Report

of the

Central Ethics Committee for Stem Cell Research (ZES)

19th Report after the Enactment of the Stem Cell Act (StZG) for the Reporting Period 1 January to 31 December 2021

1. The Central Ethics Committee for Stem Cell Research

The Central Ethics Committee for Stem Cell Research (Zentrale Ethik-Kommission für Stammzellenforschung, 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/or use of human embryonic stem cells (hES cells) according to the regulations of the Stem Cell Act, issues an opinion on every application and submits it to the Robert Koch Institute (RKI), the competent authority under the Stem Cell Act. The Committee's activities are based on the 'Act ensuring the protection of embryos in connection with the import and use of human embryonic stem cells' (Stem Cell Act – StZG) dated 28 June 2002 (BGBl. I p. 2277), most recently amended by Article 50 of the 'Act on dismantling unnecessary ordinances on the written form in Federal administrative law' (BGBl. I p. 626) dated 29 March 2017 (http://www.gesetze-im-internet.de/stzg/index.html), and on the 'Regulation concerning the Central Ethics Committee for Stem Cell Research and the competent authority pursuant to the Stem Cell Act' (ZES-Verordnung, ZESV) dated 18 July 2002 (BGBl. I p. 2663), most recently amended by Article 51 of the above-mentioned law dated 29 March 2017 (BGBl. I p. 626) (http://www.gesetze-im-internet.de/zesv/index.html).

The Committee conducts its work on an honorary basis; it 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). 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 Stem Cell Act, it is the Committee's task to examine the ethical acceptability of the applications received by 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 import and/or use hES cells meets the criteria of section 5 of the Stem Cell Act, which requires that an application must demonstrate in a scientifically substantiated manner that (a) the project pursues high-level research objectives for a gain in scientific knowledge (section 5, no. 1 of the Stem Cell Act), (b) the scientific issues have, as far as possible, been subject to a preliminary clarification in other systems, for example in animal-cell models (section 5 no. 2 letter a of the Stem Cell Act), and (c) the targeted gain in scientific knowledge can probably only be achieved by using hES cells (section 5 no. 2 letter b of the Stem Cell Act). The ZES summarizes the results of its review in a written opinion and submits it to the RKI.

Pursuant to section 14 of the ZESV, the ZES prepares an annual report which is published by the Federal Ministry of Health (BMG) and can be accessed via the websites of the BMG and the RKI (https://www.bundesgesundheitsministerium.de/service/begriffe-von-a-z/s/stammzellgesetz.html#c1091 and http://www.rki.de/DE/Content/Kommissionen/ZES/Taetigkeitsberichte/taetigkeitsbericht_node.html).

Members	Deputy members
Biology	
Prof. Dr Katja Schenke-Layland, **(Deputy Chair)** Natural and Medical Sciences Institute at Tübingen University	Prof. Dr Maria Wartenberg, Universitätsklinikum (University Hospital) Jena, Molecular Cardiology and Stem Cell Research
Prof. Dr Hans R. Schöler, Max Planck Institute for Molecular Biomedicine, Münster	Prof. Dr Martin Zenke, RWTH Aachen Helmholtz Institute for Biomedical Engineering
Medicine	
Prof. Dr Mathias Bähr, Georg-August-Universität Göttingen (Göttingen University) Neurological Clinic	Prof. Dr Wolfram H. Zimmermann, Georg-August-Universität Göttingen, (Göttingen University) Institute of Pharmacology and Toxicology
Prof. Dr Anthony D. Ho, Ruprecht-Karls-Universität, Heidelberg (Heidelberg University), Med. Universitätsklinik und Poliklinik (University Hospital and Policlinic)	Prof. Dr Beate Winner, Friedrich-Alexander-Universität Erlangen-Nürnberg, (Erlangen-Nuremberg University) Universitätsklinikum (University Hospital) Erlangen, Department of Stem Cell Biology
Prof. Dr Sonja Schrepfer, University Heart and Vascular Centre (UKE) Hamburg, Department for Cardiovascular Surgery	Prof. Dr med. Ricardo E. Felberbaum, Klinikum Kempten Oberallgäu (Kempten Hospital), Gynaecological Clinic
Ethics	
Prof. Dr Dr Sabine Salloch **(Deputy Chair)**, Medizinische Hochschule Hannover (Hanover Medical School), Institute of Ethics, History and Philosophy of Medicine	Prof. Dr Dres. h. c. Michael Quante, Westfälische Wilhelms-Universität Münster (Münster University) Department of Philosophy
Prof. Dr Silke Schicktanz, Universitätsmedizin Göttingen (Göttingen Medical School) Institute of Ethics and History of Medicine	Prof. Dr Christine Hauskeller, University of Exeter, England, Department of Sociology, Philosophy and Anthropology
Theology	
Prof. Dr Dr Antonio Autiero **(Chair)**, Westfälische Wilhelms-Universität Münster (Münster University) Faculty of Catholic Theology	Prof. Dr Dr Jochen Sautermeister, Rheinische Friedrich-Wilhelms-Universität Bonn (Bonn University), Faculty of Catholic Theology
Prof. Dr Thorsten Moos, Ruprecht-Karls-Universität Heidelberg (Heidelberg University) Theological Seminary	Prof. Dr Hartmut Kreß, Rheinische Friedrich-Wilhelms-Universität Bonn (Bonn University), Faculty of Protestant Theology

Table 1. Members and deputy members of the Central Ethics Committee for Stem Cell Research (ZES), last revised December 2021

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

In 2021, the ZES held three meetings and discussed a total of ten applications for the import and use of hES cells. In addition, the ZES had already assessed and voted on one application in 2020. Since this application was not approved by the RKI until the beginning of 2021, it is reported on in this Report. The ZES handed down favourable opinions on all the applications. In addition, three applications for extensions of already approved research projects using hES cells were assessed and voted on.

