Report

of the

Central Ethics Committee for Stem Cell Research (ZES)

15th Report after the Enactment of the Stem Cell Act (StZG) for the Reporting Period 1 January to 31 December 2017

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 connection 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), as 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 Committee conducts its work on an honorary basis and is made up of nine members and nine deputy members; 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). The fifth appointment term expired in August 2017. The following members and deputy members left the Committee: Prof. Dr Just, Prof. Dr Beckmann and Prof. Dr Hilpert. Fifteen members and deputy members were reappointed, and one member and two deputy members were appointed for the first time to the ZES for what is now the sixth appointment term (2017 to 2020). 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 Hans R. Schöler Max-Planck-Institut für Molekulare Biomedizin Münster	Professor Dr Martin Zenke Helmholtz-Institut für Biomedizinische Technik RWTH Aachen
	Prof. Dr Anna M. Wobus **(Deputy Chair)** Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK) Gatersleben	Prof. Dr Maria Wartenberg Molekulare Kardiologie und Stammzellforschung Universitätsklinikum Jena
Medicine	Prof. Dr Mathias Bähr Neurologische Klinik Georg-August-Universität Göttingen	Prof. Dr Wolfram H. Zimmermann Institut für Pharmakologie und Toxikologie Georg-August-Universität Göttingen
	Prof. Dr Marion B. Kiechle **(Deputy Chair)** Universitätsklinikum rechts der Isar Technische Universität München	Prof. Dr Ricardo E. Felberbaum Frauenklinik Klinikum Kempten Oberallgäu
	Prof. Dr Anthony D. Ho Med. Universitätsklinik und Poliklinik Ruprecht-Karls-Universität Heidelberg	Prof. Dr Beate Winner Nikolaus-Fiebiger-Zentrum für Molekulare Medizin Universitätsklinikum Erlangen
Ethics	JProf. Dr Dr Sabine Salloch Institut für Medizinische Ethik und Geschichte der Medizin Ernst-Moritz-Arndt-Universität Greifswald	Prof. Dr Ralf Stoecker Abteilung Philosophie Universität Bielefeld
	Prof. Dr mult. Nikolaus Knoepffler Lehrstuhl für Angewandte Ethik Friedrich-Schiller-Universität Jena	Prof. Dr Christine Hauskeller Department of Sociology, Philosophy and Anthropology University of Exeter, England
Theology	Prof. Dr Klaus Tanner **(Chair)** Theologisches Seminar Ruprecht-Karls-Universität Heidelberg	Prof. Dr Hartmut Kress Evangelisch-Theologische Fakultät Rheinische Friedrich-Wilhelms-Universität Bonn
	Prof. Dr Dr Antonio Autiero Katholisch-Theologische Fakultät Westfälische Wilhelms-Universität Münster	Prof. Dr Dr Jochen Sautermeister Katholisch-Theologische Fakultät Rheinische Friedrich-Wilhelms-Universität Bonn

Table 1. Members and deputy members of the Central Ethics Committee for Stem Cell Research (ZES) as of their appointment on 20 August 2017.

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

In 2017, the ZES held five meetings and discussed a total of 13 applications for the import and use of hES cells. The ZES handed down positive opinions on all the applications. In addition, two 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.	Holder of approval	Topic of approved work	Date of the positive ZES opinion
1 (118)	Prof. Dr Markus Riemenschneider, Universitätsklinikum Regensburg	Studies on the epigenetic stability of human pluripotent stem cells	13 February 2017
2 (119)	Max-Planck-Gesellschaft, Max-Planck-Institut für molekulare Biomedizin, Münster	Studies on the reprogramming of somatic cells into stem-cell-derived human brain organoids	13 February 2017
3 (120)	Max-Delbrück-Centrum für Molekulare Medizin (MDC), Berlin	Studies on early processes of spinal-cord development in humans; development of improved protocols for differentiating hES cells in motor neurons	13 February 2017
4 (121, 122)	Prof. Dr Beate Winner, Universitätsklinikum Erlangen Prof. Dr Dieter C. Li, Friedrich-Alexander-Universität Erlangen-Nürnberg	Studies on the molecular foundations of neural developmental disorders in humans	10 April 2017
5 (123)	Dr Insa S. Schröder, GSI Helmholtzzentrum für Schwerionenforschung GmbH, Darmstadt	Study of molecular and cell-biological foundations of damage to cells of the human central nervous system caused by ionizing radiation and chemotherapeutic agents	10 April 2017
6 (124)	Max-Planck-Gesellschaft, Max-Planck-Institut für Psychiatrie, München	Studies on the effects of glucocorticoids on the development and properties of neurons and brain organoids derived from human embryonic stem cells	10 May 2017
7 (125)	Max-Delbrück-Centrum für Molekulare Medizin (MDC), Berlin	Studies on the role of the human endogenous retrovirus H (HERV-H) in the regulation of the pluripotency of human embryonic stem cells	14 July 2017
8 (126)	Max-Planck-Gesellschaft, Max-Planck-Institut für molekulare Biomedizin, Münster	Differentiation of human pluripotent stem cells towards male germ cells	14 July 2017

