Report

of the

Central Ethics Committee for Stem Cell Research (ZES)

20th Report after the Enactment of the Stem Cell Act (StZG)

for the reporting period from 1 January to 31 December 2022

1. The Central Ethics Committee for Stem Cell Research

The Central Ethics Committee for Stem Cell Research (Zentrale Ethik-Kommission für Stammzellenforschung, ZES) was convened 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 (hESC) 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 pursuant to the Stem Cell Act of the applications received by the RKI for the import and use of hESC. On the basis of the documents submitted by the applicants, the Committee determines whether a research project intending to import and/or use hESC 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 hESC (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/z/zentrale-ethik-kommission-fuer-stammzellenforschung.html
and
http://www.rki.de/DE/Content/Kommissionen/ZES/Taetigkeitsberichte/taetigkeitsbericht_node.html).

Table 1. Members and deputy members of the Central Ethics Committee for Stem Cell Research (ZES), as of December 2022

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

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

In 2022, the ZES discussed a total of 9 applications for the import and use of hESC. The ZES handed down favourable opinions on all the applications. 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).

Table 2. Overview of research projects approved by the RKI during the 2022 reporting period after receiving a positive opinion from the ZES. The numbers in brackets in the left column correspond to the approval numbers in the RKI register (http://www.rki.de/DE/Content/Gesund/Stammzellen/Register/register_node.html).

Serial no.	Holder of approval	Topic of approved research	Date of the positive ZES opinion
1 (176)	Prof. Dr. Christian Schachtrup, Albert-Ludwigs-Universität Freiburg	Study of ID3-depleted neural stem cells derived from hESC to determine their suitability for cell transplantation after spinal cord injuries.	05 January 2022
2 (177)	Johann-Wolfgang-Goethe-Universität Frankfurt am Main	Characterization of risk genes of neuronal developmental disorders in the isogenic neuronal cell model.	25 March 2022
3 (178)	Helmholtz Zentrum Munich, Forschungszentrum für Gesundheit und Umwelt (GmbH) (Research Centre for Environmental Health)	Study of changes in nuclear compartments in relation to transcriptional dynamics during the differentiation of hESC.	11 April 2022
4 (179)	Max-Planck-Institut für molekulare Genetik (Institute for molecular Genetics), Berlin	Studies on a potential embryonic developmental dormancy in humans using human pluripotent stem cells and blastocyst organoids.	11 April 2022
5 (180)	Charité – Universitätsmedizin (University Hospital) Berlin	Analysis of the epigenetic regulation of human metastable epialleles in hESC.	09 June 2022
6 (181)	Medizinische Hochschule Hannover (Hanover Medical School)	Establishment of procedures for the production of pancreatic beta cells from hESC on scales required for clinical applications.	26 July 2022
7 (182)	Heidelberg University	Development of liver-cell models derived from hESC for the study of hepatitis D virus infection	25 October 2022
8 (183)	Helmholtz Zentrum Munich, Deutsches Forschungszentrum für Gesundheit und Umwelt (GmbH) (German Research Centre for Environmental Health)	Generation of functional pancreatic islet cells from hESC for research into the causes of diabetes mellitus and the development of cell-replacement therapies.	14 November 2022
9 (184)	Max Delbrück Centre for Molecular Medicine (MDC), Berlin	Pancreatic organoids derived from hESC for studying pancreatic development and associated diseases.	14 November 2022