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 Stem Cell Act and are ethically acceptable within its intendment (section 9 of the Stem Cell Act).

Serial no.	Holder of approval	Topic of approved research	Date of the positive ZES opinion
1 (163, 164, 167)	Prof. Dr Brenda Schulman, Max Planck Institute for Biochemistry, Martinsried Prof. Dr F. Ulrich Hartl, Max Planck Institute for Biochemistry, Martinsried Universitätsmedizin Göttingen (Göttingen Medical School)	Studies on the structure of α-synuclein aggregates and on the cellular mechanisms of their toxicity	09 December 2020
2 (165)	Dr Claudio Acuna Goycolea, Universitätsklinikum (University Hospital) Heidelberg	Study of the contribution of synaptic dysfunction to autism pathogenesis in human neural networks	17 March 2021
3 (166)	Universitätsklinikum (University Hospital) Erlangen,	Study of the role of myeloid cells in inflammatory processes of the central nervous system in cell and tissue models derived from hES cells	26 March 2021
4 (168)	Universitätsklinikum (University Hospital) Erlangen,	Modelling of presynaptic synaptopathies using cell and tissue models derived from embryonic stem cells	27 April 2021
5 (169)	Universitätsklinikum (University Hospital) Essen	Study of interactions and their influence on drug response in co-culture models of retinoblastoma cells and neural retina derived from human embryonic stem cells	31 May 2021
6 (170)	Leibniz-Institut DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures GmbH)	Development of an *in-vitro* model for hereditary retinoblastoma	09 August 2021
7 (171)	Helmholtz-Zentrum München GmbH (Helmholtz Centre Munich GmbH)	Studies of the influence of ferroptosis on the pluripotency and neuronal differentiation of human embryonic stem cells	09 August 2021

8 (172)	Helmholtz-Zentrum München GmbH (Helmholtz Centre Munich GmbH)	Identification and verification of genes relevant to the establishment/stabilization of neural cell identities in humans	06 September 2021
9 (173)	TWINCORE, Zentrum für Experimentelle und Klinische Infektionsforschung GmbH (Centre for Experimental and Clinical Research into Infectious Diseases GmbH)	Establishment of liver-cell models based on human embryonic stem cells for the study of infection with the hepatitis D virus	20 September 2021
10 (174)	Professor David Keays, Ludwig Maximilians University, Munich	Studies of the functions of MAST proteins in human brain development and developmental disorders	13 October 2021
11 (175)	Max Planck Institute of Molecular Physiology, Dortmund	Studies on mechanisms of molecular and cellular control of cell-differentiation rates in the embryonic stem cells of mice and humans	13 October 2021
Extensions of already approved applications			
12 Extension of approval (90)	Technische Universität (Technical University) Dresden	Differentiation of human embryonic stem cells into pancreatic beta cells and motor neurons, and their functional characterization	25 January 2021
13 Extension of approval (125)	Max Delbrück Centre for Molecular Medicine (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	08 February 2021
14 Extension of approval (120)	Max Delbrück Centre for Molecular Medicine (MDC), Berlin	Studies on early processes of spinal-cord development in humans; development of improved protocols for differentiating hES cells in motor neurons	03 May 2021

Table 2. Overview of research projects approved by the RKI during the 2021 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). For one application (serial no. 1), three identical, formally independent approvals were granted.

The aim of the first research project listed in Table 2 (163rd,164th and 167th approval in accordance with the StZG) is to gain detailed insights into the subcellular architecture of toxic protein aggregates, and into defects in cellular signalling pathways that are characteristic of some neurodegenerative diseases of the human nervous system. The focus of interest is on protein deposits that occur in connection with Parkinson's disease, so-called Lewy bodies, which consist largely of the protein α-synuclein. In addition, aspects of the selective degradation of mitochondria (mitophagy) are to be studied, since dysregulation is linked to the pathology of Parkinson's disease. In the project, hES cells are therefore to be differentiated into neurons; the formation of α-synuclein aggregates or mitophagy will be induced in the neurons, and the cells will then be comprehensively characterized with respect to the effects of protein aggregates and the mitophagy pathway, among other methods by cryo-electron tomography and mass spectrometric analyses. These studies will then also be conducted in neurons that have mutations associated with Parkinson's disease to gain insight into whether and how these mutations alter mitophagy. It is hoped that the research will yield new findings on the ways in which the formation of protein aggregates by the protein α-synuclein and disruptions in the regulation of the mitophagy pathway contribute to the pathogenesis of Parkinson's disease in humans.

