# Report

## of the

# Central Ethics Committee for Stem Cell Research (ZES)

**18th Report after the Enactment of the**
**Stem Cell Act (StZG)**
**for the Reporting Period**
**1 January to 31 December 2020**

## 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/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 sends 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 page 2277), 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 Regulation – ZESV) dated 18 July 2002 (BGBl. I p. 2663), 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). At the end of the sixth appointment term, which expired in August 2020, Prof. Dr Wobus, Prof. Dr mult. Knoepffler, Prof. Dr Stoecker and Prof. Dr Tanner retired from the ZES. Fourteen members and deputy members were reappointed, and three members and one deputy member were appointed for the first time to the ZES for what is now the seventh appointment term (2020 to 2023). 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 research objectives of high-level interest for a gain in scientific knowledge (section 5, no. 1 of the Stem Cell Act), (b) the scientific issues have already 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 sends 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,<br>**(Deputy Chair)**<br>Natural and Medical Sciences Institute at the University of Tübingen | Prof. Dr Maria Wartenberg,<br>Universitätsklinikum (University Hospital) Jena,<br>Molecular Cardiology and Stem Cell Research |
| Prof. Dr Hans R. Schöler,<br>Max Planck Institute for Molecular Biomedicine, Münster | Prof. Dr Martin Zenke,<br>RWTH Aachen<br>Helmholtz Institute for Biomedical Engineering |
| **Medicine** | |
| Prof. Dr Mathias Bähr,<br>Georg-August-Universität Göttingen (University of Göttingen),<br>Neurological Clinic | Prof. Dr Wolfram H. Zimmermann,<br>Georg-August-Universität Göttingen (University of Göttingen),<br>Institute of Pharmacology and Toxicology |
| Prof. Dr Anthony D. Ho,<br>Ruprecht-Karls-Universität, Heidelberg (Heidelberg University),<br>Medical University Hospital | Prof. Dr Beate Winner,<br>Friedrich-Alexander-Universität Erlangen-Nuremberg,<br>Universitätsklinikum (University Hospital) Erlangen,<br>Department of Stem Cell Biology |
| Prof. Dr Sonja Schrepfer,<br>University Heart and Vascular Center, UKE Hamburg, Department for Cardiovascular Surgery | Prof. Dr med. Ricardo E. Felberbaum,<br>Klinikum Kempten Oberallgäu (Kempten Hospital),<br>Gynaecological Clinic |
| **Ethics** | |
| Prof. Dr Dr Sabine Salloch<br>**(Deputy Chair)**,<br>Medizinische Hochschule Hannover (Hanover Medical School),<br>Institute of the History, Ethics and Philosophy of Medicine | Prof. Dr Dres. h. c. Michael Quante,<br>University of Münster,<br>Department of Philosophy |
| Prof. Dr Silke Schicktanz,<br>Universitätsmedizin Göttingen (Göttingen Medical School),<br>Institute of Ethics and History of Medicine | Prof. Dr Christine Hauskeller,<br>University of Exeter, England,<br>Department of Sociology, Philosophy and Anthropology |
| **Theology** | |
| Prof. Dr Dr Antonio Autiero<br>**(Chair)**,<br>University of Münster,<br>Faculty of Catholic Theology | Prof. Dr Dr Jochen Sautermeister,<br>Rheinische Friedrich-Wilhelms-Universität Bonn (University of Bonn),<br>Faculty of Catholic Theology |
| Prof. Dr Thorsten Moos,<br>Kirchliche Hochschule Wuppertal/Bethel (Bethel Ecclesiastical College, Wuppertal),<br>Institut für Diakoniewissenschaft und Diakoniemanagement (Institute for science and management of Protestant social welfare work) | Prof. Dr Hartmut Kress,<br>Rheinische Friedrich-Wilhelms-Universität Bonn (University of Bonn),<br>Faculty of Protestant Theology |

**Table 1.** Members and deputy members of the Central Ethics Committee for Stem Cell Research (ZES), last revised December 2020

