analysis of their molecular properties, and study of toxic effects of proinflammatory cytokines	03/08/2016
15 Extension of approval no. (63)	Prof. Dr. Beate Winner Friedrich-Alexander-Universität Erlangen-Nürnberg	Development and characterization of cell models for human neurodegenerative diseases using human embryonic stem cells	16/08/2016
16 Extension of approval no. (8)	Dr. Katrin Zeilinger Bioreactor Group Berlin Brandenburg Center for Regenerative Therapies (BCRT), Charité Campus Virchow-Klinikum, Berlin	Development of a 3D culture system for the expansion of human embryonic stem cells and their differentiation in liver cells	06/09/2016
17 Extension of approval no. (74)	Medizinische Hochschule Hannover	Comparative study of glycosylation during neuroectodermal differentiation in human pluripotent stem cells	25/10/2016

Table 2. Overview of research projects that were approved by the RKI during the 2016 reporting period following a positive assessment by the ZES. The numbers in brackets in the left-hand column correspond to the approval numbers in the RKI register (http://www.rki.de/DE/Content/Gesund/Stammzellen/Register/register_node.html)

The first research project listed in Table 2 (106th approval under the Stem Cell Act) deals with a nerve-cell model based on hES cells for examining molecular and cell-biological effects of genetic modifications in the synapsin-1 gene (*SYN1*), which is related in humans to autism spectrum disorders (ASD) and forms of epilepsy. To this purpose, wild-type hES cells and hES cells with a mutated *SYN1* gene are to be differentiated into neurons and, after activation, examined with an electron microscope in order to comprehensively characterize their synaptic activity at the ultrastructural level. The model system is also to be used to test selected pharmaceuticals and their influence on the structure and activities of the synapses. The project is expected to make an important contribution to clarifying the structure and functioning of human synapses, particularly with regard to the nature of vesicle release and synaptic transmission, and in this way contribute to our understanding of the molecular principles behind the above-mentioned illnesses. Furthermore, it could lay important foundations in the systematic search for active substances and therapeutic procedures for

overcoming or alleviating the symptoms of diseases such as certain forms of autism and epilepsy.

The focus of the second project (107th approval) is on studying the influence of biophysical signals on the differentiation of hES cells into mature and functional cardiomyocytes, which has hitherto not been sufficiently successful *in vitro*. A particular objective is to replicate *in vitro* conditions to which human heart cells are exposed during their maturation *in vivo*. To this purpose, hES cells are to be differentiated towards cardiac cells on optimized carrier materials in a bioreactor under 3D conditions, and comprehensively examined as regards the presence of mature cardiac cells after mechanical stress and electrical stimulation. In particular, the aim is to determine the influence of biomechanical stimulation on the differentiation of hES cells into specific cardiomuscular cell types (ventricular, atrial, nodal). The project is expected to contribute to extending our understanding of cardiac differentiation processes in humans. It can also lay important foundations for the development of efficient *in vitro* differentiation protocols, making it possible in the future to provide a sufficient quantity of mature heart-muscle cells. This is of great relevance for pharmacological-toxicological research, for disease research and for the development of regenerative therapies.

The third research project (108th approval) aims to establish a cell model for cardiomyocyte hypertrophy in humans in order to identify pro- and anti-hypertrophic substances using high-throughput methods. Pathological cardiac hypertrophy is one of the main causes of heart failure and sudden cardiac death, especially in younger patients. The first step will be to further develop and optimize the methods for differentiating hES cells into cardiomyocytes; then, building on this, the effects of anti-hypertrophic substances at the cellular and molecular level will be analysed using hES reporter-cell lines. This will also involve searching different substance libraries for pro- and anti-hypertrophic molecules and studying different aspects of the impact of pro- or anti-hypertrophic polypeptides. Finally, the studies referred to will extended to artificial heart tissue (*bioartificial cardiac tissue*, BCT), which is derived from hES cells and contains not only cardiomyocytes, but also other cardiac cells such as endothelial cells or cardiac fibroblasts. All the studies are also to be conducted using human induced stem cells (hiPS cells). The project can contribute to a better understanding of the foundations of heart-muscle hypertrophy and to identifying new active substances for treating hypertrophic heart.