The second research project (165th approval in accordance with the StZG) will explore the ways in which synaptic dysfunction can contribute to the pathogenesis of autism spectrum disorders (ASD). The focus is on studying mutations in genes for important synaptic components associated with ASD. The project will therefore initially seek to establish certain ASD-associated mutations in hES cells. The mutant hES cells will then be differentiated into neurons and astrocytes, and these will be used to establish neuronal networks; the effects of the pathogenic mutations on the development of the neurons/synapses and on their functionality will then be determined in these networks. The results are expected to yield new insights into processes that are altered as a result of mutations in genes for important synaptic components, leading to defects in the development and function of synapses and subsequently to the development of neuropsychiatric disorders such as autism.

The purpose of the third research project using hES cells (166th approval in accordance with the StZG) is to establish human cell models which can be used to study physiological functions of microglial cells and their interactions with neurons and oligodendrocytes under physiological and certain pathological conditions. The research aims to improve our understanding of the causes of neurodegenerative and inflammatory processes of different origins. The main focus is on diseases that are genetically determined (hereditary diffuse leukoencephalopathy with spheroids (HDLS)), caused by viral infections (human immunodeficiency virus 1 (HIV-1) and West Nile virus (WNV)), or triggered by toxic protein aggregates (Parkinson's disease, multisystem atrophy (MSA)). For this purpose, on the one hand, HDLS-associated mutations will be introduced into hES cells by genome editing (CRISPR/Cas9 technique), the modified hES cells will be differentiated into microglial cells and these will be co-cultured with hES cell-derived oligodendrocytes, neurons and/or CNS organoids. On the other hand, the cell models will be treated with infectious viral particles or protein aggregates. Subsequently, the effects on the properties of the neurons and glial cells in the cell models will be comprehensively characterized at the molecular, genetic and cell-biological levels. The research project aims to contribute to new insights into changes in the interactions of microglial cells with neurons and other glial cells, leading to a better understanding of the pathogenesis of inflammatory degenerative neuronal diseases. In the longer term, these findings may also be of importance for the development of new therapeutic approaches to hitherto insufficiently treatable CNS diseases.

The main emphasis of the fourth research project (168th approval in accordance with the StZG) is on the establishment of human neural cell models for studying the effects of mutations associated with so-called presynaptic synaptopathies at the molecular and cellular level. In particular, mutations in genes that encode presynaptic components involved in membrane transport, membrane sorting, and the metabolic homeostasis of the synapse will be investigated. Mutations in such genes are frequently associated with neurological diseases and developmental disorders. Each of the corresponding genes is therefore to be mutated into hES cells, the mutated hES cells characterized and subsequently differentiated towards cortical neurons, motor neurons, astrocytes and oligodendrocytes. The differentiated cells will then be used to establish more diverse co-culture systems for studying the effects of the respective mutation on the properties of the neurons. In addition to analyses of morphogenesis and functional connectivity, studies will be carried out in particular on the properties of the synapses, for example on the formation and maturation of synapses, on presynaptic membrane transport, on membrane sorting and on presynaptic metabolism. The above project is likely to provide new insights into the pathway by which mutations in genes for important presynaptic components lead to changes in the development and function of synapses, and subsequently to the development of serious diseases of the nervous system in humans.

The fifth research project (169th approval in accordance with the StZG) aims to clarify to what extent and by what means the interaction of cells of a retinoblastoma – a tumour that often occurs in early childhood – with the cells of the retina and other cells of the tumour's microenvironment change the tumour's properties and possibly make the tumours resistant to drugs. For this purpose, hES cells will first be differentiated into retinal organoids which are

then co-cultured together with human retinoblastoma cells (primary tumour cultures from patients and retinoblastoma cell lines) and microglial cells. Subsequently, the integration of retinoblastoma cells and their interaction with cells of the retinal organoid will be comprehensively studied at the cellular, molecular and genetic levels. The final aim is to determine whether, and to what extent, tumour cells show an altered sensitivity to drugs used to treat these tumours as a result of their cultivation in retinal organoids; analyses will focus primarily on determining apoptosis and cell-cycle parameters and analysing changes in the transcriptome. The above studies seem likely to provide new insights into whether and how cellular interactions in the tumour's microenvironment lead to therapy resistance in human retinoblastomas.

The topic of the sixth research project (170th approval in accordance with the StZG) is the establishment of a tissue model to study the hereditary form of retinoblastoma. The *RB1 gene* must be inactivated on both alleles before a retinoblastoma develops in humans. In this project, the gene will first be mutated in hES cells on one allele, either with artificial mutations or with mutations from patients. The hES cells mutated in this way will then be differentiated towards retinal organoids and, at different stages of differentiation, be provided with a further mutation (possibly also patient-specific) on the second allele of the *RB1 gene* by AAV-mediated transfer of the components of the CRISPR/Cas system. The cells mutated in both alleles will then be further differentiated into retinal organoids, and these will be comprehensively characterized, particularly with regard to (a) the presence and characteristics of specific retinal cell populations and the correct organization of the retinal organoid, (b) the expression of retinal marker genes, and (c) the transcriptome of specific retinal-cell populations. The results of this project may provide important clues to cellular and molecular processes that trigger the development of retinoblastoma, thus making an important contribution to our understanding of the tumour's pathogenesis. All work will also be performed comparatively using hiPS cells, where appropriate – either to confirm the equivalence of tissue models derived from the respective cell type, or to determine any differences.