The subject of the **first** research project listed in Table 2 (176th approval in accordance with the StZG) is the investigation of whether and to what extent the differentiation of human neural stem cells (NSC) into reactive astrocytes can be prevented by silencing the gene that codes for the inhibitor of DNA binding 3 (ID3). The studies will be carried out against the background that, in the case of injuries to the central nervous system of mice, the NSC at the site of the lesion develop into reactive astrocytes, which then contribute to scar formation and inhibit the axonal growth of the damaged neurons by secreting inhibitory proteins. NSC that remain at the glial progenitor cell stage, on the other hand, can contribute to the formation of a more pro-regenerative environment by secreting growth and immunomodulatory factors, thus promoting axonal growth. The project therefore aims to silence the *ID3 gene* in hESC, differentiate the genetically modified hESC into NSC, and then investigate their potential *in vitro* to either differentiate into reactive astrocytes or develop towards pro-regenerative glial progenitor cells. The NSC populations are to be transplanted into a mouse model of spinal cord injury after extensive *in-vitro* characterization and their effects on the recovery of sensory, motor and autonomic functions investigated. It is hoped that the research will yield new insights into the potential of ID3-depleted NSC for *in-vivo* spinal cord regeneration. This may be highly relevant for the future treatment of spinal cord injuries, since neuronal regeneration is insufficient in the adult CNS after a spinal cord injury.

The focus of the **second** research project (177th approval in accordance with the StZG) is on research into the molecular causes of neuronal developmental disorders that occur as a result of the functional loss of genetic regions or genes; in particular, a gene copy polymorphism is to be investigated here that affects chromosomal region 16p11.2 with 29 genes located in this region. Duplications and especially deletions of this gene segment are associated with neuropsychiatric disorders and neuronal developmental disorders, especially autism spectrum disorders. Neuronal developmental disorders are severe diseases, and the analysis possibilities and therapy options are currently limited. The research will therefore involve deleting the chromosomal region 16p11.2 in hESC, differentiating the modified cells into neural progenitor cells, mature neurons and neural organoids, and investigating the effects of the genetic modification on neuronal differentiation and the properties of the cells or organoids. Furthermore, genes in the chromosomal region 16p11.2 are to be identified that contribute to a possibly altered neuronal phenotype. These genes will then be studied in more detail in further experiments to determine their function in neural differentiation by means of genetic rescue experiments. The research work is expected to contribute to new insights into processes that lead to changes in neuronal development due to gene defects in the chromosomal region 16p11.2 and subsequently to the development of neuronal developmental disorders.

The aim of the **third** research project (178th approval in accordance with the StZG) is to investigate in detail the interplay of gene expression, epigenome and chromatin structure during the transition of human pluripotent stem cells (hPSC) from the pluripotency stage to differentiation. The main aim is to elucidate the function of nuclear membraneless organelles (MLOs) in altering the spatial organization of the genome and in remodelling chromatin. In the project, changes in the transcription dynamics, the chromatin structure and the epigenome of hESC during the transition to early differentiation will first be recorded in detail and corresponding cell atlases created. In addition, changes in the morphology of nuclear MLOs during early differentiation will be detected using imaging techniques; subsequently, the activities of genes whose products are involved in the maintenance of MLO homeostasis will be specifically modulated. Finally, the relationship between MLO homeostasis and chromatin organization during differentiation will be investigated. These studies are expected to provide new insights into the molecular function of nuclear MLOs in controlling changes in gene expression and chromatin organization during differentiation of human cells.

In the **fourth** research project (179th approval in accordance with the StZG), hESC are to be used to investigate whether the cells of the human blastocyst have the ability to enter a developmental dormancy, as is known (as a diapause) from the embryonic development of

numerous other mammalian species, and by what means such a pause could be regulated in humans. For this purpose, hESC will first be transferred into naïve pluripotent stem cells, which will be treated with inhibitors of the mTOR signalling pathway, and the potential entry into dormancy will be studied by determining various critical parameters at the morphological and molecular level. In a second step, hESC will then be used to establish human blastocyst organoids, so-called blastoids, and these will be investigated with regard to their ability to enter a dormancy phase, also by specifically inhibiting the mTOR pathway. Furthermore, extensive analyses of the transcriptome will be carried out before, during and after developmental dormancy in order to identify the signalling pathways involved in the establishment, maintenance and/or exit of dormancy. The aim is for the research to lead to new insights into the ability of hPSC and their derived blastoids to enter a developmental dormancy. This can contribute towards an understanding of the molecular and cell-biological basis of such a developmental pause in humans. The work will also be carried out using human-induced pluripotent stem cells (hiPSC) and the results compared with those obtained using hESC.