The subject of the fourth research project (109th and 110th approval) is the establishment of cellular models from hES cells as customized systems for the study of viral infections (hepatitis C virus (HCV) and respiratory syncytial virus (hRSV)). Studying these viral infections at the cellular level requires human cells (liver or lung cells), and, at present, the required quality of such cells can only be obtained from human pluripotent stem cells. The first part of the project is to be devoted to studying the relation between HCV-associated cellular lipoproteins and the persistence of the virus. The intention is to analyse the cellular lipoproteins with which HCV interacts and how the incorporation of certain lipids and lipoproteins into the HCV capsid modulates the binding of the virus to cell-surface receptors and the effectiveness of antibodies against HCV. To this purpose, hES cells are first to be differentiated into liver-cell-like cells (hepatocyte-like cells, HLC). The differentiated cells are to be comprehensively characterized, and in particular components of fat metabolism are to be subjected to a quantitative and qualitative examination. The second part of the project will focus on analysing early events in hRSV infection. This will involve differentiating hES cells into lung epithelial cells and analysing the hRSV infection at the molecular level, particularly with regard to the interaction of the virus with potential receptors, as well as the cell entry of the virus. All the research work will also make comparisons with hiPS cells. The project is expected to contribute to extending our knowledge and understanding of the interactions between the studied viruses and their host cells, and is thus of importance for clarifying the pathogenesis of the diseases caused by these viruses. This can help create the basis for new therapeutic procedures for use in humans, e.g. in the development of a prophylactic vaccine against HCV and, in addition, to the validation of substances that prevent the hRSV infection.

The focus of the fifth research project (111th approval) is on studying the function of certain chromatin-modifying proteins which are associated with *Polycomb-group* (PcG) and *Trithorax-group* (TrxG) proteins during early differentiation processes of human cells. Mutations in the affected genes are sometimes associated with serious diseases in humans. These include various forms of leukaemia and other tumours, as well as severe embryonal and infantile development disorders. To date, the role played by these proteins in the epigenetic control of pluripotency and differentiation processes has not been fully clarified. Therefore, the respective genes are to be changed into hES cells in such a way that proteins are formed which are no longer functional or whose function has been modified, and the effects – particularly on the ability of the modified hES cells to differentiate into neural and cardiac cells, as well as into mesenchymal precursor cells – are to be comprehensively investigated. In addition, further genes that are associated with the investigated genes are to be identified and analysed. All the studies are also to be carried out using hiPS cells that have been derived both from corresponding patients and from healthy subjects. The research work can contribute to gaining new insights into the hitherto insufficiently researched role of chromatin-modified proteins, which are associated with *Polycomb group* (PcG) and *Trithorax group* (TrxG) proteins, in the early stages of human development. Furthermore, the project can also make a contribution to clarifying the pathogenesis of the associated diseases.

The sixth research project (112th approval) concentrates on establishing new strategies for the stem-cell-based regeneration of damaged heart tissue and on testing them in different animal models. Cardiovascular diseases are often accompanied by a massive loss of heart-muscle cells and are among the most common causes of death. The first step will be to produce cardiac cells and cardiac tissue from hES cells. These are then to be applied in a number of animal models, inter alia to examine which is the best form of administration in order to restore the heart function. In addition, hES cells are to be provided with reporter genes in order to facilitate the *in-vitro* enrichment of different cardiac-cell types, to analyse the further development and maturation of the various cell types within the framework of the three-dimensional tissue produced *in vitro,* and to track the fate of specific cells types after transplantation. The work mentioned will also make use of hiPS cells. This research work is expected to yield new fundamental findings, e.g. on the reproducible extraction of transplantable human heart tissue, and on the most suitable cellular source for the production of the cardiac replacement tissue.

The long-term aim of the seventh research project (113th approval) is the development of new stem-cell-based therapies for diseases of the lungs and the development of *in vitro* testing systems for use in pharmacological/toxicological screening systems. With the help of specific differentiation protocols and using the influence of extracellular matrix factors, the intention is to generate from hES cells pulmonary cell types that are as mature as possible, an area where success has so far been limited. The first step will be to establish reporter-cell lines for different lung cell types and, building on this, to develop an *in vitro* model system for the further maturation of the precursor cells. The correspondingly modified hES cells are also to be used to search low-molecular substance libraries in order to identify hitherto unknown factors that promote pulmonary differentiation. A further focus of the work is on achieving a functional maturation of lung (precursor) cells derived from hES cells *in vitro* using decellularized lung tissue. The resulting pulmonary tissue thus artificially extracted is also to be examined to determine its suitability for use in pharmacological/toxicological screening procedures. Finally, there are to be studies on the further maturation and the engraftment of hES-cell-derived lung (precursor) cells *in vivo* by transplantation into rodent models for lung damage. The work is also to be carried out with hiPS cells in order, inter alia, to examine whether hES and hiPS cells are equally suitable for differentiation into pulmonary cells. The research project is expected to contribute to extending our understanding of pulmonary differentiation processes in humans, and to gaining knowledge that can be of considerable importance for a targeted future tissue-replacement therapy in diseases of the lung.