The focus of the seventh research project (171st approval in accordance with the StZG) is to investigate to what extent and by what means ferroptosis, a form of regulated cell death caused by the iron-dependent peroxidation of membrane phospholipids, affects pluripotency and the neural differentiation of hES cells. Recent findings on the involvement of ferroptosis in the development of various neurodegenerative diseases form the background for the suspected relevance of ferroptosis, especially in the case of neural differentiation processes in humans. In this project, therefore, the ferroptosis signalling pathway is to be modulated in hES cells that are at the pluripotency stage – as well as in various neuronal cell types and brain organoids derived from hES cells – using known pharmacological agents; any changes in pluripotent stem cells and the differentiated or differentiating cells/organoids will be determined. In addition to effects on morphology, the particular aim here is to determine the influence of ferroptosis on the transcriptome of the undifferentiated stem cells, and of the respective neuronal (progenitor) cells and cerebral organoids. Furthermore, the project aims to determine whether there are changes in the lipidome, metabolome and in lipid peroxidation. The results of the research should yield insights into what specific changes are caused at the cellular and molecular level by the modulation of ferroptosis – in hES cells, in neuronal cells differentiating from them, and in terminally differentiated neurons or brain organoids. These findings may contribute to our understanding of the importance of ferroptosis for maintaining pluripotency and for the neuronal differentiation of human pluripotent stem cells; it may also be important for elucidating the pathogenesis of diseases in which ferroptotic processes play a role in their development and progression.

The aim of the eighth research project (172nd approval in accordance with the StZG) is to research the molecular principles of neural transprogramming processes in humans and to identify factors that determine human neuronal cell identities. For this purpose, hES cells that have already been extensively genetically modified abroad will first be differentiated into astrocytes. Genes in astrocytes derived from hES cells will then be activated and possible

changes in glial cell identity towards a neuronal cell identity will be determined. This approach is also to be extended to include neurons of different specificities derived from hES cells and to neural organoids in order, among other things, to identify genes/factors that determine the cellular identity of different neuronal subtypes. Another aim is to test whether inducing the expression of specific genes can stabilize the glial identity of astrocytes, alter the properties of neurons towards a more glial identity, and inhibit the transprogramming of astrocytes to neurons. Finally, genome-wide approaches are to be used to identify factors that trigger, promote or inhibit neuronal transprogramming. The planned research is highly relevant for the long-term development of basic principles for new therapeutic procedures for application in humans to regenerate nervous systems damaged by injury or disease.

The research in the ninth research project (173rd approval in accordance with the StZG) aims to establish a cell model derived from hES cells for infecting human hepatocytes with the hepatitis D virus (HDV); the aim is to investigate hitherto unresolved questions regarding the biology and immunology of this virus. HDV is responsible for one of the most severe forms of viral hepatitis: delta hepatitis. In the project, hES cells will first be differentiated towards hepatocyte-like cells (HLCs); these will be comprehensively characterized, and an analysis conducted of the permissiveness of HLCs and their progenitor cells for infection with HDV. Where appropriate, the host-cell factors required for infection by HDV will also be identified and their relevance confirmed. In addition, a study will be conducted as to whether and to what extent certain pathways of the innate immune response, by which viral infection can be controlled, are functionally active in HLCs and can be activated by an HDV infection in these cells. Finally, another focus of the work will be to establish a more authentic HLC-based cell model for infection with HDV in which co-infection occurs with a helper virus (specifically hepatitis B virus, HBV) required for the formation of infectious particles. The cell models based on hES cells are expected to help expand our understanding of the interactions between the hepatitis D virus and the hepatic host cell, and could be important for elucidating the virus's pathogenicity as well as for developing principles for novel therapeutic approaches.

The tenth research project (174th approval in accordance with the StZG) will investigate the role of the microtubule-associated proteins (MAPs) MAST1-4 (microtubule-associated serine/threonine kinase 1-4) during neuronal development in humans. The background of the research is that mutations in genes for MAST are frequently associated with neurodevelopmental disorders/diseases in humans. The genes for different MAST proteins in hES cells will therefore first be either *knocked out* or mutated in a patient-specific manner. The mutant cells will then be developed into brain organoids, which will be analysed comprehensively with respect to their morphological and electrophysiological properties during different developmental phases. In order to determine the effects of the *MAST* gene mutations at the molecular level, the transcriptome, proteome and phosphoproteome will be analysed at different stages of organoid development; changes in the kinase activity of the MAST proteins will be determined, and cellular interaction partners of the MAST proteins identified. Subsequently, the signalling pathways involving the interaction partners of the MAST proteins will be studied in more detail. The results of the research are expected to deepen our understanding of the processes that are disrupted as a result of mutations in genes encoding important protein kinases, causing changes in neuronal development and subsequently triggering neurodevelopmental disorders/diseases.