The subject of the **fifth** research project (180th approval in accordance with the StZG) is a study of the influence of the presence of certain C1 metabolites on the methylation patterns of metastable epialleles during the early development of hESC. Changes in these methylation patterns can lead to a dysregulation of gene expression and thus to an increased risk of developing diseases in childhood and adulthood, e.g. obesity. In the project, hESC will first be transferred to the more pristine naïve state, then further developed into a formative stage (which corresponds to the state of embryonic cells in the pre-gastrulation phase of the embryo) and finally differentiated into different cell types. At each of these stages, potential metastable epialleles will be investigated with regard to the status of DNA methylation and with regard to chromatin modifications, and the binding of specific transcription factors to the metastable epialleles will be determined. In addition, the gene expression patterns of the cells will be analysed at the RNA and protein level at different points in development. Subsequently, the significance of the presence of C1 metabolites for the establishment of methylation in genetic regions of metastable epialleles will be investigated. Furthermore, the activity of enzymes related to C1 metabolism will be determined. These studies are primarily intended to provide new insights into possible causes of the methylation variability of human metastable epialleles and the associated risk of the development of certain diseases.

The **sixth** research project (181st approval in accordance with the StZG) using hESC aims to develop efficient and robust methods for the reproducible provision of functional insulin-producing beta cells in high quality and large quantities, as required for future regenerative treatments of type 1 diabetes mellitus (T1D). Currently, about 373,000 people in Germany live with T1D, which is about 0.4 percent of the total German population. No cure for T1D is hitherto possible. Within the framework of the research work, the conditions for the cultivation and propagation of hESC, for their differentiation into pancreatic progenitor cells and for the further development of these progenitor cells into mature pancreatic beta cells are to be established respectively in suspension cultures using specific bioreactors and optimized with regard to critical parameters; this will also involve a gradual increase in the culture volumes. The identity, quantity and quality of each of the intermediates and the terminally differentiated pancreatic cells will be examined on the basis of different parameters (including gene expression patterns, cell surface markers, etc.). Finally, the overall process of obtaining large quantities of pancreatic beta cells from hESC under 3D conditions and on a large scale will be established and transferred to Good Manufacturing Practice (GMP) conditions. Furthermore, various hiPSC lines will also be investigated with regard to their ability to develop into pancreatic beta cells, and compared with hESC in this respect. It is expected that this work will increase the yields of pancreatic progenitor cells and pancreatic beta cells and significantly improve the reproducibility and robustness of the differentiation process. Moreover, it is hoped that the work will lead to the establishment of reliable procedures that can be used to provide comprehensively characterized GMP-certified beta cells in sufficient quantities. This represents an important prerequisite for the use of pluripotent stem-cell-derived pancreatic

cells for the cell-therapy treatment of people suffering from diabetes, which is conceivable in the future.

The focus of the **seventh** research project (182nd approval in accordance with the StZG) is on the development of an *in-vitro* model that can be used to study processes of infection of human liver cells with the hepatitis D virus (HDV). Hepatitis D is a global health problem: according to the WHO, 15-20 million people worldwide are infected with HDV. Safe and effective therapies that specifically target hepatitis D are not currently available. The project first aims to determine which of the hitherto established protocols for generating human liver-cell-like like cells (HLC) from hESC can be used to obtain the HLC best suited for HDV infection, as well as which intermediate cellular stages of liver-cell differentiation are permissive for HDV infection, and whether the infection rate can be increased by ectopically expressing the gene for the HDV entry receptor in HLC. Subsequently, it will be examined whether and to what extent the entire life cycle of HDV can be recapitulated in HLC in the presence of the S antigens of the hepatitis B virus (HBV) after infection with HDV, and whether and to what extent HLC-based cell models are suitable for identifying antivirally active substances. Over a longer-term perspective, it is hoped that the cell models based on hESC will make it possible to expand our understanding of important steps in the life cycle of the hepatitis D virus and might be important for elucidating the pathogenesis of the virus.