The focus of the eighth research project (114th approval) is on studying the functions of the cell-adhesion and cell-recognition molecule L1, whose functional *knockout* causes serious development defects in the human nervous system and can lead to recessively inherited X-chromosomal L1 syndrome. The first part of the work aims to characterize the cellular pathogenicity of L1-syndrome-associated point mutations in the *L1* gene. To achieve this, initially hES-derived L1-deficient neurons are to be used to develop a human *in vitro* model, with which the pathogenicity of *L1* genetic defects can be evaluated. Another study will investigate the extent to which L1 is involved in neurodegenerative processes (e.g. via the interactions between neurons and T-lymphocytes) and the role played by L1 as regards the integrity and survival of neurons under ischemic conditions, such as those caused by a stroke or craniocerebral trauma. The results of the work could generate new insights into the extent to which cell-adhesion molecules are relevant for protection and regeneration processes in the nervous system. It could also clarify molecular principles of development-related deficits that are related to L1-syndrome.

The overarching objective of the ninth research project (115th and 116th approval) is to identify the evolutionary, development-biological and functional changes that led in the course of human evolution to a differentiation of the human brain and to neurobiological foundations of the cognitive abilities of *homo sapiens sapiens*. The main emphasis of the work is on the functional analysis of human-specific genes that are strongly expressed in the cortical germ zones and could thus play a role in the development of the human neocortex. In this context, corresponding genes are to be functionally deleted, and the effects of these genetic alterations studied *in vitro* in a stem-cell-based cerebral organoid system. Genes of relevance to the development of the cortex will also be modified in such a way that their sequence corresponds to the corresponding genes in Neanderthals or great apes. The research work is likely to make an important contribution to our understanding of the cell-biological and genetic foundations which, from an evolutionary point of view, led to new functions and specifically human abilities such as language and complex sociality. They could also lead to new findings relating to diseases that impair those skills.

The tenth research project (117th approval) aims to improve our understanding of the processes that take place at the molecular and cellular level in the development of psychiatric disorders. To this purpose, initially mutations that are (potentially) associated with such psychiatric disorders as schizophrenia and autism spectrum disorders are to be generated in hES cells. The genetically modified hES cells are to be differentiated into various types of neural cells; these – in comparison with neural cells differentiated from wild-type hES cells – will be comprehensively characterized at the levels of the transcriptome, the epigenome and the (phospho)proteome, as well as in relation to their morphological, biochemical, electrophysiological and pharmacological properties. Finally, neurons differentiated from hES cells are to be transplanted into rodents and the cells comprehensively characterized at different points in time after the transplantation. The experiments will also make comparisons with (disease-specific) hiPS cells. If they are successful, the results might lead to new insights into the molecular and cellular pathogenic mechanisms of psychiatric disorders, which can then form the basis for the development of therapeutic agents.

During the reporting period, applications were made for the extension of research work relating to seven approvals on which the ZES commented (see nos. 11 to 17 in Table 2).

The objective of the research project listed under no. 11 is to clarify molecular and cellular processes involved in the development of Huntington's disease and, where possible, to identify substances that can influence the expression of the disease. Originally, the approved work was only to be conducted on cells of the cell population most affected by Huntington's disease, so-called medium-spiny neurons (MSN). Now, the work is also to be extended to other types of neural cells that are likewise affected by Huntington's disease. In addition, hES cells are to be used in which the Huntington gene (HTT) contains CAG trinucleotide repeats of different lengths ('HTT allele series') and which, after neural differentiation, are expected to provide good images of the cellular phenotype of Huntington's disease at the cellular level.

The research project is expected to contribute to a better understanding of the pathogenesis mechanisms in the genesis of Huntington's disease.