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

In 2020, the ZES discussed a total of eight applications for the import and use of hES cells at in-person meetings or, due to the Covid pandemic, video conferences. 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 (154) | Max Delbrück Center for Molecular Medicine (MDC), Berlin | Study on the role of circular RNAs in the regulation of splicing in human embryonic stem cells and during neuronal differentiation | 15/01/2020 |
| 2 (155) | Max Delbrück Center for Molecular Medicine (MDC), Berlin | Effect of a specific mutation in the *KCNQ1* gene on the development and function of pancreatic beta cells derived from human embryonic stem cells | 23/03/2020 |
| 3 (156) | Prof. Dr Alexander Kleger,<br>Universitätsklinikum Ulm (Ulm University Hospital) | Characterization of the gastrointestinal manifestation of SARS-CoV-2 infection in intestinal organoids derived from human embryonic stem cells | 24/04/2020 |
| 4 (157) | Klinikum rechts der Isar (Rechts der Isar Hospital) Technische Universität München (Technical University, Munich) | Study of cardiovascular progenitor cells derived from human embryonic stem cells to examine their suitability for treating cardiac diseases in large-animal models | 29/05/2020 |
| 5 (158) | Dr Claudio Acuna Goycolea,<br>Universitätsklinikum Heidelberg (Heidelberg University Hospital) | Study of specific functions of human astrocytes and their role in the development of neurological diseases using human embryonic stem cells | 11/06/2020 |
| 6 (159, 160) | Dr Claudio Acuna Goycolea,<br>Universitätsklinikum Heidelberg (Heidelberg University Hospital)<br>Dr Varun Venkataramani,<br>Universitätsklinikum Heidelberg (Heidelberg University Hospital) | Modelling of human glioblastomas and brain metastases using human neural cells derived from embryonic stem cells | 26/06/2020 |
| 7 (161) | Dr Jacob J. Metzger,<br>Max Delbrück Center for Molecular Medicine (MDC), Berlin | Differentiation of human embryonic stem cells into organoids for the quantitative study of cortical development and associated human diseases | 29/09/2020 |
| 8 (162) | Prof. Dr Wolfram-H. Zimmermann,<br>Universitätsmedizin Göttingen (Göttingen Medical School) | Use of human embryonic stem cells for the development of human tissues for pharmaceutical research | 14/10/2020 |

| Extensions of already approved applications | | | |
|---|---|---|---|
| 9<br>Extension of approval (125) | Max Delbrück Center 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 | 06/01/2020 |
| 10<br>Extension of approval (102) | Dr Ulrike Nuber,<br>Technische Universität Darmstadt (Technical University, Darmstadt) | Studies on the role of MECP2 in the development of Rett syndrome. Development of methods for isolating and enriching neural progenitor cell populations | 16/01/2020 |
| 11<br>Extension of approval (123) | Dr Insa Schröder,<br>GSI Helmholtz Centre for Heavy Ion Research, 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 | 19/03/2020 |

**Table 2.** Overview of research projects that were approved by the RKI during the 2020 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 registry (http://www.rki.de/DE/Content/Gesund/Stammzellen/Register/register_node.html).
For one application (serial no. 6), two identical, but formally independent approvals were granted.

The aim of the first research project listed in Table 2 (154th approval under the Stem Cell Act) is to clarify whether and in what way human-specific circular RNAs (circRNAs) are involved in the regulation of early developmental processes in human cells, particularly in maintaining pluripotency and in neuronal differentiation. In particular, research is to be conducted into whether and to what extent circRNAs can influence the process of RNA splicing via direct trans-RNA-RNA interactions between circRNAs and immature mRNAs. In the project, RNA-Seq libraries are to be produced and sequenced from naïve and primed hES cells, as well as from neuronally differentiating hES cells; furthermore, linear and circular splice products as well as their trans-RNA-RNA interaction partners are to be identified using bioinformatic methods of analysis. It is hoped that the research will contribute to new insights into the function of circRNAs during splicing in human pluripotent stem cells and during neuronal differentiation processes in human cells. In the longer term, these findings may also be important for explaining diseases where non-coding RNAs play a key role in development and progression, for example in various types of cancer or in neurodegenerative diseases.