The focus of the **eighth** research project (183rd approval in accordance with the StZG) is to optimize procedures for the *in-vitro* harvesting of hormone-producing, mature pancreatic islet cells, thus gaining a better understanding of regulators of differentiation, maturation and authentic function, and of factors that influence the metabolism of pancreatic cells. To this end, the project aims to use genome editing to introduce precise genetic changes in genes for relevant factors for transcription, splicing and metabolic processes in hESC, and to comprehensively investigate the effects on the properties of pancreatic cells derived from these hESC. These studies include, among others, the analysis of differentiation and maturation behaviour, analyses of the transcriptome, proteome and metabolism, as well as a functional characterization of the pancreatic cells *in vitro* and *in vivo*. In this way, the aim is, on the one hand, to provide more mature, functional islet and beta cells for future cell-replacement therapy and, on the other hand, to contribute to reaching a deeper understanding of the pathogenesis of diabetes mellitus. In order to be able to use hiPSC as starting material for the production of islet and beta cells in the future, it is furthermore planned to also carry out the work using hiPSC, with hESC serving as reference material.

The subject of the **ninth** research project (184th approval in accordance with the StZG) is the *in-vitro* production of human pancreatic organoids from hESC, which will then be used to investigate questions of pancreatic development and maturation, to analyse interactions of different pancreatic cell types in the context of the organoids, to develop model systems particularly for type 2 diabetes (T2D), and to identify potential active substances for treating T2D. To this end, the role of certain transcription factors in beta-cell development will first be investigated in detail *in vitro*. The next step will be to develop pancreatic organoids that, in addition to hESC-derived endocrine cells, also contain primary or hESC-derived endothelial cells/tissues. The properties of the endocrine cells in a complex tissue environment will then be investigated and their interactions with other cells in the organoid analysed. In addition, the influence of various forms of metabolic and immunological stress, as also occurs in T2D, on the survival and properties of the pancreatic cells in the organoid will be determined. Finally, pancreatic organoids will be used to establish *in-vitro* models for genetic forms of diabetes mellitus. After comprehensive analysis of their cell-biological, molecular and functional properties, these *in-vitro* models will then be used to screen substance libraries with the aim of identifying substances that increase in particular the survivability of beta cells under stress conditions. The research aims to contribute to a better understanding of the molecular and cellular mechanisms of human pancreas development, and of fundamental mechanisms of the pathogenesis of diabetes mellitus.

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 justifying the high-ranking status of the research projects, the sufficient preliminary clarification of the respective research questions, and the need to use hESC were also included in the assessment of the research projects by the RKI.

Of the applications discussed during the reporting period (Table 1, serial nos. 2-9), six 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 20 years of activity, the ZES has submitted opinions to the RKI on a total of 178 applications for the import and/or use of hESC. 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.[1]

Since the Stem Cell Act came into force, the RKI has issued 184 approvals, some of which have been extended. Forty-five of these approvals have expired to date. Currently, 98 groups at 55 research institutions in Germany are in possession of approvals in accordance with the StZG and can conduct research using hESC (as of 31 December 2022).

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

3.1. Research topics in the reporting period

3.1.1 Generation of therapeutically useful cells from hPSC

Of the nine research projects evaluated during the reporting period, three projects (Table 2, serial nos. 1, 6, 8) deal with the establishment and optimization of methods for the *in-vitro* production of potential starting materials for a clinical application. Two research projects focus on diseases of the pancreas; methods for the efficient production of hormone-producing, mature pancreatic islet and beta cells from hPSC are needed for future treatments of such diseases (Table 2, serial nos. 6, 8). Another of these projects (Table 2, serial no. 1) aims to provide neural stem cells suitable for the *in vivo* regeneration of the spinal cord; in the future, these may be the starting material for the production of cell therapeutics treating spinal cord injuries. The development of procedures for the provision of cells for clinical applications is highly relevant in view of the international developments in this field.