A need for further work connected with the generation of pancreatic beta cells from hES cells became necessary during the implementation of the research project listed under no. 12, which aims, among other things, to extract pancreatic beta cells from hES cells. This additional work includes in particular the transfer of reporter genes into the loci of genes whose products can serve as indicators of a successful differentiation towards pancreatic beta cells or other cell types, as well as additional transplantation experiments into the eye chambers of mice, in order to be able to analyse the maturation of pancreatic progenitor cells *in vivo*. The research work is expected to contribute to an improved understanding of the molecular foundations of the processes that take place during the maturation of pancreatic precursor cells into viable pancreas cells.

Different cardiomyocyte subtypes (ventricular, atrial and nodal cardiomyocytes) are to be incorporated into the project referred to under no. 13, in which comparative studies are being carried out on the glycosylation patterns of hiPS and hES cells and cardiac cells derived from them. Furthermore, the analyses are be extended to the secretome of cells differentiating into cardiac cells; another aim is to identify and comprehensively characterize cell-type-specific (glycosylated) surface proteins that could be used as markers both for specific types of cardiac cell and for differentiation states during cardiac differentiation. The research work is expected to lead to a deeper understanding of glycosylation processes in cardiac differentiation, as well as (perhaps) to improved cardiac differentiation protocols, which will be needed for a future use of human pluripotent stem cells in tissue-replacement therapy.

The project listed under no. 14, which deals with the differentiation of hES cells into pancreatic cells, is now to be extended to include the study of the *in vivo* maturation of the differentiated cells after transplantation in mouse and rat models of type I diabetes mellitus. In this context, various materials for the encapsulation of the differentiated cells before transplantation are to be tested. The research work is expected to generate new insights into the characteristics of pancreatic cells derived from hES cells and might lead to new therapeutic methods in the treatment of diabetes mellitus.

The project listed under no. 15 aims to establish and characterize an hES-cell-based nerve-cell model for examining degenerative motor neuron diseases such as amyotrophic lateral sclerosis (ALS) or hereditary spastic spinal paralysis (HS). In an extension of the work approved hitherto, further genes will now be examined whose products are involved in processes of neural development and neurodegeneration. The work on the project is expected to make an important contribution to our understanding of the pathogenesis mechanisms in the development of human motor neuron diseases for which there are currently no therapeutic approaches.

In the project specified under no. 16, which aims to develop and establish a three-dimensional culture system for the cultivation of hES cells and their differentiation into cells similar to liver cells, additional analyses are to be carried out on the glycosylation patterns of hES cells and liver-cell-like cells differentiated from hES cells, also in comparison to primary human hepatocytes. These studies could generate knowledge on why *in vitro* differentiation strategies have not hitherto led to mature human hepatocytes, and on how the corresponding procedures need to be modified in order to obtain such cells. Furthermore, the identification of specific glycosylation patterns of hepatic cells could lead to the establishment of quality markers for a successful hepatic differentiation of human pluripotent stem cells.

The research projects listed under no. 17 deal with comparative studies of the glycosylation pattern of hiPS and hES cells, in order to determine possible differences between the two cell types. The projects will examine how the glycome, transcriptome and proteome of both cell types change in the course of neuroectodermal differentiation. The focus of the study is on hiPS cells of patients suffering from a hereditary glycosylation defect (congenital disorder of glycosylation Ia, CDG Ia). This disease can be expressed in serious neuro-mental disorders. The corresponding research work is now to be intensified in the context of an

extension. Hepatic differentiation is to be incorporated into the studies in addition to neuronal differentiation, since the disease CDG Ia also leads to disturbances of liver metabolism. On the other hand, the project will make a closer study of issues involving the role of C-mannosylation in human pluripotent stem cells and neural and hepatic cells derived from them. The results of this work could help to deepen our understanding of the role of specific glycosylations into pluripotent and differentiating cells as well as our understanding of a modified glycosylation in pathological situations.

Further information on the content of the research projects is available from the RKI's register (http://www.rki.de/DE/Content/Gesund/Stammzellen/Register/register-inhalt.html). In each case, the essential arguments made by the ZES justifying the high-ranking status of the research projects, their adequate preliminary clarification and the necessity to use human ES cells were also included in the RKI's assessment of the research projects.

Five of the new applications discussed during the reporting period were submitted by researchers or institutions that had not yet previously received an approval under the Stem Cell Act. Five applications were made by working groups at an institution that had already received approvals under the Stem Cell Act in the past. All the applications were approved by the RKI after review by the ZES. During its 14 years of activity, the ZES has submitted opinions to the RKI on a total of 117 applications for the import and/or use of hES cells. In addition, a total of 34 applications for extensions of already approved projects, on which the ZES issued an opinion, have been approved by the RKI to date. In its decisions on the eligibility of applications for approval, the RKI has followed the ZES's recommendations in all cases up to now.