The second research project (155th approval under the Stem Cell Act) intends to investigate whether and in what way a specific mutation in the *KCNQ1* gene, which codes for a potassium channel protein, could impair the differentiation of pluripotent stem cells into pancreatic beta cells and thus contribute to the development of diabetes. This mutation has led to permanent neonatal diabetes mellitus in one patient. To decode the mechanisms underlying the developments of permanent neonatal diabetes mellitus, the patient-specific mutation will first be introduced into hES cells. The genetically modified cells will then be differentiated towards pancreatic beta cells and their properties comprehensively studied at the molecular and functional levels. The results of this work will most likely provide new insights into the molecular and cell-biological principles of the pathogenesis of the permanent neonatal diabetes mellitus observed here. The research may also contribute to a better understanding of a potential role of KCNQ1 in human pancreas development.

The focus of the third research project (156th approval under the Stem Cell Act) is to study the hitherto unknown molecular and cell-biological principles of infection of cells in the human gastrointestinal tract caused by the novel coronavirus SARS-CoV-2; human intestinal organoids derived from embryonic stem cells are to be used as an infection model. The background for the research work are numerous reports that an infection with SARS-CoV-2, which causes severe respiratory diseases, possibly with a lethal outcome, also leads to

symptoms of gastrointestinal diseases in some patients. Based on published protocols, hES cells will first be differentiated into intestinal organoids and infected with SARS-CoV-2. The next step will be to determine the intestinal cell types that are permissive for the virus or show damage as a result of infection; the effects of the viral infection, as well as the viral replication in intestinal cells, will be thoroughly studied. Tests will be carried out subsequently to determine whether and to what extent antiviral agents already approved for the treatment of humans can inhibit the infection of intestinal cells or the replication/assembly of the virus in these cells. Overall, the applied-for research can contribute to understanding the molecular and cell-biological principles of the infection of cells in the human digestive tract with SARS-CoV-2 and, on this basis, possibly to developing new therapeutic approaches to treating diseases in the gastrointestinal tract caused by SARS-CoV-2.

In the fourth research project (157th approval under the Stem Cell Act), a large-animal model (pig) is to be used to examine whether cardiac progenitor cells derived from hES cells are suitable for regenerating the heart in cases of cardiac insufficiency. Cardiac (myocardial) insufficiency is one of the main causes of death in western industrialized countries. Severe cardiac insufficiency leads to a high mortality rate (approx. 50%) within 5 years of diagnosis as a result of congestive heart failure or malignant cardiac arrhythmias. No efficient therapies for cardiac regeneration are available to date. The intention in this project is therefore to differentiate hES cells according to established procedures into cardiac progenitor cells and transplant them into pigs in which a tissue lesion of the heart has been induced by radiofrequency ablation (RFA) or myocardial ischaemia. Furthermore, transplantation is also to be performed in pigs with genetically caused cardiomyopathies. Subsequently, various parameters will be used to determine the extent to which the transplanted cardiac progenitor cells cause a regeneration of the damaged myocardium. One aim will be to investigate the survival, proliferation and maturation of the cells, another to assess therapeutic effects. Since permanent immunosuppression in humans is associated in some cases with considerable side effects, the above-mentioned studies will also be carried out using an already published multiple transgenic and hypoimmunogenic hES cell line. These studies aim primarily to provide new insights into the potential of cardiac precursor cells produced from human pluripotent stem cells in the *in-vivo* regeneration of the heart. This may be highly relevant for future treatments of cardiac insufficiency.

The fifth research project (158th approval under the Stem Cell Act) focuses on the production and use of functional hES cell-derived astrocytes. The human astrocytes are to be produced from hES cells based on published procedures and used to answer a number of research questions. In the first part of the project, astrocytes derived from hES cells will be used as a backing layer/trophic support for co-cultivation with neurons in order to establish purely human functional neural networks, avoiding the use of murine astrocytes, and to determine whether and to what extent more authentic cell models can be developed by using human astrocytes for neural network formation. A second subproject focuses on studying the contribution of human astrocytes and factors secreted by them to the ability of human neurons to form synapses. Finally, a third subproject will aim to determine whether and in what way mutations associated with neuropsychiatric diseases that lead to altered astrocyte function contribute to the initiation and development of the corresponding diseases. As a result of the work, new insights may emerge into the function of human astrocytes for neural networks and their influence on the formation and properties of synapses. In addition, insights are expected into processes that lead to changes in synaptogenesis and synaptic functionality as a result of altered astrocyte function, which can contribute to the development of neuropsychiatric disorders such as autism.