3.1.2 In-vitro disease models using hPSC

A large proportion of the research projects approved during the reporting period aim to provide human cell models for clarifying molecular and cellular causes of diseases, and to determine the relation between modifications in genes and/or genetic regions and the pathogenesis of human diseases at the cellular level. In this context, research projects are carried out on questions of neuronal developmental disorders and diseases related to the methylation variability of human metastable epialleles (Table 2, serial nos. 2 and 5). Two further research projects are directed towards the provision of cell models for the study of hepatitis delta infection and diabetes mellitus type 2 respectively (Table 2, serial no. 7 and 9). Some of these projects aim to generate (epi)genetic modifications in disease-relevant genes or genetic regions in hESC to determine the consequences for the differentiation of the cells, and for the

[1] The discrepancy between 178 favourable opinions of the ZES and 184 approvals of the RKI to date is due to the occasional joint application by more than one person. In these cases, two or three approvals were granted on the basis of only one opinion on the respective application.

functionality of the differentiated cells. Insights are expected here into changes in processes that take place at the cellular level in the respective disease of interest, hopefully leading to a better understanding of the molecular pathogenesis of the diseases in question than has been the case to date. 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 treating these severe and sometimes inadequately treatable diseases.

3.1.3 Regulation of differentiation processes and early human embryonic development

Two of the research projects approved during the reporting period aim to clarify research questions relating to the importance of specific molecular processes in differentiation processes of hESC and early embryonic development. The aim is to gain new insights into the molecular function of nuclear membraneless organelles in controlling changes in gene expression and chromatin organization during the differentiation of hESC (Table 2, serial no. 3). The aim of another research project is to clarify whether and to what extent pluripotent cells of humans (and blastoids derived from them) have the ability to enter a developmental dormancy. The molecular and cell-biological basis of such a developmental pause in humans is to be investigated (Table 2, serial no. 4). In this project, which involves the use of blastoids, it becomes particularly clear that research on hESC can address and possibly clarify important questions about early human embryonic development which could only have been answered using human embryos a few years ago.

The findings of these research projects may contribute to a deeper understanding of the control of basic development-biological processes in humans.

3.2. Comparative studies on hiPSC and hESC

Comparative studies using hiPSC and hESC are the subject of three of the nine research projects approved during the 2022 reporting period (Figure 1). In one project (Table 2, no. 4), in which the aim is to clarify the extent to which hPSC have the capacity for an embryonic developmental dormancy and what the molecular basis of such a developmental pause in humans might be, hESC and hiPSC are to be studied comparatively, since no comprehensive knowledge is yet available for either type of cell. In two other projects, hESC are to be used as reference material to assess the potential of hiPSC to differentiate into insulin-producing islet and beta cells (Table 2, nos. 6 and 8). Different hiPSC 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 and the factors used in this context for reprogramming, or by a possible epigenetic memory of the cells.