Since the Stem Cell Act came into force, the RKI has issued 117 approvals, some of which were extended. Eighteen of these approvals have expired to date. At present, 72 groups at 48 research institutions are conducting approved research work with hES cells.

3. Developments and trends in research using human embryonic stem cells in Germany

1. In the period under review, some of the research projects applied for aim to optimize the differentiation of hES cells into specific cell types and their functional *in vitro* and *in vivo* analysis. They aim to explain basic molecular mechanisms, which lead, for example, to the maturation and functionality of the cardiac and pulmonary cell types generated *in vitro*. Further objectives are to examine the influence of biophysical stimuli, the matrix or certain carrier materials and, using high-throughput methods in substance libraries, to identify new low-molecular substances that have a differentiation-promoting effect. This is a prerequisite both for the development of efficient pharmacological/ toxicological *in vitro* testing systems with human cells, and for future cell-replacement therapies in humans. On the other hand, the aim is to establish cell models from hES cells for genetic diseases, in order to contribute in this way to understanding the molecular foundations of the respective disease and, in the long term, to develop new therapeutic procedures. In addition, the projects evaluated in the reporting period deal with the establishment of cell models for cardiac hypertrophy and also with the establishment of cellular models from hES cells for the study of viral infections (hepatitis C virus (HCV) and respiratory syncytial virus (hRSV)). Such hES-cell-based cell models are also to be used for screening active substance libraries. They can contribute to identifying potential active substances and ultimately to the development of new therapeutic methods for the treatment of certain forms of autism and epilepsy, cardiomyocyte hypertrophy and viral infections. The evaluated research projects also aim to attain new knowledge of the epigenetic control of pluripotency and differentiation processes, and to identify the evolutionary, development-biological and functional changes that led in the course of human evolution to a differentiation of the human brain and to neurobiological foundations of the cognitive abilities of *homo sapiens sapiens*.

2. Comparative studies on hiPS cells and hES cells are again the subject of numerous research projects in 2016. 7 of the 12 newly approved research projects study hiPS cells and hES cells in parallel (Figure 1). In some projects, hES cells will be used as reference material for assessing the differentiation potential of hiPS cells. The various hiPS cell lines differ considerably in terms of their ability to differentiate. The reasons for this can be found in the genetic background of the donors, the properties of the somatic cells used for reprogramming, the reprogramming method (e.g. retroviral or lentiviral vectors), the factors used for reprogramming, or a possible epigenetic memory of the cells. Another aim of these studies is to clarify which human pluripotent stem-cell lines will be most suitable in the future for potential cell and tissue therapies. In other projects aiming to model diseases *in vitro* and to clarify pathogenesis mechanisms at the cellular level, hiPS cells are derived from cells of ill people and compared with hES cells in which the mutation causing the disease has been generated, as well as with non-modified hES cells. Particularly in the case of diseases caused by singular genetic modifications, hES-cells represent valuable reference material, because they enable the effects of the respective genetic modification to be studied and compared with non-modified hES cells against an otherwise identical genomic background. These studies can contribute to our understanding of the molecular causes of these diseases and help identify targets for the development of therapeutic agents.