9 (127)	Dr Sabina Tahirovic, Deutsches Zentrum für Neurodegenerative Erkrankungen, München	Studies on the role of microglia-relevant genes in the properties of monocytes and microglial cells differentiated from hES cells	14 July 2017
10 (128)	Dr Michelle Vincendeau, Helmholtz Zentrum München	Study of possible functions of retrotransposons in the neural differentiation of human pluripotent stem cells	21 August 2017
11 (130)	Universitätsklinikum Essen Institut für Transfusionsmedizin	Directed differentiation of pluripotent stem cells to progenitor cells of the cornea epithelium and the retinal pigment epithelium	18 October 2017
12 (131)	Klinikum rechts der Isar der Technischen Universität München	Identification of gene regulatory elements of cardiac development in human cardiovascular progenitor cells	18 October 2017
13 (132)	Prof. Dr James Adjaye Universitätsklinikum Düsseldorf, Institut für Stammzellforschung und Regenerative Medizin (ISRM)	Transplantation of mesenchymal stem cells derived from hES cells into a rat model of Crigler-Najjar syndrome	24 November 2017
Extensions of already approved applications			
14 Extension of approval (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	10 May 2017
15 Extension of approval (84)	Dr David Vilchez, Universität Köln	Study of the regulation of proteostasis in human embryonic stem cells in order to understand the molecular principles of cellular ageing	8 June 2017

Table 2. Overview of research projects that were approved by the RKI during the 2017 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 (118th approval under the Stem Cell Act) is the molecular analysis of human induced pluripotent stem cells (hiPS cells) obtained from somatic cells of patients with idiopathic Parkinson's disease at the levels of the transcriptome and epigenome in comparison with hES cells as reference material. This is particularly important because some studies describe an epigenetic memory that survives reprogramming, which would adversely affect the therapeutic use of the hiPS cells derived from the patients. By determining the RNA expression patterns and methylation signatures, the approved research project aims to explore whether, and to what extent, an epigenetic memory survives in the hiPS cells after reprogramming compared to hES cells, what differences exist between individual hiPS cell clones, and what additional changes, if any, take place as a result of the reprogramming process. The results of the work could generate new insights into the extent to which hiPS cells from patients with idiopathic Parkinson's disease might be suitable for a treatment of Parkinson's disease based on cell therapy. In view of the fact that there is no causal therapy available for treating this disease, great hopes are being placed in a cell-based therapy.

The focus of the second research project (119th approval) is on establishing a human stem-cell-based organoid model of the brain as an experimental platform for researching and optimizing the direct *in vivo/in situ* reprogramming of tissue cells in somatic progenitor cells. The long-term objective is that the procedure will contribute towards supporting the regenerative capacity of injured, diseased and/or aged tissue. Such tissue often exhibits a

reduced number of progenitor-cell populations; its capacity for regeneration is therefore limited. One focus of the work is the direct transdifferentiation of non-neuronal cells to neural progenitor cells by means of the viral transfer of genes for reprogramming factors. For example, the project aims to examine – by direct reprogramming – possibilities of regeneration and healing or self-repair which are not normally a given natural ability of the brain. This work will also make use of hiPS cells. If successful, the findings might lead to new insights into the molecular and cellular mechanisms on which stem-cell-dependent tissue renewal is based, particularly in the brain. In addition, the work could also lay the foundations for the development of new therapy approaches for treating neurodegenerative diseases such as amyotrophic lateral sclerosis, Parkinson's disease or Huntington's disease.

The third research project (120th approval) aims to clarify the biological mechanisms that underlie the development of the neuromuscular system in humans. At a certain point in time, bipotent neuromesodermal progenitor-cell populations (NMP cells) can be found in the developing embryo. These cells differentiate both to nerve cells (motor neurones) and to mesodermal (muscle) cells of the spinal cord. NMP cells have only been partially characterized up to now. The first step will be to produce bipotent NMP cells from hES cells and to thoroughly characterize them at the molecular level, also in a three-dimensional spinal-cord organoid model. In this context, the project will study the role of the Wnt signalling pathway and its participating factors in the identity and differentiation decisions of hES-cell-derived NMP cells. Another focus of the work lies in the study of suitable conditions under which NMP cells can efficiently develop into spinal motor neurons of different regional identities. The expected results could lead to new insights into the biological mechanisms underlying the development of the spinal cord in the very early phase of embryonic development, insights that might also form the basis for the development of new therapies for spinal-cord injuries and degenerative diseases.