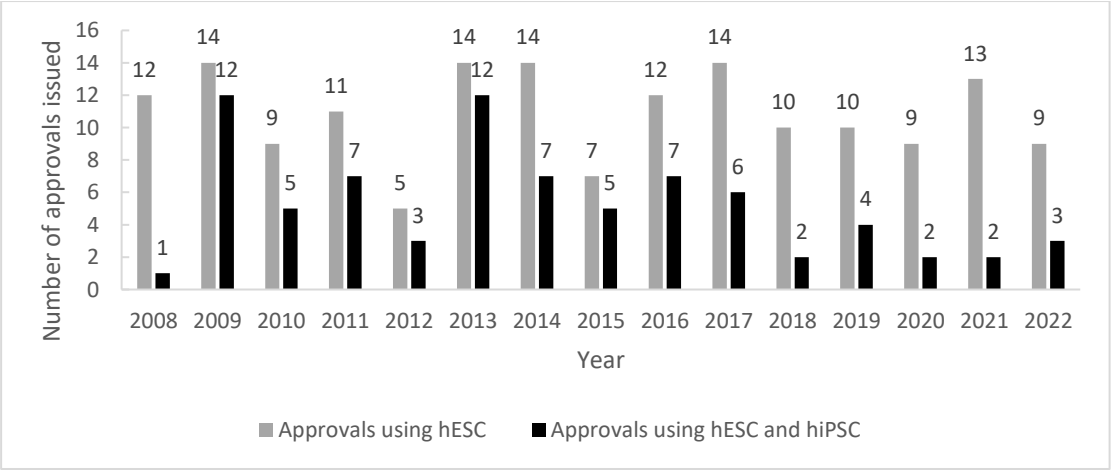

Figure 1. Use of hESC and hiPSC in approved research projects 2008-2022. The chart shows the total number of approved research projects (grey) and the number of research projects in which not only hESC but also hiPSC are used (black).

3.3. Increase in the number of clinical trials using hPSC

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 continuously moving in the direction of clinical applications. Table 3 provides an overview of the hPSC-based clinical trials conducted worldwide in the period from 2010 to 2022. Their number now totals 112 studies (as of the end of 2022). In such studies, cells derived from hPSC are being tested to determine their suitability for treating diseases for which no adequate therapy options currently exist. Their safety and tolerability are also being examined in long-term studies in the meantime. Furthermore, over time there has also been a continuous expansion in the spectrum of diseases for whose treatment cell therapeutics derived from hPSC are being clinically tested. For example, clinical trials based on hPSC were initiated for the first time in the reporting period, targeting the therapy of left heart failure, acute and subacute liver failure, multiple myeloma and B-cell lymphoma.

The clinical trials listed in Table 3 are conducted predominantly using cell therapeutics derived from hESC or hiPSC in approximately equal measure (hESC 52/112, hiPSC 57/112). Two studies use cells based on human, parthenogenetically produced pluripotent stem cells (hpPS cells), and one study uses cells (NT-hESC) derived from embryos created by nuclear transfer (SCNT).

Most of the studies conducted with cells derived from hESC (52) focus on the treatment of diseases of the eye and adnexa (25/52 studies). Other studies treat diseases of the metabolic system (9/52 studies), the nervous system (5/52 studies), the circulatory system (3/52), the genitourinary system (2/52), the digestive system (1/52), the musculoskeletal system (1/52), as well as the treatment of malignant neoplasms (1/52), spinal cord injuries (3/52) and COVID-19 infections (2/52). The studies using hiPSC focus on the treatment of (malignant) neoplasms (18/57), diseases of the circulatory system (14/57) and diseases of the eye and adnexa (9/57). Clinical studies using cells derived from NT-hESC or hpPSC are in the minority and focus on the treatment of age-related macular degeneration and Parkinson's disease.

Table 3. Overview of clinical trials conducted or initiated worldwide on the basis of hPSC (2010-2022), 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. Source: 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 2022.

	Disease (according to ICD-10)	Number of studies	Participants (does not correspond to the number of patients treated)*
hESC	**Endocrinal, nutritional and metabolic diseases**	**9**	**427**
	Type 1 diabetes mellitus	7	394
	Primary ovarian insufficiency	1	28
	Urea cycle disorders	1	5
	Diseases of the eye and adnexa	**25**	**357**
	Age-related dry macular degeneration	12	186
	Retinitis pigmentosa	2	22
	Stargardt's disease	5	53

	Other eye disorders	6	96
	Diseases of the circulatory system	**3**	**58**
	Ischaemic heart disease	1	10
	Cerebral infarction, unspecified	1	30
	Left heart failure	1	18
	Diseases of the digestive system	**1**	**5**
	Acute and subacute liver failure	1	5
	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	**5**	**106**
	Motor neuron disease	1	16
	Primary Parkinson's disease	2	20
	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**	**8**
	Malignant neoplasm of the bronchi and lungs	1	8
	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
	hESC studies: Total	***52***	***1078***
hiPSC	**Diseases of the eye and adnexa**	**9**	**146**
	Age-related wet macular degeneration	2	7
	Age-related dry macular degeneration	1	20
	Hereditary retinal dystrophy: retinitis pigmentosa	1	2
	Retinal affection, unspecified	2	100
	Other corneal affections described in more detail	1	4
	Bullous keratopathy	1	3