The aim of the sixth research project (159th and 160th approval under the Stem Cell Act) is to develop a better understanding of interactions between tumour cells and nerve cells and, in particular, to investigate the role of synapses between neurons and tumour cells in the progression of brain tumours. The research comes against the backdrop of new evidence that neurons interact with developing tumours in the brain via newly formed synapses, which

evidently leads to increased tumour growth. The molecular causes of tumour progression enhanced by synaptic interactions are poorly understood at present. The project will therefore initially establish different co-culture models with neural cells derived from hES cells and glioma or brain-metastasis cell lines. The effect of this co-culture on tumour-cell growth and properties will then be determined and the synapses forming between the different cell types will be comprehensively characterized at the morphological, molecular-biological and functional levels. Another focus of the work is on identifying new therapy targets for brain tumours and on clarifying whether and to what extent the newly established co-culture models are suitable for testing the effect of ionizing radiation and chemotherapeutic agents on tumour growth. The results of the work may provide new insights into the molecular and cellular mechanisms underlying the initiation and progression of brain tumours, which may form a basis for developing new therapies for brain tumour diseases.

The subject of the seventh research project (161st approval under the Stem Cell Act) is the establishment of methods for the reproducible generation of human cortical organoids, which can be used to answer questions on the development of the human cortex and to study the molecular and cellular principles of neurodevelopmental disorders and neurodegenerative diseases *in vitro*. First, the procedures for generating cortical organoids from hES cells will be further developed and optimized with the aim of achieving a high degree of reproducibility of the organoid properties by using predefined microstructures for differentiation. After extensive characterization of the organoids, the further maturation of these cells in the cortical organoids, especially with respect to synaptogenesis and the formation of the multilayered structure of the cortex, will be analysed over a longer period of time. Finally, the cortical organoids will be used to model neurodevelopmental disorders and neurodegenerative diseases; the mutations associated with the corresponding diseases/developmental disorders will be generated in hES cells, among others, which will then be the starting point for the production of correspondingly mutated cortical organoids. The work will also make comparisons using human-induced pluripotent stem cells (hiPS cells). As a result of the work, new *in-vitro* model systems based on human neurons can be created that mimic cortical development in humans, making it possible to study complex developmental processes and changes associated with neurodevelopmental disorders and neurodegenerative diseases, as well as the mechanisms underlying these processes. This can provide new insights into the molecular and cellular processes underlying human cortex development and elucidate pathogenesis mechanisms of neurodevelopmental disorders and neurodegenerative diseases. The work may also lay the foundation for the future development of new pharmacological test systems in the development of active ingredients, which may be significant for the development of new therapies.

The central objective of the eighth research project (162nd approval under the Stem Cell Act) is to establish 3D tissue models based on human cells for skeletal-muscle tissue, connective tissue, nerve tissue and liver tissue as pharmacological and toxicological *in-vitro* test systems, and to study their maturation and functionality. In addition to applications in drug development, the tissue models to be established here will also be developed and analysed with regard to their potential use in future tissue-replacement therapies in humans. Both tissue-engineering techniques and organoid approaches are to be used for the production of the human 3D tissue models. It is expected that test methods which – in contrast to two-dimensional cell cultures or animal models – are based on 3D tissue models with human cells can predict the effects and side effects of drugs better than has been possible up to now. All work will also make comparisons using hiPS cells to confirm the equivalence of tissue models derived from the respective cell type or to determine any differences.

During the reporting period, applications were made for extensions of research work relating to three approvals on which the ZES commented in the run-up to approval (see serial nos. 9, 10 and 11 in Table 2).