The purpose of the eleventh research project (175th approval in accordance with the StZG) is to study the molecular mechanisms regulating the species-specific dynamics of cell differentiation. This is to be done by comparative analyses of murine and human embryonic stem cells. To this end, the first part of the project will aim to establish a standardized experimental system for measuring both the dynamics of cell differentiation and the cellular energy turnover under comparable conditions in pluripotent stem cells from mice and humans. In the second part of the project, this system will then be used to identify and examine possible mechanisms of species-specific differentiation dynamics, with the aim of identifying and validating genes whose products can influence the rate of differentiation by modulating

metabolic activity. In the future, pluripotent stem cells from other species (mainly iPS cells) may also be included in the studies, and the results of the research with murine and human ES cells will be checked for their validity in other species. The research findings are expected to provide new insights into the genetic and molecular causes of different developmental dynamics in different mammalian species.

For three projects that have already been approved, additional research using hES cells was applied for during the reporting period, for which the ZES was required to issue an opinion in advance of approval (Table 2, serial nos. 12, 13 and 14).

Within the scope of the research project listed under no. 12, efficient procedures for the generation of pancreatic beta cells are to be developed to gain a more profound understanding of the molecular processes involved in the *in-vitro* differentiation of hES cells into pancreatic beta cells. The objective of the originally approved research project remains unchanged. The only addition is that further research is to be carried out which, in the course of the research approved to date, has been deemed necessary to achieve the research objectives. In order to assess the differentiation efficiency and the physiological function of beta cells derived from hES cells, genes whose products are involved in specific physiological functions of beta cells will now also be fused with reporter genes in hES cells. In addition, hES cells will now also be used to produce endothelial cells, and their influence on beta-cell maturation and function will be investigated in the context of pancreatic clusters. The research findings are expected to provide deeper insights into molecular and cell-biological processes involved in particular in the maturation of pancreatic progenitor cells and their development into functional cells of the pancreas.

It remains the aim of the research project listed under no. 13 to clarify the potential role of human endogenous retrovirus H (HERV-H) and HERV-H-regulated genes in the maintenance of human embryonic stem-cell pluripotency and in the induction of naïve pluripotency. However, in the course of the project the need arose to conduct further control experiments to further verify the research results. The research remains committed to improving our understanding of the ways in which HERV-H is involved in the regulation of human stem-cell pluripotency.

The research project listed under no. 14 aims at a better understanding of the molecular principles of the processes involved in the diversification of human motoneurons. An additional objective is to develop and test improved procedures for the efficient *in-vitro* differentiation of human pluripotent stem cells into specific types of motor neurons. In an extension of the originally approved research project, the role of various HOX genes in the development of position-specific motoneurons is now also to be studied. Furthermore, the influence of vascularization or the presence of endothelial cells on the development and functional maturation of spinal-cord organoids is to be clarified, and the processes involved in motor endplate development characterized in more detail. The findings are expected to provide new insights into the biological mechanisms underlying neuromuscular development, which may also form the basis for the development of new therapies for neuromuscular diseases.

Further information on the content of the research projects is available (in German) from the RKI's register (http://www.rki.de/DE/Content/Gesund/Stammzellen/Register/register-inhalt.html). The main arguments of the ZES, which justify the high-ranking status of the research projects, the sufficient preliminary clarification of the respective research questions as well as the need to use human ES cells were also included in the assessment of the research projects by the RKI.

Of the new applications discussed during the reporting period (Table 1, serial nos. 2-11), seven were submitted by researchers or institutions that had not yet previously received an approval in accordance with the Stem Cell Act. Three applications were made by researchers or institutions 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 19 years of activity, the ZES has submitted opinions to the RKI on a total of 169 applications for the

import and/or use of hES cells. In addition, a total of 42 applications for extensions of already approved projects have been approved by the RKI to date. The ZES issued an opinion on each of these. 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 175 approvals, some of which have been extended. Forty-five of these approvals have expired to date. Currently, 97 groups at 58 research institutions in Germany are in possession of approvals in accordance with the StZG and can conduct research using hES cells (as of 31 December 2021).

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

3.1. Research topics in the reporting period

3.1.1 In-vitro disease models using hPS cells

A large proportion of the research projects approved during the reporting period aim to provide human cell models for the study of pathogenesis mechanisms of human diseases, and to use them to study the molecular pathology of the respective disease. Neurological diseases and developmental disorders remain at the focus of interest (Table 2, serial nos. 1, 2, 3, 4, 10). Other projects aim to establish cell models for the study of the pathogenesis mechanisms of the hereditary or therapy-resistant form of human retinoblastoma and of delta hepatitis (Table 2, serial nos. 5, 6, 9). Some of the research also seeks to identify potential targets for pharmacological interventions and thus ultimately to contribute to the development of new therapeutic methods for the treatment of these severe and sometimes inadequately treatable diseases.