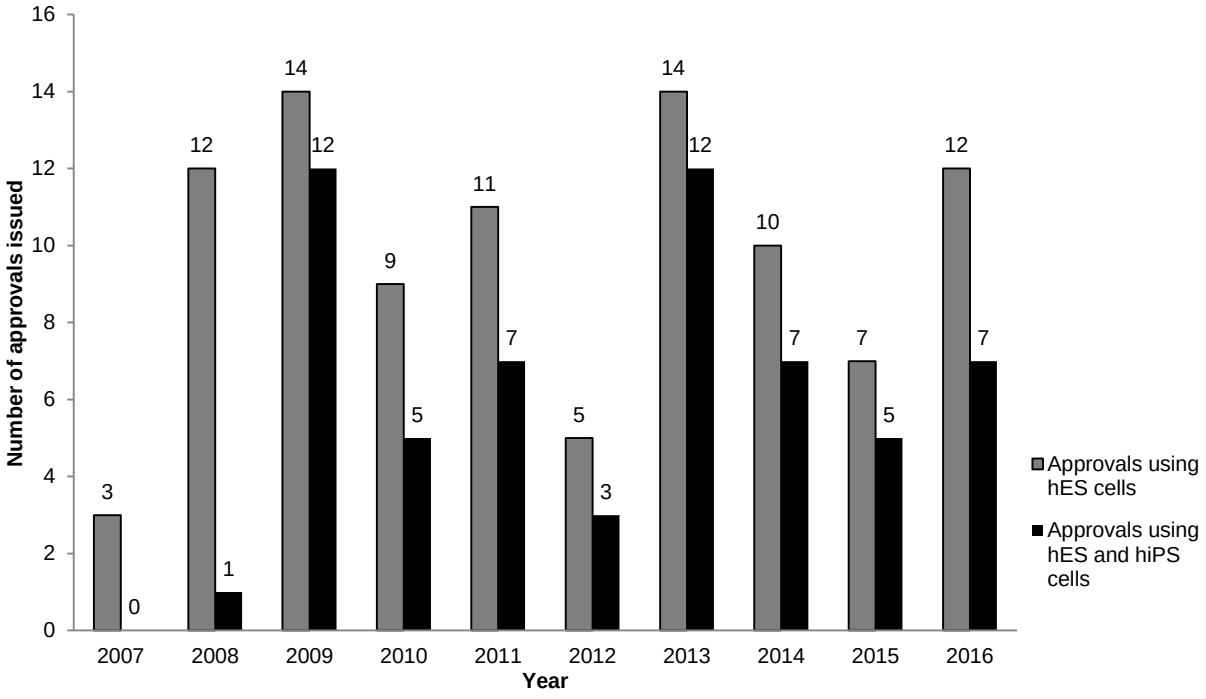

Figure 1. Use of hES and hiPS cells in approved research projects, 2007-2016. The chart shows the total number of approved research projects (grey) and the number of research projects in which not only hES but also hiPS cells are used (black).

3. At its 86th meeting on 13 June 2016, the ZES was informed about novel methods for producing cellular organoids, which are also the subject of research with hES cells in Germany. In recent years, a number of new procedures have been developed for producing microstructures, known as organoids, which imitate the structure of various organs in the human body. As a rule, their size is limited to a few millimetres, since they lack the supporting stroma (e.g. blood vessels). The model systems are made in the cell culture from pluripotent stem cells under the influence of suitable growth factors. Pluripotent stem cells have the ability to differentiate into any cell in the body; they also have a high potential for self-organization, following their natural development programme. Both are properties that can be used in organoid formation. Adult stem cells are also used. This has made it possible to replicate a large number of organoids representing different types of human tissue in a cell culture dish. These include in particular organoids of the intestine, the kidney, the brain, the retina, the liver and the heart. What they all have in common is their three-dimensional structure, giving them a comprehensive complexity that cannot be achieved in a two-dimensional culture. This stem-cell-based technology opens up new possibilities for researching diseases and developing drugs and new treatment plans, e.g. in the field of tissue-replacement and cancer therapy. Experiments with mice, for example, have shown that organoids generated from single stem cells of the intestine can integrate into living tissue and repair damaged intestinal mucosa. Similar results have been reached with liver organoids. There has already been a first practical application of organoids in the context of personalized cancer therapy: Hans Clevers, who was awarded the Körber Prize in 2016, and his team of researchers succeeded in generating patient-specific colorectal cancer organoids. Using an automated system, they were able to examine how pharmacologically effective substances influenced the growth of the cancer organoids. In the last few years, organoids have also been increasingly used in the chip-based multi-micro-organoid culture system, in which organ-like tissues can be combined as in the human or animal body, nurtured by a form of circulation and connected with each other.

4. At its 88th meeting on 16 November 2016, the ZES dealt with the legal expert opinion by Prof. Dr. Ralf Müller-Terpitz on embryo definitions in German and European law, which was commissioned by the Ethical, Legal and Social Scientific Competence Network on Stem-Cell Research in North Rhine-Westphalia. Several central questions of interpretation have already been the subject of detailed discussions by the ZES in the past when specific inquiries have been made or applications filed.