The focus of the research project listed under no. 9 lies on the study of the potential role of the human endogenous retrovirus H (HERV-H) in maintaining the pluripotency of human embryonic stem cells. The objective of the research projects remains fundamentally

unchanged. However, the need to conduct complementary research work has arisen in the course of the project. On the one hand, this work will first aim to clarify whether increased LINE-1(L1) transposition – which involves a higher incidence of DNA double-strand breaks and is associated with increased apoptosis activity – is suppressed by HERV-H, which could contribute to maintaining a (possibly naïve) pluripotent stem-cell population. On the other hand, the research is aimed at demonstrating a hitherto unknown, possible function of the product of the *ESRG* gene, whose expression is controlled by HERV-H, in the regulation of telomere length. The corresponding experiments aim to provide clues to the mechanistic principles of a possible regulation of pluripotency by HERV-H and to allow a deeper understanding of functions of *ESRG* in the regulation of pluripotency in human cells. Overall, the results of the additional work may provide new insights into the role of HERV-H during the development of early human embryonic cells.

One of the aims of the research project listed under no. 10 is to develop procedures for separating and enriching different subpopulations of neural progenitor cells differentiated from pluripotent human stem cells on the basis of specific surface markers. The enriched neural progenitor cells will then be comprehensively characterized *in vitro*. Extending the previously approved work, characterization *in vivo* (after transfer into chicken embryos) is now planned in addition. Overall, the work may provide a basis for the provision of better characterized and purer populations of neural progenitor cells for differentiation into the respective neural cell type that is of interest. This could also make clinically relevant neural cell types available in greater quantity and purity than is currently possible.

The project listed under no. 11 will study the consequences of ionizing radiation – alone or in combination with tumour drugs – on populations of specific neurons or cerebral organoids. An additional aim is to establish more realistic models for the evaluation of possible toxic effects of radiation/tumour drugs on human neural cells by culturing the neurons or cerebral organoids with tumour cells and vascularizing the organoids. Furthermore, tumours are also to be induced by the targeted overexpression of specific oncogenes (or by inhibiting the expression of tumour-suppressor genes) in the cells of cerebral organoids and subsequently also used to study possible damaging effects of radiation/tumour drugs on neural cells. Another aim of the research work is to further contribute to the understanding of the cell-biological and molecular processes in nerve cells during the radiation and/or drug treatment of brain tumours and in this way to lay the foundation for establishing cell models for predicting possible neurotoxic effects of radiation and tumour drugs in the human brain.

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

Moreover, at its 103rd regular meeting on January 15, 2020, the ZES reviewed the latest research on the self-organization of the early mammalian embryo. Recently, there have been new research approaches in this field which applied embryo cultures and artificial embryo models. In this way, very early phases of embryonic development can be imaged and studied in detail *in vitro* for the first time. In the context of the increasing use of artificial embryo models from stem cells in research and their rapid further development, the ZES discussed the associated legal and ethical problems.

Two of the new applications discussed during the reporting period were submitted by researchers or institutions that had not previously received approval under the Stem Cell Act. Six 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 18 years of activity, the ZES has submitted opinions to the RKI on a total of 158 applications for the import and/or use of hES cells. In addition, a total of 39 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 162 approvals, some of which have been extended. Thirty-two of these approvals have expired to date. At present, 88 groups at 53 research institutions are conducting approved research work with hES cells (figures for 31 December 2020).

## 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 significant proportion of the research projects approved during the reporting period continues to aim at providing human cell models for clarifying molecular and cellular causes of diseases, and at determining functional consequences of genetic defects for the pathogenesis of human diseases at the cellular level. Neurological diseases and developmental disorders are at the focus of interest (Table 2, serial nos. 5, 6, 7). Other projects aim to establish cellular models to study the cellular and molecular principles of permanent neonatal diabetes mellitus (Table 2, serial no. 2) and coronavirus disease 2019 (COVID-19, Table 2, serial no. 3). Some of the work also seeks to identify potential targets for pharmacological interventions and thus ultimately to contribute to developing new therapeutic methods for treating severe and sometimes inadequately treatable diseases.