3.1.2 Regulation/control of pluripotency and differentiation processes

Some of the research projects approved during the reporting period aim to clarify research questions related to the importance of specific molecular processes for pluripotency and differentiation processes in human cells. The aim of one project is to gain a more profound understanding of the influence of ferroptosis on maintaining pluripotency and on the differentiation of hES cells into human neuronal cells (Table 2, serial no. 7). Another project aims to research the molecular principles of neural transprogramming processes in humans and to determine factors critical for human neuronal cell identities (Table 2, serial no. 8). A further project focuses on clarifying which molecular mechanisms regulate the species-specific dynamics of neuroectodermal differentiation (Table 2, serial no. 11). The findings of this research may contribute to a deeper understanding of the control of basic development-biological processes and, in the longer term, may be significant for explaining or treating neurological/neurodegenerative diseases.

3.1.3 Research with transgenic hES cell lines

In two of the research projects evaluated during the reporting period (Table 2, serial nos. 8 and 11), the need to use hES cells was also justified by the fact that achieving the research objectives required the use of transgenic pluripotent stem cells in which extensive genetic modifications had already been carried out abroad. These include, for example, reporter-cell lines or cell lines containing components of a specific CRISPR/Cas system. These (sometimes multiple) transgenic cell lines had already been established and characterized on the basis of hES cells, but were not available on the basis of hiPS cells. The ZES regards the availability of already established and well-characterized transgenic hES cell lines as a viable and sufficient argument for the necessity of using hES cells if such lines are not available at the time of application on the basis of hiPS cells. On the one hand, transgenic pluripotent stem cells often have unique properties, and it is not easy to make reliable predictions about the

effects of an identical genetic modification in different cell types (for example hES and hiPS cells). On the other hand, the applicant's presentation (and consequently also the assessment of whether the requirements of section 5 of the StZG have been met) must be based on the *current* state of knowledge. The applicants cannot be required to first establish a new state of knowledge, e.g. by establishing genetically modified hiPS cells, before their application is approved.

3.2. Comparative studies on iPS cells and hES cells

Comparative studies using iPS cells (human and from other species) and hES cells are the subject of two of the thirteen research projects approved during the 2021 reporting period (Figure 1). In one project, hES cells are used as reference material for assessing the differentiation potential of hiPS cells in retinal cell types/organoids (Table 2, serial no.1). Different hiPS cell lines vary considerably in terms of their differentiation ability. This can be caused by the genetic background of the donors, the properties of the somatic cells used for reprogramming, the reprogramming method, the factors used for reprogramming, and/or by a possible epigenetic memory of the cells. In another project (Table 2, serial no. 11), which aims to study cellular and molecular mechanisms that regulate the species-specific dynamics of cell differentiation, comparative analyses are initially to be performed on murine and human embryonic stem cells. This research will also be extended to the pluripotent stem cells of other species (mainly iPS cells), and hES cells will also be used as reference material here.