   This relates in particular to

   - the importance for criminal law of different legal definitions of the embryo in different regulatory contexts, in particular in the administrative Stem Cell Act (StZG, 2002) and the Embryo Protection Act (ESchG, 1990),
   - the assessment as to whether parthenotes are embryos within the meaning of the StZG, and
   - the implications of the statements made by the ECJ on the definition of the embryo under patent law in the decision dated 18 October 2011, Rs.C- 34/10 (known as the Brüstle Patent), including the limits of European and national legal regimes (harmonized patent law vs. national-state-specific regulations [e.g. in the ESchG], and on reservations on research [e.g. in the StZG]).

   The ZES shares the expert's view both on the relevance of the ability to develop when assessing whether an entity is an embryo within the meaning of the ESchG or StZG, and on the historically determined regulation of reproductive medicine in the context of a criminal law, which leads to certain implications and limitations. The ZES also has a positive view of the fact that the expert opinion defined the respective scopes of the embryo terms and distinguished them from one another, identified possible areas of conflict and illustrated the legal impacts. The ZES also underlines the importance of the expert's statement that there is a need for clarification and, in some cases, modification

of the ban on cloning under section 6 of the ESchG in the light of new scientific and technological possibilities.

5. Since 2010, research on pluripotent stem cells has not only related to basic research; at the international level it also continues to move ever closer towards a clinical application. Table 3 gives an overview of the clinical trials conducted worldwide with pluripotent stem cells in the period from 2010 to 2016. In the approved studies, cells derived from pluripotent stem cells are being tested to determine their suitability for treating diseases for which no adequate therapy options are currently available. Their safety and tolerability in particular are being reviewed. The majority of the clinical trials between 2010 to 2016 were carried out using cell types differentiated from hES cells (23), followed by studies of cell types differentiated from hiPS cells (3) and from parthenogenetic stem cells (1). Most of the studies conducted with cells derived from hES cells (15) focus on the treatment of different forms of macular degeneration. Other studies target the treatment of different eye diseases (4), type I diabetes mellitus (1), ischaemic heart diseases (1), and injuries to the spinal cord (2). In the studies using hiPS cells that were begun only recently, the focus is on the treatment of age-related macular degeneration ((2), one of the studies was suspended in 2015 as a result of genetic changes in the hiPS cells) or on graft-versus-host disease (1). To date, the only study using parthenogenetic stem cells, in which parthenogenetic neural stem cells are transplanted, serves the treatment of Parkinson's disease.

Summary of clinical trials with pluripotent stem cells (2010-2016)

	Disease	Number of studies	Participants
hES cells	Diseases of the eye and the appendages of the eye	19	295
	Age-related macular degeneration (AMD)	9	166
	Stargardt's disease (hereditary juvenile form of macular degeneration)	5	59
	Myopic macular degeneration	1	12
	Retinitis pigmentosa	1	10
	Other eye disorders	3	48
	Endocrinal, nutritional and metabolic diseases	1	40
	Type 1 diabetes mellitus	1	40
	Diseases of the circulatory system	1	6
	Ischemic heart disease	1	6
	Diseases of the nervous system	2	40
	Spinal-cord injuries	2	40
hiPS cells	Diseases of the eye and the appendages of the eye	2	16
	Age-related macular degeneration (AMD)	2	16
	Injuries, poisonings and certain other consequences of external causes	1	16
	Graft-versus-host disease	1	16
hpPS cells	Diseases of the nervous system	1	12
	Parkinson's disease	1	12
	Total	27	425

Table 3. Phase I/II clinical trials with cells developed from pluripotent stem cells, sources: ClinicalTrials.gov, a service of the U.S. National Institutes of Health (NIH), and International Clinical Trials Registry Platform (ICTRP) of the World Health Organization (WHO); data last revised 31 December 2016

See Table 4 for an overview of the countries in which the studies are being carried out. In summary, it can be said that in the period from 2010 to 2016 the USA was the leading nation when it came to the use of hES cells with a total of 10 clinical trials; it was followed by China with a total of 5 clinical trials also using hES cells.

Overview of the countries where clinical trials with pluripotent stem cells are carried out (2010 -2016)

	Country	Number of studies
hES cells	USA	10
	China	5
	UK	3
	Korea	2
	Brazil	1
	France	1
	Israel	1
	Canada	(1)*
hiPS cells	Australia	1
	UK	1
	Japan	1
hpPS cells	Australia	1
	Total	27

Table 4. Overview of the countries participating in clinical trials. 1* Canada together with USA. Last revised: 31 December 2016

The 14th Report was adopted at the 89th ordinary meeting of the ZES on 13 February 2017.