#### *3.1.2 Preclinical research with cells derived from hES cells*

Research that can be classified as preclinical research was also evaluated during the reporting period (Table 2, serial no. 4). The aim of this work is to gain new insights into the potential of cardiac precursor cells obtained from human pluripotent stem cells for the *in-vivo* regeneration of the heart by using a large animal model. This is highly relevant for the future treatment of myocardial damage caused by infarctions, myocardial inflammation or genetic diseases.

#### *3.1.3 Cell and tissue models for toxicological and pharmacological testing*

Another subject of the research projects applied for in 2020 (Table 2, serial no. 8) was the generation of cells derived from human pluripotent stem cells (hPS cells) for the development of human three-dimensional cell and tissue models to form the basis for the future development of predictive toxicological and pharmacological *in-vitro* test systems for drug research. This project aims to make new human cell and tissue models available that reproduce the human *in-vivo* environment better than the test systems used up to now. In future, for example, it might be possible to test new active substances in an environment that is much closer to the situation in humans than two-dimensional human cell cultures or laboratory animals. This is important for a more precise assessment of the risks and efficacy of new active substances.

#### *3.1.4 Molecular principles of pluripotency and differentiation processes*

Research into the molecular principles of pluripotency and differentiation processes in human cells continues to be of interest (Table 2, serial no. 1). This project aims to clarify questions about the function of circRNA during splicing in human pluripotent stem cells and during neuronal differentiation processes in human cells. In the longer term, the expected findings may be important for the clarification of diseases in which the development and progression of non-coding RNAs play a decisive role.

### 3.2. Comparative studies on hiPS cells and hES cells

Comparative studies on hiPS cells and hES cells were also subject of some research projects in 2020. Two of the nine research projects approved in the reporting period study hiPS cells and hES cells in parallel (Figure 1). In one project (Table 2, serial no.1), hES cells are used as reference material for assessing the potential of hiPS cells to differentiate into the cell types that are the subject of interest. The various hiPS cell lines differ considerably in terms of their differentiation potential. Such differences may be due to the origin of hiPS cells from genetically different donors, each of whom has a specific genetic background, but it may also be due to the donor's age. Even where the donor is the same, considerable line-specific differences may be caused by reprogramming artefacts such as incomplete reprogramming or reprogramming-related *de-novo* mutations, by the somatic cell type used for reprogramming (blood cells, fibroblasts, keratinocytes, etc.), or by the method chosen for reprogramming. In another project (Table 2, serial no. 7), which aims to model developmental disorders and neurodegenerative diseases (autism spectrum disorders and Alzheimer's disease) *in vitro* and to clarify pathogenesis mechanisms at the cellular level, hiPS cells are compared with hES cells in which the mutation causing the disease has respectively been generated, as well as with corresponding isogenic wild-type controls. The aim here is to identify disease-specific signatures in order to be able to include in the studies cases of illness that are not based on known individual mutations and for which, therefore, no isogenic cell lines can be produced and used for the study. These studies can improve our understanding of the molecular causes of these diseases and contribute to the development of new therapeutic processes.