The subject of the fourth research project (approvals 121 and 122) is the identification of possible common molecular foundations of neural developmental disorders which, although associated with mutations in different genes, are similar in their respective phenotypical expression. The project focuses on the Coffin-Siris syndrome (CSS), the Nicolaides-Baraitser syndrome (NBS), and the Pitt-Hopkins syndrome (PHS), which involve considerable mental retardation. The aim is to introduce precise genetic mutations into the disease-associated genes in hES cells using genome editing, and to thoroughly determine the effects on the properties of hES-cell-derived cortical and hippocampal nerve cells, as well as three-dimensional cortical organoids. These studies will involve, for example, the analysis of cell viability and proliferation, the differentiation and migration behaviour, and functional, epigenetic and proteomic analyses, as well as a pharmacological or genetic characterization of selected signalling pathways. In addition, fluorescence-protein and luciferase-based hES reporter cell lines will be generated for selected markers, in order to visualize cell types, cell compartments and biological processes. The project is expected to contribute to broadening our understanding of dysregulated signalling pathways or processes that play a role in the pathogenesis of mental retardation syndromes of different genetic etiology.

The focus of the fifth research project (123rd approval) is on establishing an *in vitro* system for assessing damage caused in cells of the human central nervous system (CNS) or in stem and progenitor cells differentiating to such cells by ionizing radiation and chemotherapeutic agents. Combinations of radiation and chemo- and immunotherapy and/or other medications are increasingly being used for treating paediatric tumours of the CNS and have made a considerable contribution to raising the survival rate of children with tumours of the CNS. However, apart from acute side effects, the therapies may also have serious negative long-term effects. These include, among other things, reduced cognitive skills and neurological dysfunctions, leading to a progressive deterioration of brain performance. Yet the mechanisms underlying these combination therapies' injurious effect on healthy CNS tissue are largely unknown, and no systematic analysis has been carried out to date. Therefore, the first step will be to differentiate hES cells to different types of neuronal and glial cells and

three-dimensional brain organoids and to thoroughly characterize them in order to check their integrity. These cell systems will then be exposed to ionizing radiation – alone or in combination with selected substances – and subsequently thoroughly characterized at the molecular, morphological, genetic, epigenetic and functional levels to determine any changes compared to untreated cells. The research work could contribute towards extending our understanding of the molecular and cellular mechanisms that lie behind the neurotoxic effects of ionizing radiation in combination with medication. In addition, such *in vitro* cell systems could be used to predict the likely neurotoxic effect of radiation and pharmaceuticals better than hitherto. If successful, this work could lay the foundations for optimized treatment plans and ultimately contribute to avoiding radiation damage.

The sixth research project (124th approval) intends to examine what influence glucocorticoids have on the development of neuronal cells and cortical organoids from hES cells. The background of these studies is a wide range of findings indicating that an increased and long-lasting glucocorticoid secretion as a result of stress can cause changes in early brain development and represents a potential risk of development of psychiatric disorders. To this purpose, hES cells are to be differentiated to neural cells and cortical organoids with functional glucocorticoid receptors according to largely established protocols, and the integrity of the cells/organoids confirmed by extensive analyses. Subsequently, the differentiation will be carried out in the presence of different doses of glucocorticoids and possible changes determined in the differentiated or differentiating cells/organoids. In addition to effects on proliferation, survival rate, migration and dendrite formation, the particular aim is to determine the influence of glucocorticoids on the transcriptome and the epigenome of the respective neuronal (progenitor) cells and cerebral organoids. Another aim is to find out whether exposure to glucocorticoids leads to changes in the binding profile of the glucocorticoid receptor. The results of the work could lead to new insights into whether – and if so which – changes in neuronal development are caused by increased glucocorticoid concentrations at the cellular and molecular level. Since glucocorticoids are an important stress hormone, the desired results, if achieved, could contribute to a deeper understanding of the role played by stress as a risk factor for psychiatric disorders.