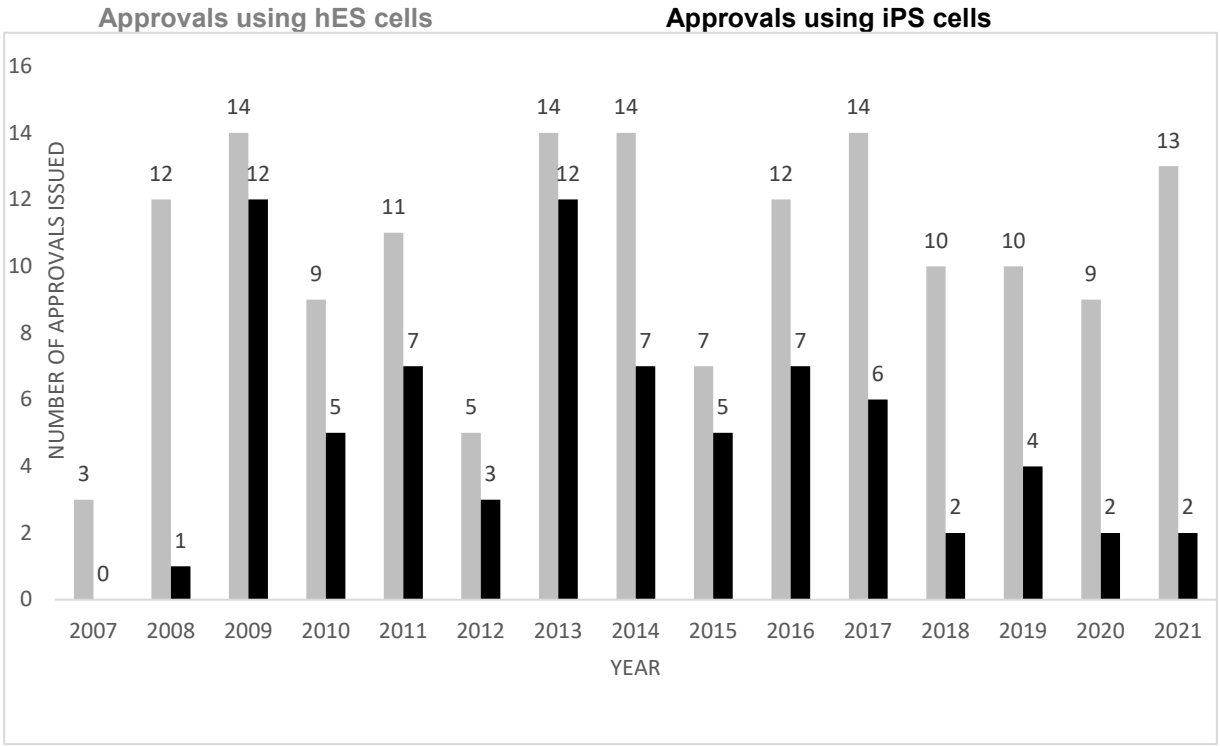

Figure 1. Use of hES and iPS cells in approved research projects 2007-2021. The chart shows the total number of approved research projects (grey) and the number of research projects in which not only hES but also iPS cells are used (black).

3.3. Increase in the number of clinical trials using hPS cells

It can be observed that there was a marked increase in the number of clinical trials conducted using cells derived from hPS cells in 2021 compared to the previous year – rising from a total of 74 studies worldwide up to the end of 2020 to a total of 96 studies up to the end of 2021. Furthermore, there was also a significant expansion in the spectrum of diseases for whose treatment cell therapeutics derived from hPS cells were being clinically tested. For example, clinical trials based on hPS cells were initiated during the reporting period targeting the treatment of cerebral infarction, Parkinson's syndrome, multiple sclerosis, epilepsy, bullous keratopathy and non-Hodgkin's lymphoma. In other studies, cell therapeutics based on hPS cells were clinically tested for the treatment of malignant neoplasms of the mammary gland and genital organs, as well as for retinal affections. Table 3 provides an overview of clinical studies based on hPS cells that had been conducted since 2010 and/or were still ongoing at the end of 2021.

The clinical trials listed in Table 3 are conducted predominantly with cell therapeutics derived from hES cells or hiPS cells, using them in approximately equal measure (hES cells 47/96, hiPS cells 46/96). Two studies use cells based on human, parthenogenetically produced pluripotent stem cells (hpPS cells), and one study uses cells (NT-hES cells) derived from embryos created by nuclear transfer (SCNT).

Studies using cells derived from hES cells, which in 2021 increased in number from 40 to 47 studies compared to the previous year, still predominantly target the treatment of diseases of the eye and adnexa (24/47 studies); several studies focus on the treatment of metabolic diseases (8/47 studies). The number of studies based on hiPS cells also increased markedly compared to the previous year – from 31 studies up to the end of 2020 to 46 studies up to the end of 2021. Most of these studies aim to develop therapies for (malignant) neoplasms and diseases of the circulatory system and the eye (33/46). Most of the clinical trials conducted with hiPS cells are based on allogeneic cells. The prediction originally made for cell therapeutics derived from hiPS cells – that such therapeutics could be produced and used on an autologous basis, thereby at least alleviating the need for immunosuppression – has not yet been realized. Clinical studies using cells derived from NT-hES cells or hpPS cells are still in the minority and focus on the treatment of age-related macular degeneration and Parkinson's disease.

The increase in the number of clinical trials based on hES cells – from 6 studies (as of the end of 2012) to 47 studies (as of the end of 2021) – indicates that, in some cases, steps towards translation into the clinic are increasingly being taken in recent years based on successful basic research using hES cells.

Overview of clinical trials based on human pluripotent stem cells conducted worldwide (2010-2021)

	Disease (according to ICD-10)	Number of studies	Participants (does not correspond to the number of patients treated)*
hES cells	**Endocrinal, nutritional and metabolic diseases**	**8**	**467**
	Type 1 diabetes mellitus	6	434
	Primary ovarian insufficiency	1	28
	Urea cycle disorders	1	5
	Diseases of the eye and adnexa	**24**	**292**
	Age-related dry macular degeneration	11	118
	Retinitis pigmentosa	2	22
	Stargardt's disease	5	53
	Other eye disorders	6	99
	Diseases of the circulatory system	**2**	**40**
	Ischaemic heart disease	1	10
	Cerebral infarction, unspecified	1	30
	Diseases of the musculoskeletal system and connective tissue	**1**	**18**
	Meniscus damage due to an old tear or old injury	1	18
	Diseases of the nervous system	**4**	**96**
	Motor neuron disease	1	16
	Primary Parkinson's disease	1	10
	Multiple sclerosis	1	30
	Epilepsy	1	40
	Diseases of the urogenital system	**2**	**35**
	Interstitial cystitis (chronic)	1	3
	Intrauterine synechiae	1	32
	Neoplasms	**1**	**48**
	Malignant neoplasm of the bronchi and lungs	1	48
	Injuries, poisonings and certain other consequences of external causes	**3**	**35**
	Injury of the spinal cord, number unspecified	3	35
	Provisional classifications of diseases with unclear aetiology and unassigned code numbers	**2**	**29**
	COVID-19	2	29
	hES studies: Total	***47***	***1060***