Degeneration of the macula and posterior pole	1	10
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	**15**	**221**
Ischaemic cardiomyopathy	6	56
Cerebral infarction, unspecified	2	24
Cardiac insufficiency, unspecified	5	135
Dilated cardiomyopathy	2	6
Diseases of the nervous system	**3**	**24**
Primary Parkinson's disease	3	24
Neoplasms	**18**	**2239**
Malignant neoplasms	5	636
Head, face and neck	1	9
Myeloid leukaemia	1	234
B-cell chronic lymphatic leukaemia	2	849
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	3
Carcinoma in situ of other and unspecified genital organs	1	18
Thrombocytopenia, unspecified	1	10
Multiple myeloma	1	168
B-cell lymphoma, unspecified	1	75
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, level unspecified	1	4
Provisional classifications of diseases with unclear aetiology and unassigned code numbers	**2**	**29**
COVID-19	2	29
Endocrinal, nutritional and metabolic diseases	**2**	**50**

	Type 1 diabetes mellitus	1	20
	Unspecified diabetes mellitus – with diabetic foot syndrome	1	30
	Diseases of the musculoskeletal system and connective tissue	**2**	**444**
	Gonarthrosis, unspecified	2	444
	hiPSC studies: Total	***57***	***3192***
NT-hESC	**Diseases of the eye and adnexa**	**1**	**3**
	Age-related dry macular degeneration	1	3
	NT-hESC studies: Total	***1***	***3***
hpPSC	**Diseases of the nervous system**	**2**	**62**
	Primary Parkinson's disease	2	62
	hpPSC studies: Total	***2***	***62***
	Studies using hPSC: Total	**112**	**4335**

See Table 4 for an overview of the countries in which the clinical trials were/are being carried out. It becomes clear here that between 2010 and 2022 clinical trials based on hESC were carried out mainly in the USA, China, the UK and Canada; trials using cell products derived from hiPSC 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, France, Germany) have no or very little research activity in this field, although the different national regulations for clinical trials may also play a role here. In Germany, one clinical trial based on hiPSC is currently being conducted with the aim of treating cardiac insufficiency.

Table 4. Overview of the countries in which clinical trials based on hPSC have been or are being conducted or initiated (2010-2021; 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 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 2022.

	Country	Number of studies
hESC	USA	22
	China	12
	UK	7
	Canada	5
	Korea	3
	Israel	2
	France	2
	Brazil	1
	Japan	1
	Sweden	1
	unknown	1
hiPSC	Japan	23
	USA	16
	China	12
	Australia	4
	UK	1
	Iran	1
	Germany	1
NT-hESC	Korea	1
hpPSC	Australia	1
	China	1
	Total	**118***

3.4. Final remarks

As stated in the 2021 Report, it should be emphasized that the remarks made in previous ZES reports on the desiderata of the Stem Cell Act remain valid. Twenty-one years after the law came into force, the problems include the following:

- the cut-off date, now 16 years ago, which prevents imports of newer stem-cell lines relevant to research,
- the inadmissibility of important research whose sole aim is to develop procedures that could make it possible to reduce the number of animal experiments or replace them,
- the contradiction inherent in the Stem Cell Act's exemption for research, i.e. that hESC may be used in research projects but not for the subsequent application of research results. If hESC are required for this purpose, results of hESC research developed in this country may not be implemented in Germany for pharmacological and toxicological purposes or for the production of therapeutics for use in clinical practice.

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

The 20th Report was adopted at the 111th ordinary meeting of the ZES on 12 April 2023.