The focus of the seventh research project (125th approval) lies in the study of the function of the human endogenous retrovirus H (HERVH) in maintaining the pluripotency of human embryonic stem cells. On the basis of transcriptome comparisons, the project will begin by determining the role played by the products of specific genes – which are differentially expressed in human pluripotent stem cells when HERVH is present and when it is absent – in the pluripotency of human cells. To this purpose, these genes will be overexpressed, or their expression diminished or switched off, in hES cells and the respective effects on the properties of hES cells relevant for pluripotency will be studied. Furthermore, interaction partners of the corresponding gene products in hES cells will be identified and their function in the metabolism of hES cells determined using high-throughput methods. Another aim is to examine in greater detail how HERVH-controlled pluripotency is regulated by the LBP9 transcription factor and what the significance of the TFCP2 transcription factor is for the maintenance of pluripotency. If successful, the results could generate new knowledge about molecules and signalling pathways and processes that determine the self-renewal and differentiation of human pluripotent stem cells.

The eighth research project (126th approval) aims to clarify the molecular processes that occur during the differentiation of male human germ cells. To this purpose, hES cells are to be differentiated *in vitro* to primordial germ cells (PGCs) or PGC-like cells (PGCLC) and the further maturation to male gonocytes achieved using testicular organoids; the PGCs developed from hES cells are to be co-cultivated with foetal testicular cells from rats (xeno-organoid system) or with somatic gonad cells derived from hES cells (allogenic organ system). The influence of the testicular microenvironment on germ-cell development will be comprehensively investigated with methods of cell and molecular biology, including the use of suitable hES reporter cell lines. In addition, spermatogonial stem cells (SSC) will also be

produced from hES cells by direct differentiation; their properties will then be tested in the xeno-organoid system and in the allogenic organoid system. Moreover, the early processes of male germ-cell formation and participating signal transduction pathways will be studied *in vitro*, and the role played by participating gene products clarified. All work is to be carried out based on a comparison between hES and hiPS cells. The research work could contribute to clarifying molecular and cell-biological principles of the development and specification of male germ cells and, in the longer-term, also lay the foundations for new therapies in the treatment of male infertility.

The objective of the ninth research project (127th approval) is to study inflammatory processes that take place at the molecular and cellular level in the development of neurodegenerative diseases, particularly Alzheimer disease. To this purpose, genes whose products are relevant for microglia function and show mutations in affected patients are to be either functionally switched off or specifically mutated in hES cells and the effects on the properties of hES-cell-derived microglia or monocyte cells studied, particularly at the functional level (e.g. phagocytosis of β-amyloid plaques). The studies will also be carried out in comparison with hiPS cells from patients with neurodegenerative disorders and mutations in the corresponding genes. This research work could lead to new insights into the molecular mechanisms that cause changes in the function of microglia and monocytes in neurodegenerative diseases.

The focus of the tenth research project (128th approval) is on studying the functions of retrotransposons integrated into the human genome and the role of these coded gene products in maintaining pluripotency and in the neural differentiation of human cells. The first step will be to examine whether, and to what extent, the expression of the retrotransposons changes during the neural differentiation of hES cells. To this purpose, the expression of retrotransposons will be specifically activated or inhibited in hES cells using a modified CRISPR/Cas9 system, and the effects on the transcriptome and epigenome of the neurally differentiating cells studied. Furthermore, the project will study the functions of retrotransposons, cellular genes regulated by retrotransposons, and genes which themselves regulate retrotransposons. The corresponding genes are to be overexpressed, modified or switched off in hES cells and the effects on the properties of cells differentiated from the corresponding hES cells determined. In this context, the interest is on possible changes of the transcriptome and the epigenome, as well as other molecular and functional properties of the cells. Moreover, the genetically modified hES cells produced in the context of the two sub-projects are to be used to produce brain organoids, the intention being to determine the effects of a modified expression of retrotransposons and changes in gene functions on the properties of the respective brain organoids. The above-mentioned studies are also to be carried out in comparison with hiPS cells. The project could contribute to our understanding of retrotransposons' role during neural differentiation.

The eleventh research project (130th approval) aims to clarify the molecular processes that occur during differentiation to stem cells of the cornea epithelium and the retinal pigment epithelium, as well as epithelial cells derived from them. In addition, protocols will be established that enable an efficient, directed *in vitro* differentiation of pluripotent stem cells to the above-mentioned cell types. To this purpose, genes for transcription factors that are involved in the respective differentiation processes are to be introduced into hES cells and ectopically expressed – or, alternatively, the expression of these genes is to be reduced or switched off in hES cells. The aim is for the comparative differentiation of genetically modified and wild-type cells – and subsequent comprehensive analyses of the differentiated cells at the morphological, immunohistochemical and molecular levels – to enable conclusions to be drawn on the function of the respectively altered genes in the differentiation process. Analysis of the transcriptomes will then make it possible for further genes to be identified whose activity has been altered as a result of the genetic modifications and which may be involved in differentiation processes in retinal and corneal cells. Such genes will then also be ectopically expressed or switched off in hES cells, and the effects on