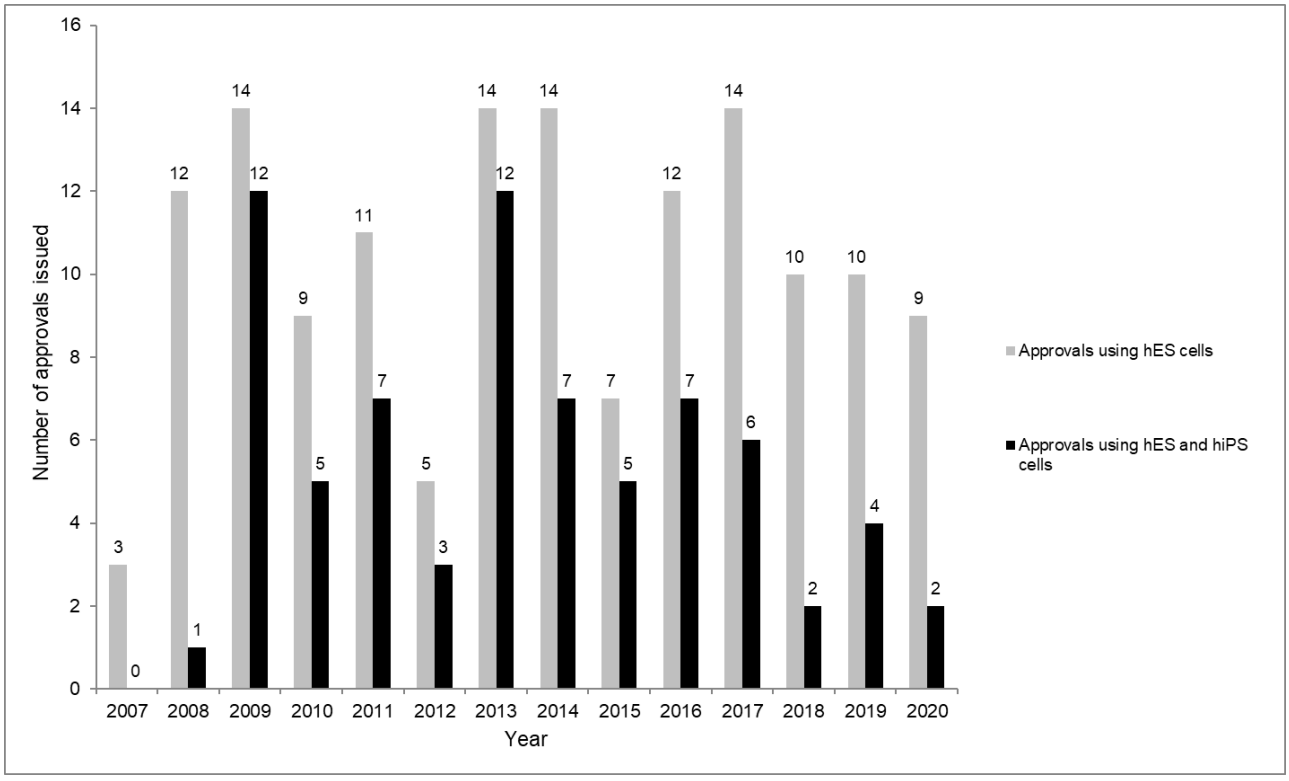


**Figure 1.** Use of hES and hiPS cells in approved research projects 2007-2020. The chart shows the total number of approved research projects (grey) and the number of research projects in which not only hES but also hiPS cells are used (black).

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

It can be observed that, compared to the previous year, in 2020 there was a marked increase in the number of clinical trials conducted using cells derived from hPS cells – rising from a total of 54 studies at the end of 2019 to 74 studies at the end of 2020. Furthermore, the spectrum of diseases to be treated using hPS-cell-derived cell therapeutics also expanded significantly. For example, clinical trials with hPS cell derived cells targeting the treatment of urea cycle disorders, intrauterine synechiae, interstitial cystitis, spinal cord injuries/diseases, coronavirus disease 2019 (Covid-19), (malignant) neoplasms (including head, face, neck, mammary gland), knee-cartilage injuries, aplastic anaemia and gonarthrosis were initiated. Table 3 provides an overview of clinical studies based on hPS cells that have been conducted since 2010 and/or were still being conducted.

The majority of the clinical trials listed in Table 3 were still being conducted using cell-therapeutic agents derived from hES cells (40/74 studies). 31 clinical studies are currently using material derived from hiPS cells. In two studies, cells based on human parthenogenetically produced pluripotent stem cells (hpPS cells) are applied, and in one study cells derived from embryos created by nuclear transfer (SCNT) (NT-hES cells) are used.