hiPS cells	**Diseases of the eye and adnexa**	**7**	**86**
	Age-related wet macular degeneration	2	7
	Age-related dry macular degeneration	1	20
	Hereditary retinal dystrophy: retinitis pigmentosa	1	2
	Retinal affection, unspecified	1	50
	Other corneal affections described in more detail	1	4
	Bullous keratopathy	1	3
	Diseases of the blood and blood-forming organs and certain disorders involving the immune system	**3**	**15**
	Beta thalassemia	2	14
	Other aplastic anaemias	1	1
	Diseases of the circulatory system	**11**	**141**
	Ischaemic cardiomyopathy	5	31
	Cerebral infarction, unspecified	2	24
	Cardiac insufficiency, unspecified	3	83
	Dilated cardiomyopathy	1	3
	Diseases of the nervous system	**3**	**24**
	Primary Parkinson's disease	3	24
	Neoplasms	**15**	**1165**
	Malignant neoplasms	4	368
	Head, face and neck	1	9
	Myeloid leukaemia	1	72
	B-cell chronic lymphatic leukaemia	2	420
	Malignant neoplasms of the mammary gland [mamma]	1	32
	Acute myeloblastic leukaemia (AML)	2	155
	Other and unspecified types of non-Hodgkin's lymphoma	1	50
	Malignant neoplasm of other and unspecified female genital organs	1	31
	Carcinoma in situ of other and unspecified genital organs	1	18
	Thrombocytopenia, unspecified	1	10
	Injuries, poisonings and certain other consequences of external causes	**3**	**24**
	Graft-versus-host disease	1	16
	Tear of the knee-joint cartilage, acute	1	4
	Injury of the spinal cord, number unspecified	1	4
	Provisional classifications of diseases with unclear aetiology and unassigned code numbers	**2**	**36**
	COVID-19	2	36
	Endocrinal, nutritional and metabolic diseases	**1**	**20**
	Type 1 diabetes mellitus	1	20
	Diseases of the musculoskeletal system and connective tissue	**1**	**440**
	Gonarthrosis, unspecified	1	440
	hiPS studies: Total	***46***	***1951***

NT-hES cells	**Diseases of the eye and adnexa**	**1**	**3**
	Age-related dry macular degeneration	1	3
	NT-hES studies: Total	***1***	***3***
hpPS cells	**Diseases of the nervous system**	**2**	**62**
	Primary Parkinson's disease	2	62
	hpPS studies: Total	***2***	***62***
	Studies using pluripotent stem cells: Total	**96**	**3076**

Table 3. Clinical trials with cells derived from human pluripotent stem cells (including long-term studies with patients from previous clinical trials). *The term 'participants' refers to the number of people who have been or are to be recruited for the respective study. The number of people who are already being treated or have been treated is currently only partly known to the public. Robert Koch Institute, unpublished data from various sources, *inter alia*: 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 on 31 December 2021.

See Table 4 for an overview of the countries in which the clinical trials are carried out. It can be stated here that between 2010 and 2021 clinical trials based on hES cells were carried out mainly in the USA, China and the UK; trials using cell products derived from hiPS cells took place mainly in Japan, the USA and China. It is striking that the findings of stem-cell research are frequently translated into clinical practice in countries where legislation is less restrictive and/or there is extensive funding of pluripotent stem-cell research. Numerous clinical trials are being conducted in Western industrialized countries with liberal regulations on stem-cell research (e.g. USA, UK, Canada) or extensive research funding (Japan), whereas important industrialized countries with restrictive framework conditions (e.g. Italy, Germany) have no or very little research activity in this field. In Germany, one clinical trial based on hiPS cells is currently being conducted with the aim of treating cardiac insufficiency.

Overview of the countries where clinical trials with human pluripotent stem cells are carried out (2010-2021)

	Country	Number of studies
hES cells	USA	21
	China	11
	UK	6
	Canada	4
	Korea	3
	Israel	2
	France	2
	Brazil	1
	Japan	1
hiPS cells	Japan	19
	USA	13
	China	9
	Australia	3
	UK	1
	Iran	1
	Germany	1
NT-hES cells	Korea	1
hpPS cells	Australia	1
	China	1
	Total	**101***

Table 4. Overview of countries in which clinical trials based on hPS cells had been or were being conducted or initiated up to the end of 2021 (including long-term studies with patients from previous clinical trials). * Some studies are being carried out in several countries. Robert Koch Institute, unpublished data from various sources, *inter alia*: 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 on 31 December 2021.

3.4. Final remarks

As stated in the 2020 Report, it should be emphasized that the remarks made in previous ZES reports on the desiderata of the Stem Cell Act remain valid. Twenty years after the law came into force, the problems include:

- the cut-off date, now 15 years ago, which prevents imports of newer stem cell lines relevant to research,
- the restriction of research objectives, insofar as research directly aimed at replacing animal testing methods may not be approved,
- the contradiction inherent in the Stem Cell Act's exemption for research, i.e. that hES cells may be used in research projects but not for the subsequent application of research results. If hES cell lines are required for this purpose, according to the Stem Cell Act the results of hES cell research may not be implemented for pharmacological or toxicological purposes or in clinical practice in Germany.

There is an ongoing need for clarification and reform on such points.

The 19th Report was adopted at the 109th ordinary meeting of the ZES on 11 April 2022.