differentiation determined. Furthermore, relevant signal transduction pathways are to be characterized more precisely using pharmacologically active substances. The project is expected to make an important contribution to clarifying the transcriptional processes that are essential for the differentiation of human pluripotent stem cells to cells of the cornea epithelium and the retinal pigment epithelium. In the longer-term perspective, however, it could also be relevant for the development of new cell therapies for treating eye diseases in humans, e.g. macular degeneration or corneal defects.

Within the framework of the twelfth research project (131st approval), hES cells are to be used to clarify the function of gene regulatory elements during early stages of cardiovascular differentiation in humans. To this purpose, particularly so-called superenhancers are to be identified in cardiovascular progenitor cells of the first heart field which are derived from a specific hES reporter cell line. In order to investigate their role in cardiac development more closely, these enhancer elements will then be completely deleted or specifically mutated, and the respective effect on the cardiac differentiation of the genetically modified hES cells determined. Furthermore, in the context of this work further genes that are specifically expressed in human cardiac progenitor cells of the first heart field will also be identified and their function examined in order to find new markers for this cell type. The above-mentioned studies can be expected to primarily generate knowledge of transcriptional networks that are active during human cardiogenesis. In addition, the results could also contribute to our understanding of the molecular causes of congenital heart defects, since such diseases can also be caused by changes in the non-coding genome (which makes up 95% of the human genome).

The subject of the thirteenth research project (132nd approval) is the differentiation and generation of mesenchymal stem cells (MSCs) from hES cells in order to study new strategies for treating Crigler-Najjar syndrome type 1 (CN1) and test them in animal models. CN1 syndrome is a congenital disorder in bilirubin metabolism for which no effective therapy is available. Initially, hES cells are to be differentiated to MSCs and their cell-typical properties thoroughly examined. They will subsequently be transplanted into partially hepatectomized Gunn rats, a well characterized animal model for this disease, to determine on the basis of different parameters whether, and to what extent, MSCs derived from hES cells have the potential to reverse the CN1 phenotype. The above-mentioned studies will also be carried out in comparison with hiPS cells. These studies are expected to lead to new insights into the potential of MSCs derived from human pluripotent stem cells in the *in vivo* regeneration of the liver. This is of great relevance to the treatment of CN1 syndrome, but potentially also to the future treatment of other metabolic disorders that are manifested in the liver.

During the reporting period, applications were made for the extension of research work relating to two approvals on which the ZES commented in advance to the approval (see nos. 14 and 15 in Table 2).

The focus of the research project listed under no. 14 is on studying the various (patho-) physiological functions of the L1 gene in cell-culture models with human neurons, not all aspects of which are currently known. In this context, the role of L1 is to be studied, especially in relation to L1-syndrome-associated point mutations in the *L1* gene, and questions answered on the pathophysiological function of L1 in *in vitro* models of neuroinflammation and ischaemia. In the course of the project, the need to conduct further work has now arisen: to examine whether L1 agonists have similar effects in neurons as the restoration of L1 expression in L1-deficient neurons. A further intention is to study questions of the proteolytic processing of L1 under ischaemic conditions in the presence of different pharmacologically active substances. And another aim is to determine the effects of neuroprotective and anti-inflammatory factors on human neurons in a complex *in vitro* model of traumatic brain injury and ischaemia. The approved research work can be expected to contribute to deepening our molecular understanding of acutely neurodegenerative diseases such as stroke and craniocerebral injury, and to identifying suitable therapeutic substances.

The research project listed under no. 15 deals with the study of proteostasis in hES cells. The aim of the work is to identify the mechanisms and peculiarities involved in the maintenance of protein integrity in hES cells and in this way to get new insights into the molecular principles of how the lifespan of cells and cellular senescence are regulated. The research focuses in particular on studying the different components of the ubiquitin-proteasome system and its regulation. In an extension of the work approved hitherto, further aspects will now be studied in addition. Among other things, it was discovered in the course of the previously approved research work that the expression of genes for certain E2 enzymes is increased in hES cells. Such enzymes have essential functions in the regulation of protein ubiquitination and are involved to some extent in the differentiation of hES cells. They also bind to specific histones and regulate their ubiquitination, which indicates a function of E2 enzymes in the maintenance or transformation of the epigenome. The aim of expanding the project is to study in more detail the role played by E2 enzymes in the differentiation of hES cells in various somatic cell types, which genes are affected by changes in histone methylation as a result of the switching off of E2 enzymes, and what molecular processes lie behind the regulation of histone modification by E2 enzymes. In addition, hES cells are to be used to examine whether regulators of the epigenome previously identified in *C. elegans* are also functional in hES cells. The research work might help gain new insights into ways in which the epigenome is regulated in hES cells and how important it is for pluripotency, differentiation and cell ageing.