Most of the studies using cells derived from hES cells (which increased in number from 32 studies at the end of 2019 to 40 studies at the end of 2020) are still predominantly for the treatment of diseases of the eye and adnexa (24/40 studies), followed by studies on treating metabolic diseases (6/40 studies). The number of studies based on hiPS cells also increased markedly compared to the previous year – from 19 studies at the end of 2019 to 31 studies at the end of 2020. These studies are mainly aimed at the development of therapies for diseases of the circulatory system, of the eye and for (malignant) neoplasms (18/31). An almost equal number of the studies conducted with hiPS cells are based on autologous and allogeneic cells. 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 at the end of 2012 to 40 studies at the end of 2020 – is an indication that in several cases steps have increasingly been taken in recent years towards translation into clinical research on the basis of successful basic research using hES cells.

**Overview of clinical trials based on human pluripotent stem cells conducted worldwide (2010-2020)**

| | 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** | **6** | **380** |
| | Type 1 diabetes mellitus | 4 | 347 |
| | 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** | **1** | **10** |
| | Ischaemic heart disease | 1 | 10 |
| | **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** | **1** | **21** |
| | Motor neuron disease | 1 | 21 |
| | **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** | **2** | **30** |
| | Injury of the spinal cord, height unspecified | 2 | 30 |
| | **Provisional classifications of diseases with unclear aetiology and unassigned code numbers** | **2** | **29** |
| | COVID-19 | 2 | 29 |
| | **Total** | **40** | **863** |

| | | | |
|---|---|---|---|
| hiPS cells | **Diseases of the eye and adnexa** | **5** | **33** |
| | Age-related wet macular degeneration | 1 | 5 |
| | Age-related dry macular degeneration | 2 | 22 |
| | Hereditary retinal dystrophy: retinitis pigmentosa | 1 | 2 |
| | Other corneal affections described in more detail | 1 | 4 |
| | **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** | **8** | **101** |
| | Ischaemic cardiomyopathy | 4 | 21 |
| | Cerebral infarction, unspecified | 2 | 24 |
| | Cardiac insufficiency, unspecified | 1 | 53 |
| | Dilated cardiomyopathy | 1 | 3 |
| | **Diseases of the nervous system** | **3** | **24** |
| | Primary Parkinson's disease | 3 | 24 |
| | **Neoplasms** | **5** | **356** |
| | Malignant neoplasms | 2 | 152 |
| | Head, face and neck | 1 | 9 |
| | Myeloid leukaemia | 1 | 72 |
| | B-cell chronic lymphatic leukaemia | 1 | 123 |
| | **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, height 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 |
| | **Total** | **31** | **1049** |

| | | | |
|---|---|---|---|
| NT-hES cells | **Diseases of the eye and adnexa** | **1** | **3** |
| | Age-related dry macular degeneration | 1 | 3 |
| | **Total** | **1** | **3** |
| hpPS cells | **Diseases of the nervous system** | **2** | **62** |
| | Primary Parkinson's disease | 2 | 62 |
| | **Total** | **2** | **62** |
| | **Total studies** | **74** | **1977** |

**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 31/12/2020.

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 2020 clinical trials based on hES cells were carried out mainly in the USA, China and the UK, while trials using hiPS cells took place mainly in Japan, China and the USA. In Germany, a clinical trial based on human induced pluripotent stem 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 (2010-2020)**

| | Country | Number of studies |
|---|---|---|
| hES cells | USA | 15 |
| | China | 11 |
| | UK | 6 |
| | Canada | 4 |
| | France | 2 |
| | Israel | 2 |
| | Korea | 3 |
| | Brazil | 1 |
| | Japan | 1 |
| hiPS cells | Japan | 13 |
| | China | 8 |
| | USA | 6 |
| | Australia | 3 |
| | UK | 1 |
| | Germany | 1 |
| NT-hES cells | Korea | 1 |
| hpPS cells | Australia | 1 |
| | China | 1 |
| | **Total** | **80*** |

Table 4. Overview of countries in which clinical trials based on hPS cells had been or were being conducted or initiated by the end of 2020 (including long-term studies with patients from previous clinical trials) *Some trials were 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 31/12/2020.

### 3.4. Final remarks

The remarks made in the previous ZES reports on the desiderata of the Stem Cell Act remain valid. The problems include:

- the cut-off date, now 14 years ago, which prevents the import 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 authorized,
- 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 18th Report was adopted at the 106th ordinary meeting of the ZES on 17 March 2021.