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.

Seven 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. Seven 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 receipt of the ZES's opinion. During its 15 years of activity, the ZES has submitted opinions to the RKI on a total of 131 applications for the import and/or use of hES cells. In addition, a total of 36 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 132 approvals, some of which were extended. Twenty five of these approvals have expired to date. At present, 77 groups at 51 research institutions are conducting approved research work with hES cells.

3. Event to mark the 15th anniversary of the ZES

The summer of 2017 marked fifteen years since the coming into force of the German Stem Cell Act (StZG), which regulates the import and use of hES cells for research purposes. The Central Ethics Committee for Stem Cell Research (ZES) also came into being 15 years ago. The body is appointed by the Federal Government for a term of three years respectively; it reviews and assesses the ethical acceptability of research projects involving hES cells within the framework of the respective approval procedures. To mark this occasion, a symposium entitled 'Making Ethical Judgements in Stem Cell Research – 15 years of work by the Central Ethics Committee for Stem Cell Research (ZES)' was held at the Berlin-Brandenburg Academy of Sciences and Humanities (BBAW) (see programme in Table 3). It aimed not only to provide an opportunity to shed some light on the ZES's role in German stem-cell research, but also to discuss the tasks and significance of ethics committees. Invitations were extended to elected officials and staff of the Bundestag, members of the German Ethics

Council and the Academy for Ethics in Medicine, as well as representatives of the ministries, representatives of various scientific foundations and organizations, scientists conducting research projects with hES cells according to the Stem Cell Act, as well as former members of the ZES.

Programme

	Presenter Prof. Dr Marion Kiechle Klinikum der Technischen Universität München
Welcoming speeches	
14.30	Prof. Dr Lothar H. Wieler President of the Robert Koch Institute Prof. Dr Klaus Tanner Theologisches Seminar, Universität Heidelberg Chair of the Central Ethics Committee for Stem Cell Research (ZES)
Lectures	
14.50	**Introduction to the event** Prof. Dr Ralf Stoecker Abteilung Philosophie, Universität Bielefeld
15.05	**Human pluripotent stem cells in research and biomedicine** Prof. Dr Alexander Meissner Max-Planck-Institut für Molekulare Genetik, Berlin
15.50	**Break**
16.05	**Blurred boundary lines as a challenge for medical ethics** Prof. Dr Geert Keil Institut für Philosophie, Humboldt-Universität zu Berlin
16.50	**Ethics in commissions: fig leaf or civilizing force?** Prof. Dr Claudia Wiesemann Institut für Ethik und Geschichte der Medizin, Universitätsmedizin Göttingen
17.35	**Commentary and introduction to the discussion** Prof. Dr Klaus Tanner Theologisches Seminar, Universität Heidelberg Chair of the Central Ethics Committee for Stem Cell Research (ZES)
17:50	**Panel discussion**

Table 3. Programme of the event to mark the 15th anniversary of the ZES

More information on the event is available (in German) on the RKI website: https://www.rki.de/DE/Content/Kommissionen/ZES/Symposium2017/symposium2017_node.html.

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

1. Some of the new applications filed during the reporting period were concerned with the differentiation and maturation of hES cells to certain cell types. They aim to explain basic molecular and cell-biological mechanisms that are important for efficient differentiation to motor neurons, male germ cells, cells of the corneal epithelium, and the retinal pigment epithelium. Furthermore, *in vivo* studies will examine the extent to which human pluripotent stem cells derived from MSCs by transdifferentiation to functional hepatocytes can contribute to the regeneration of the liver. Other evaluated research projects aim to differentiate hES cells to neural cells. It is clear that the recently successful establishment of three-dimensional organoid models of the brain from hES cells is also of considerable importance for stem-cell research in Germany. For example, such organoids can serve as an experimental platform for researching and optimizing the direct *in vivo and in situ* reprogramming of tissue cells into somatic progenitor cells. Another project is to study the influence of a significant stress hormone (glucocorticoids) on the development of cortical organoids in order to attain a deeper understanding of the role of stress as a risk factor in psychiatric disorders. The brain organoid models based on hES cells are also to be used in the *in vitro* testing of ionizing radiation, in order to predict its neurotoxic effect better than hitherto. Furthermore, brain organoids are to be established 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. As reported in its 14th Report for 2016, the ZES was informed at its 86th meeting on 13 June 2016 about novel processes for producing cellular organoids. In addition, it can be clearly seen that research into the molecular foundations of pluripotency and differentiation processes in human cells is still of considerable interest even nearly 20 years after the establishment of the first hES cells. Aspect to be investigated in this context by the research projects approved during the reporting period include gaining new insights into possible functions of retrotransposons during neural differentiation; decoding the role of the human endogenous retrovirus H (HERVH) in the regulation of pluripotency of hES cells; and identifying gene regulatory elements of cardiac development in human cardiovascular progenitor cells and analysing their role during early differentiation processes.

2. Comparative studies on hiPS cells and hES cells were again the topic of the research projects in 2017. Six of the 14 new approvals study hiPS cells and hES cells in parallel (Figure 1). In some projects, hES cells are used as reference material for assessing the differentiation potential of hiPS cells in the respective cell types that are the subject of interest. The various hiPS cell lines differ considerably in terms of their differentiability. This can be caused by the different genetic background or the donor's age, the cell type used for the reprogramming (blood cells, fibroblasts, keratinocytes, etc.), the reprogramming method (e.g. retro- or lentiviral vectors), or the factors used for reprogramming. Furthermore, epigenetic differences have often been observed between hiPS and hES cells which can be attributed, for example, to incomplete reprogramming. The possibility cannot be excluded, moreover, that new mutations take place during the reprogramming process whose effects on differentiation processes cannot be predicted. Another aim of the research work approved during the reporting period is to clarify which type of human pluripotent stem-cell lines will in the future be most suitable 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 to the development of new therapeutic processes.

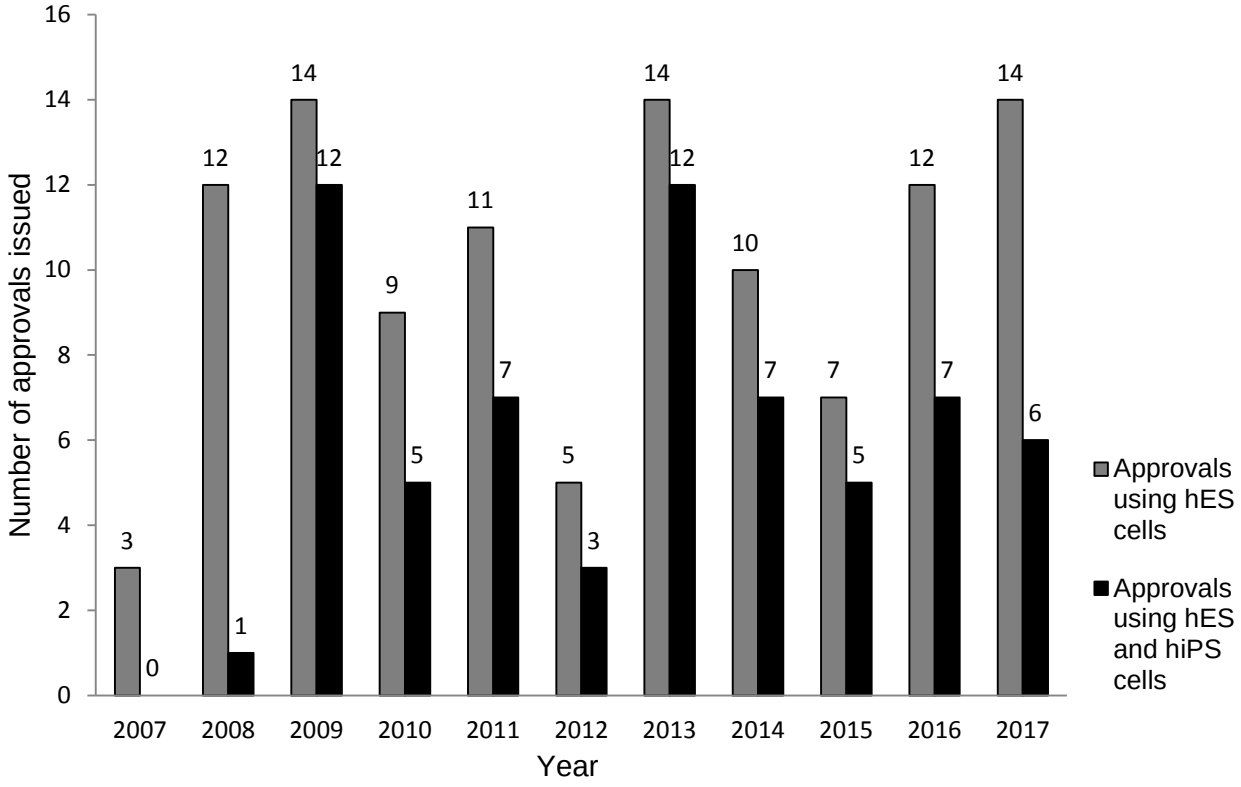

Figure 1. Use of hES and hiPS cells in approved research projects, 2007-2017. The chart shows the total number of approved research projects (grey) and the number of research projects in which not only hES but also hiPS cells are used (black).

3. As shown in the Committee's reports over recent years, research on pluripotent stem cells since 2010 does not only involve basic research; at the international level it is also increasingly moving in the direction of clinical applications. Table 4 gives an overview of the clinical trials that were – or are being – carried out worldwide with cells derived from pluripotent stem cells between 2010 and 2017. In such studies, cells derived from pluripotent stem cells are being tested outside of Germany to determine their suitability for treating diseases for which no adequate therapy options currently exist. In the meantime, their safety and tolerability are also being examined within the framework of long-term studies. The majority of clinical trials listed in Table 4 are being carried out using cells that have been derived from hES cells (28 studies). Material derived from hiPS cells is currently being used in three clinical trials, while one study respectively is using cells based on parthenogenetic stem cells or stem cells (NT-hES cells) derived from embryos produced by nuclear transfer (SCNT). 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 development of therapies for different eye diseases (5), type I diabetes mellitus (1), ischaemic heart diseases (1), and diseases of the nervous system (3). In the studies using hiPS cells, 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). It is striking that none of the studies currently being conducted with hiPS cells are being carried out on the basis of autologous cells; all the studies are using cell products obtained from allogenic hiPS cells. The clinical study using cells derived from NT-hES

cells targets the treatment of age-related macular degeneration, while the only study to date using human parthenogenetic pluripotent stem cells (hpPS cells) in which neural stem cells are transplanted targets the treatment of Parkinson's disease.

Summary of clinical trials with pluripotent stem cells (2010-2017)

	Disease	Number of studies	Participants
hES cells	**Diseases of the eye and adnexa**	**20**	**286**
	Age-related macular degeneration (AMD)	10	139
	Stargardt's disease (hereditary juvenile form of macular degeneration)	5	52
	Retinitis pigmentosa	1	10
	Other eye disorders	4	85
	Endocrinal, nutritional and metabolic diseases	**4**	**335**
	Type 1 diabetes mellitus	4	335
	Diseases of the circulatory system	**1**	**6**
	Ischaemic heart disease	1	6
	Diseases of the nervous system	**3**	**90**
	Spinal-cord injuries	2	40
	Parkinson's disease	1	50
hiPS cells	**Diseases of the eye and adnexa**	**2**	**11**
	Age-related macular degeneration (AMD)	2	11
	Injuries, poisonings and certain other consequences of external causes	**1**	**16**
	Graft-versus-host disease	1	16
NT-hES cells	**Diseases of the eye and adnexa**	**1**	**3**
	Age-related macular degeneration (AMD)	1	3
hpPS cells	**Diseases of the nervous system**	**1**	**12**
	Parkinson's disease	1	12
	Total	**33**	**759**

Table 4. Clinical trials with cells developed from pluripotent stem cells (including long-term studies with patients from previous clinical trials). Source: Robert Koch Institute, unpublished data. Data last revised: 15 November 2017

See Table 5 for an overview of the countries in which the studies are being carried out. In summary, it can be stated that clinical trials using hES cells were carried out mainly in

the USA and China between 2010 and 2017. In Germany, no clinical trials were or are being carried out on the basis of pluripotent stem cells.

Overview of the countries where clinical trials with pluripotent stem cells are carried out (2010-2017)

	Country	Number of studies
hES cells	USA	12
	China	7
	UK	5
	Korea	2
	Brazil	1
	France	1
	Israel	1
	Canada	3
hiPS cells	Australia	1
	UK	1
	Japan	2
NT-hES cells	Korea	1
hpPS cells	Australia	1
	Total	33*

Table 5. Overview of the countries participating in clinical trials (including long-term studies with patients from previous clinical trials). * Some studies are being carried out in several countries. Source: Robert Koch Institute, unpublished data. Data last revised: 15 November 2017

The 15th Report was adopted at the 94th ordinary meeting of the ZES on 17 January 2018.